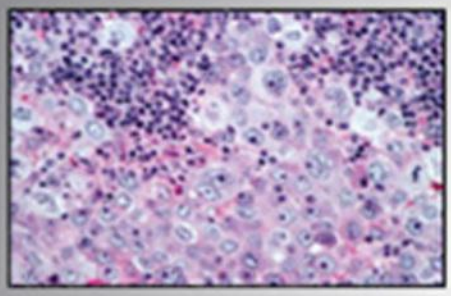
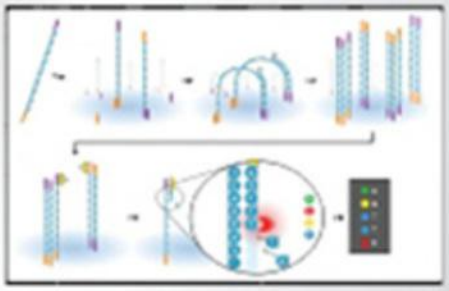
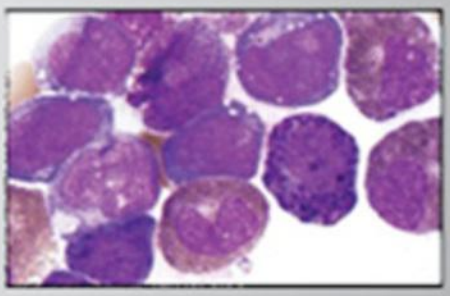
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To my teachers and mentors who inspired me,
our trainees who constantly challenge and question,
and my family who makes it all worthwhile.

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FOREWORD

As any practicing pathologist knows, it is not possible for any single individual to master all of the skills and knowledge required to perform all areas of surgical pathology at the highest level. Providing a correct diagnosis is challenging enough, but the standard of care has now far surpassed simply providing an accurate pathologic diagnosis. We are now asked to provide an enormous amount of ancillary information, both diagnostic and prognostic, often on minute tissue fragments, a task that can be daunting even for the most skilled and experienced pathologists.

Although general surgical pathology textbooks remain useful resources, they cannot possibly cover many of the detailed aspects that pathologists need to know and include in their diagnostic reports. For this reason, *Foundations in Diagnostic Pathology* was initiated. This series of books is designed to cover the major areas of surgical pathology, with each volume focusing on one major topic. The goal of every book in this series, which is now in its third edition, is to provide the essential information that any pathologist—whether general or subspecialized, in training or in practice—would find useful in the evaluation of virtually any type of specimen encountered.

My colleague of many years at Cleveland Clinic, Dr. Eric Hsi, has edited this outstanding book on hematopathology that fulfills the goals and philosophy behind *Foundations in Diagnostic Pathology*. This third edition cuts to the essentials of what all practicing pathologists want and need to know about this rapidly evolving group of neoplastic and non-neoplastic disorders. As always, the list of contributors is impressive and includes a who's who of hematopathologists, all of whom were extremely generous in providing their time and expertise to this book. By design, there is uniformity in the organization of these chapters, each of which includes practical information and numerous photographs that emphasize the essential diagnostic points. Importantly, there is seamless integration of ancillary diagnostic techniques including immunohistochemistry, cytogenetics, and molecular techniques, which are essential to the modern practice of hematopathology.

The book is organized into four major sections: Non-Neoplastic Disorders; Lymphomas; Leukemias, Chronic Myeloproliferative Neoplasms, and Myelodysplasia; and finally Disorders of Histiocytes, Mast Cells, Plasma Cells, Spleen, and Ancillary Techniques. This edition features 24 chapters written by leaders in the field, and each chapter cuts to the heart of what practicing pathologists need to know.

I would like to extend my deepest appreciation to Dr. Eric Hsi and all of the authors who have contributed to this outstanding third edition of the *Foundations in Diagnostic Pathology* series. I strongly believe this edition improves on what was already an exceptional volume in this series, and I am confident you will enjoy this edition of the *Foundations in Diagnostic Pathology* series.

John R. Goldblum, MD

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PREFACE

Since the first edition of this text in 2007, classifications have evolved to incorporate much more information that has an impact on the diagnosis and therapy of hematologic diseases. I am honored to have been asked to embark on a third edition of this book. New authors have been added and chapters updated and revised. I am pleased to have incorporated a more complete treatment of coagulation that serves to differentiate this book as one that now covers the spectrum of non-neoplastic hematopathology, neoplastic hematopathology, and coagulation. I believe this text will give the reader a solid foundation for learning and practicing hematopathology. Importantly, it incorporates information and classifications relevant to the long-awaited 2016 WHO update. As in previous editions, I am indebted to the numerous expert contributors who have given of themselves to produce excellent and concise chapters (with a minimal amount of nagging from me …). They are the true resource and talent for this book, and it has been an honor to work with this group of outstanding pathologists and educators.

Eric D. Hsi, MD

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SECTION 1

Non-Neoplastic Disorders

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1

Red Blood Cell/Hemoglobin Disorders*

■ Juehua Gao, MD, PhD ■ Sara A. Monaghan, MD

■ INTRODUCTION

The disorders of red blood cells can be divided into those of decreased red blood cell (RBC) mass (anemias) and those of increased RBC mass (erythrocytoses). Most of this chapter is devoted to various types of anemias, with only a short discussion of erythrocytosis.

From the pathophysiologic standpoint, anemias can be divided into three categories (Box 1.1): anemias of blood loss, anemias of decreased RBC production, and anemias caused by increased RBC destruction (hemolysis). Each of these categories can be subdivided further, as illustrated in Box 1.1 and subsequent sections.

This chapter focuses on commonly encountered conditions. Various rare causes of anemia and some disorders lacking characteristic changes in peripheral blood or bone marrow are omitted.

■ ANEMIAS OF BLOOD LOSS

Blood loss, the most common cause of anemia, can occur as obvious bleeding from trauma, occult bleeding (e.g., gastrointestinal ulcers, uterine fibroids), induced bleeding secondary to surgery, hemodialysis, or repeated diagnostic testing. Blood loss can be acute or chronic. The anemia of acute blood loss, such as that associated with major trauma, is not immediately evident based on complete blood cell count (CBC) parameters because losses consist of whole blood, and the vascular system contracts on the decreased blood volume. However, with intravascular volume repletion, the erythrocytes will be diluted and the degree of RBC and hemoglobin (Hb) loss will be manifest on the CBC.

Chronic, low-level blood loss is initially compensated by increased marrow RBC production; therefore no anemia will be present, although reticulocytosis may be evident. With prolonged blood loss, body iron stores become depleted, the iron-deficient marrow is no longer able to compensate, and iron deficiency anemia (IDA) develops. Thus the anemia of chronic blood loss is essentially IDA (see the following section).

■ ANEMIAS OF DECREASED RED BLOOD CELL PRODUCTION

Anemias of decreased red blood cell production can be divided into those due to ineffective erythropoiesis and those due to a decrease in effective RBC production.

INEFFECTIVE ERYTHROPOIESIS

Anemias of ineffective erythropoiesis are characterized in general by a failure of the marrow to produce adequate mature erythrocytes, despite erythroid hyperplasia in the marrow, and often demonstrate morphologic abnormalities in erythroid precursors, mature RBCs, or both. The defect may be primarily one of nuclear maturation (megaloblastic anemia) or cytoplasmic maturation (iron deficiency). Myelodysplastic syndromes, although often characterized by ineffective erythropoiesis, are discussed in Chapter 18.

MEGALOBLASTIC ANEMIA

The megaloblastic anemias are macrocytic anemias associated with characteristic morphologic features of hematopoietic cells. Cytopenias are due to impaired deoxyribonucleic acid (DNA) synthesis, which leads to abnormal nuclear maturation of hematopoietic precursors and ineffective hematopoiesis. The major causes of megaloblastic anemia are folate deficiency and cobalamin (Cbl; vitamin B_{12}) deficiency. Folate in the form of tetrahydrofolate (THF) is a transporter of 1-carbon units, used for synthesis of purines, thymidine, and methionine

*We gratefully acknowledge the contribution of Steven H. Kroft, MD, who co-authored this chapter in previous editions.

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Box 1.1 Classification of Red Blood Cell Disorders

Anemias

- Anemias of blood loss
  - Acute
  - Chronic
- Anemias of decreased red blood cell production
  - Ineffective erythropoiesis
    - Megaloblastic anemia
    - Iron deficiency
    - Myelodysplastic syndromes
    - Sideroblastic anemia
    - Congenital dyserythropoietic anemia
  - Decreased effective erythropoiesis
    - Stem cell defects
    - Marrow replacement processes
    - Anemia of chronic kidney disease
    - Anemia of chronic disease
- Hemolytic anemias
  - Intrinsic red cell defects
    - Hemoglobin disorders
      - Structural hemoglobin disorders
        - Sickling diseases
        - Hemoglobin C disease
      - Thalassemias
    - Red cell membrane disorders
      - Hereditary spherocytosis
      - Hereditary elliptocytosis
      - Hereditary pyropoikilocytosis
    - Red cell enzyme disorders
      - Glucose-6-phosphate dehydrogenase deficiency
    - Paroxysmal nocturnal hemoglobinuria
  - Extrinsic red cell defects
    - Autoimmune hemolytic anemia
      - Warm
      - Cold
    - Microangiopathic hemolytic anemia
    - Parasitic hemolysis
      - Malaria
      - Babesia

Erythrocytosis

- Relative erythrocytosis (decreased plasma volume)
- Absolute erythrocytosis (polycythemia)
  - Primary
    - Polycythemia vera
    - Primary familial polycythemia
  - Secondary
    - Chronic hypoxia
      - Cardiac disorders
      - Pulmonary disorders
      - Sleep apnea
      - High-altitude residence
    - Hemoglobin abnormalities
      - High-oxygen-affinity hemoglobins
      - Increased carboxyhemoglobin (smokers)
    - Aberrant erythropoietin production by tumors
    - Other rare congenital causes of secondary polycythemia (e.g., Chuvash polycythemia)

(Fig. 1.1). Cbl is a required intermediate in an intracytoplasmic reaction that converts methyltetrahydrofolate, the main form of folate transported in plasma and taken up by cells, into THF while transferring the methyl group to homocysteine to generate methionine. Therefore either folate or Cbl deficiency impairs thymidine and methionine synthesis. Cbl also serves as a cofactor for intramitochondrial conversion of methylmalonyl-coenzyme A to succinyl-coenzyme A (not illustrated).

Because Cbl is produced in nature only by microorganisms, humans must obtain it from their diet. Food sources include red meat, seafood, and dairy products. After ingestion, Cbl is released from food in the stomach in the presence of pepsin and gastric acid and then binds to haptocorrins (R-proteins) secreted in saliva. However, in the alkaline milieu of duodenum, haptocorrins are degraded by pancreatic enzymes, permitting Cbl to bind to intrinsic factor secreted by gastric parietal cells. Intrinsic factor–Cbl binds to receptors specific for this complex on ileal enterocytes and is absorbed. A small proportion of Cbl can also passively diffuse through gastrointestinal mucosa. A fraction of Cbl is bound to the transporter protein, transcobalamin II (TC II), in the plasma and is the biologically active component available for receptor-mediated cellular uptake; however, a larger proportion of plasma Cbl is bound to haptocorrins. In summary, the Cbl absorption depends on adequate dietary intake, gastric acid and pepsin, pancreatic proteases, gastric-derived intrinsic factor, and ileal receptors for intrinsic factor–Cbl complex. Disruption of any factor along this pathway may lead to Cbl deficiency.

The body stores a large amount of Cbl; therefore it may take several years for deficiency to develop, even with the onset of severe dietary insufficiency or malabsorption. Cbl deficiency is most often caused by malabsorption. Autoimmune gastritis resulting in a lack of intrinsic factor (i.e., pernicious anemia) is a common cause, but malabsorption of Cbl may also be due to other gastric disease (e.g., atrophic gastritis, *Helicobacter pylori* infection), ileal disease (e.g., inflammatory bowel disease, tropical sprue), postsurgical malabsorption, pancreatic insufficiency, drugs (e.g., proton pump inhibitors, histamine H_2–receptor agonists, biguanides), chronic alcoholism, and competition for dietary Cbl (e.g., intestinal bacterial overgrowth, fish tapeworm). Overt Cbl deficiency is rarely due to isolated dietary insufficiency, but a devastating exception to this occurs among exclusively breast-fed infants of mothers who have subclinical Cbl insufficiency that may be related to a vegan diet or occult malabsorption. These infants have increased risk for neural tube defects including spina bifida and anencephaly. In rare cases, Cbl deficiency can be caused by congenital deficiency of TC II.

In contrast to Cbl, the amount of folate body stores is sufficient for only about 4 months, and, thus folate deficiency is more often related to a poor diet. Important dietary sources for folate include leafy vegetables, fruit, cereals, and fortified flour and grains. Other causes of folate deficiency are increased demand (e.g., pregnancy, prematurity, chronic hemolytic anemia, severe exfoliative dermatitis, leukemias), loss due

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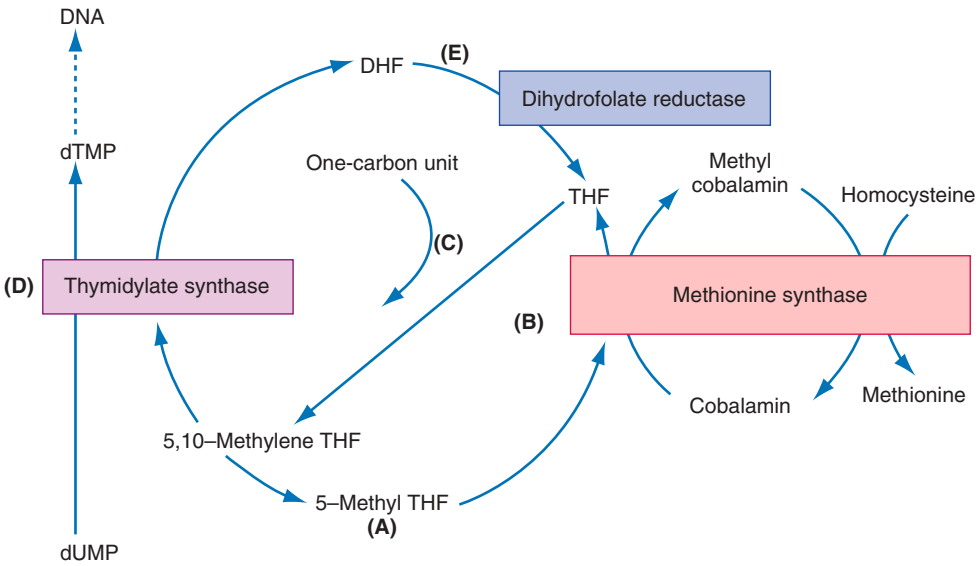


FIG. 1.1

Folate and cobalamin in the synthesis of DNA and methionine. Folates conjugated with multiple glutamic acid residues (folate polyglutamates) are deconjugated during absorption in the duodenum and jejunum. Folate monoglutamates then undergo conversion to N5-methyl tetrahydrofolate *(N5-methyl THF),* the main form of folate transported in plasma and taken up by cells (A). Demethylation of N5-methyl THF (B) yields tetrahydrofolate *(THF),* which undergoes polyglutamation needed for cellular retention of folate. This methyltransferase reaction requires cobalamin as an intermediate with transfer of the methyl group to homocysteine to generate methionine. THF, as an intermediate, accepts a one-carbon unit (C). The folate derivative N5,N10-methylene THF donates the one-carbon unit to the reaction catalyzed by thymidylate synthase (D). Both thymidylate *(dTMP),* a required component for DNA synthesis, and dihydrofolate (DHF) are produced. Dihydrofolate reductase reduces DHF back to THF (E).

to hemodialysis, drugs, and competition for dietary folates (intestinal bacterial overgrowth). Malabsorption of folate is rare unless there is extensive intestinal disease (e.g., gluten-induced enteropathy, tropical sprue).

Clinical Features

The hematologic manifestation of Cbl and folate deficiency is megaloblastic anemia. The onset of anemia is gradual, and the severity at presentation is extremely variable. Some patients exhibit profound macrocytic anemia with Hb levels as low as 2 g/dL, whereas other patients may have a more subtle manifestation or present with symptoms other than anemia. Due to defective DNA synthesis, ineffective erythropoiesis or intramedullary hemolysis can lead to jaundice, markedly increased lactate dehydrogenase, and increased indirect bilirubin. The ineffective hematopoiesis and hemolysis may be so pronounced that haptoglobin is reduced and hemosiderinuria occurs. The mean corpuscular volume (MCV) is characteristically high, with clinically overt megaloblastic anemia, but may also become elevated before the development of detectable anemia. However, the MCV may be in the normal range if there is concurrent iron deficiency anemia or thalassemia. The reticulocyte count is low. Leukopenia and thrombocytopenia are often present because impairment of DNA synthesis affects all proliferating cells.

The effect of Cbl deficiency on epithelial cells may result in the classic "beefy red smooth tongue" (atrophic glossitis). More important, Cbl deficiency is often associated with a peripheral neuropathy and demyelinization of the dorsal and lateral columns of the spinal cord (i.e., subacute combined degeneration), leading to paresthesias, loss of position and vibration sense, spastic ataxia, and extensor plantar reflexes. Cognitive impairment may be seen; psychosis is a rare complication. Neurologic complications of Cbl deficiency may be only partially reversible, making early recognition of this disorder essential.

Pathologic Features

In clinically overt megaloblastic anemia, the peripheral blood film demonstrates marked variability in red cell size (anisocytosis), with large, oval red cells (macroovalocytes), teardrop cells, fragments, microcytes, and other nonspecific abnormally shaped red cells (poikilocytes; Fig. 1.2). Polychromasia is characteristically absent. With increasing severity, there is increased red cell fragmentation. Howell-Jolly bodies, nucleated RBCs with megaloblastic morphology, and basophilic stippling are often observed (Fig. 1.3). Hypersegmented neutrophils with six or more nuclear lobes, in combination with macrocytic anemia, are highly specific for megaloblastic anemia (Fig. 1.4).

Bone marrow aspirate smears typically demonstrate erythroid hyperplasia and a shift to immaturity, as well as characteristic cytologic changes in all three myeloid lineages. Maturing cells are larger than normal, with asynchronous nuclear and cytoplasmic maturation. The chromatin of the erythroid precursors appears more granular than during normal

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MEGALOBLASTIC ANEMIA—FACT SHEET

Definition

- Macrocytic anemia due to folate deficiency, cobalamin deficiency, and other causes, resulting in impaired DNA synthesis, abnormal nuclear maturation, and ineffective hematopoiesis

Incidence and Location

- Folate deficiency is rare in countries with mandatory food fortification
- Dietary cobalamin deficiency is rare, except among vegans and populations with low intake of animal-source foods

Morbidity and Mortality

- Increased risk of fetal neural tube defects in folate/Cbl-deficient pregnancy
- Peripheral neuropathy, subacute combined degeneration of the spinal cord, and cognitive abnormalities that may be only partially reversible (cobalamin deficiency)
- Increased risk of gastric adenocarcinoma and carcinoid tumor (patients with pernicious anemia)

Prevalence

- Prevalence estimates based on low red blood cell (<140 ng/mL) and serum (<3 ng/mL) folate levels among women of childbearing age in the United States is 4.5% and 0.3%, respectively (2005–2006 National Health and Nutrition Examination Survey)
- Increased incidence of cobalamin deficiency in breast-fed infants and young children among populations at risk of dietary insufficiency (strict vegans and developing countries)
- Prevalence of cobalamin deficiency owing to malabsorption increases with age

Clinical Features

- Gradual onset of macrocytic anemia
- Evidence of hemolysis (jaundice, elevated lactate dehydrogenase, elevated total bilirubin, low haptoglobin)
- Atrophic glossitis
- Neuropsychiatric effects (cobalamin deficiency)
  - Paresthesias
  - Loss of position and vibration sense
  - Ataxia
  - Cognitive impairment (impaired memory, dementia)
  - Psychosis (rare)

Prognosis and Therapy

- Treatment of the underlying cause of the deficiency
- Intramuscular injection (or large oral doses) of vitamin B_{12}
- Oral folate supplementation (parenteral administration needed in some cases)

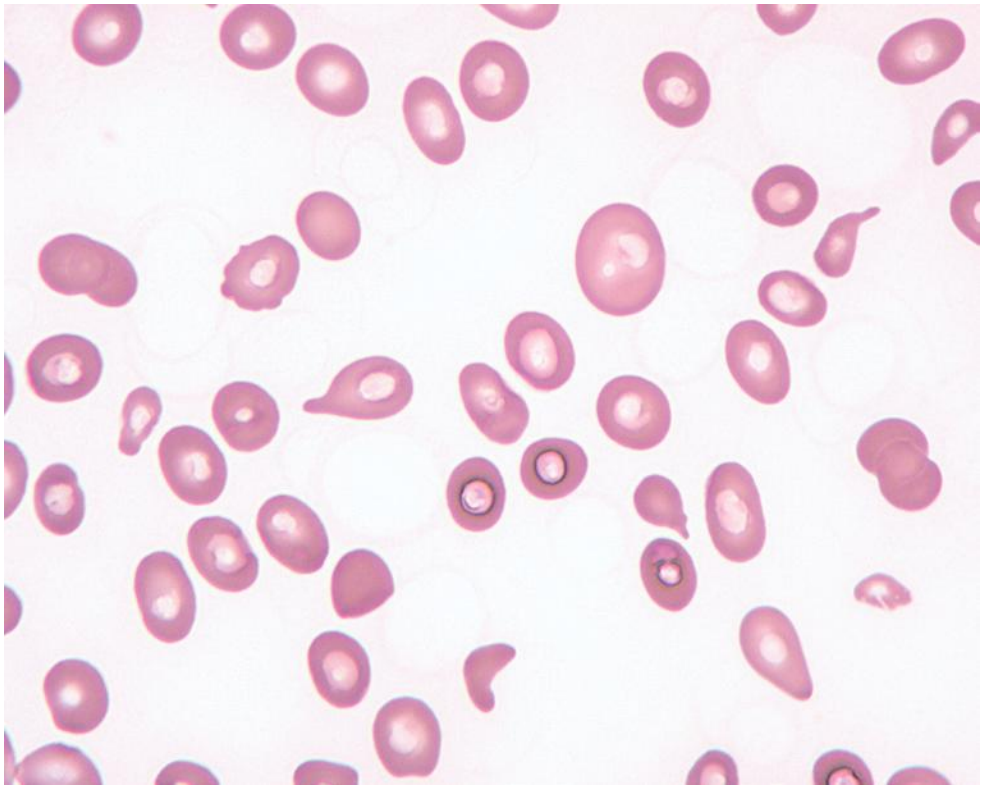


FIG. 1.2

Megaloblastic anemia, peripheral blood findings. This blood smear from a patient with severe megaloblastic anemia demonstrates prominent anisopoikilocytosis, including large oval cells (oval macrocytes), microcytes, and teardrop cells.

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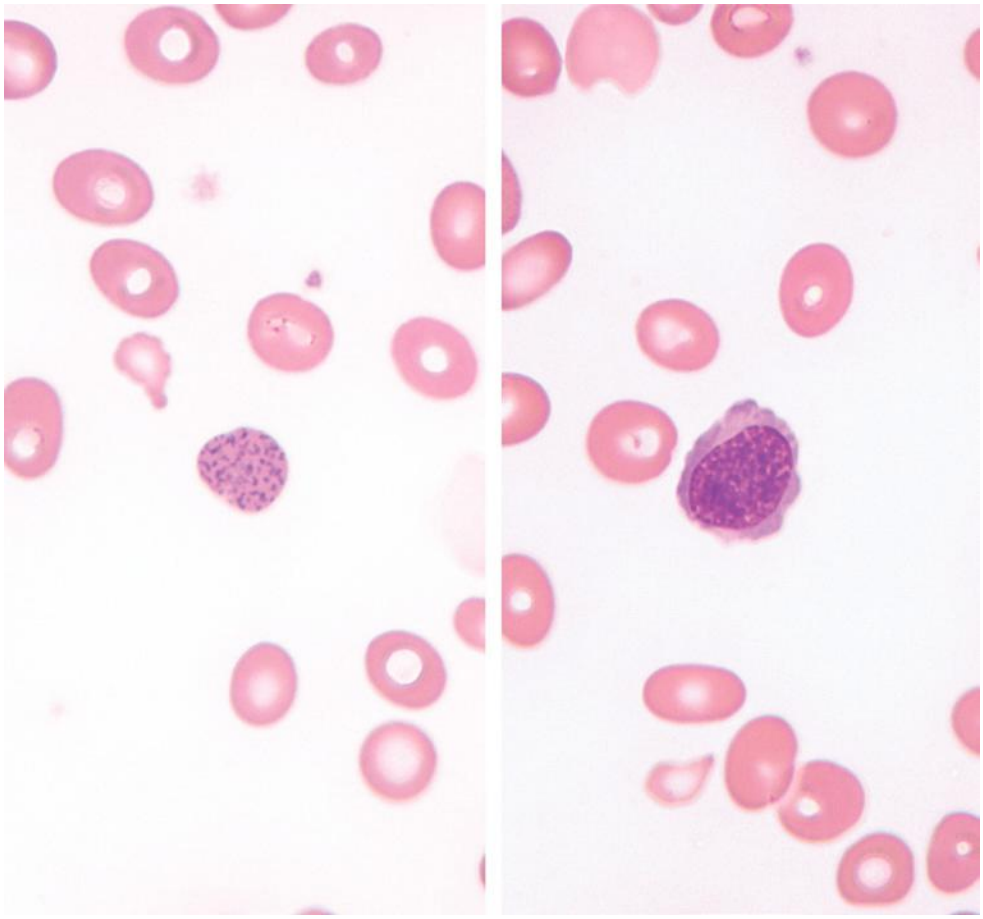


FIG. 1.3

Megaloblastic anemia, peripheral blood findings. Coarse basophilic stippling *(left)* and circulating nucleated red cells *(right)* are common in megaloblastic anemia. The red cell precursor is relatively mature, judging by the pink-gray quality of the cytoplasm. However, the chromatin is quite open, with an abnormal stippled appearance, indicating megaloblastic change.

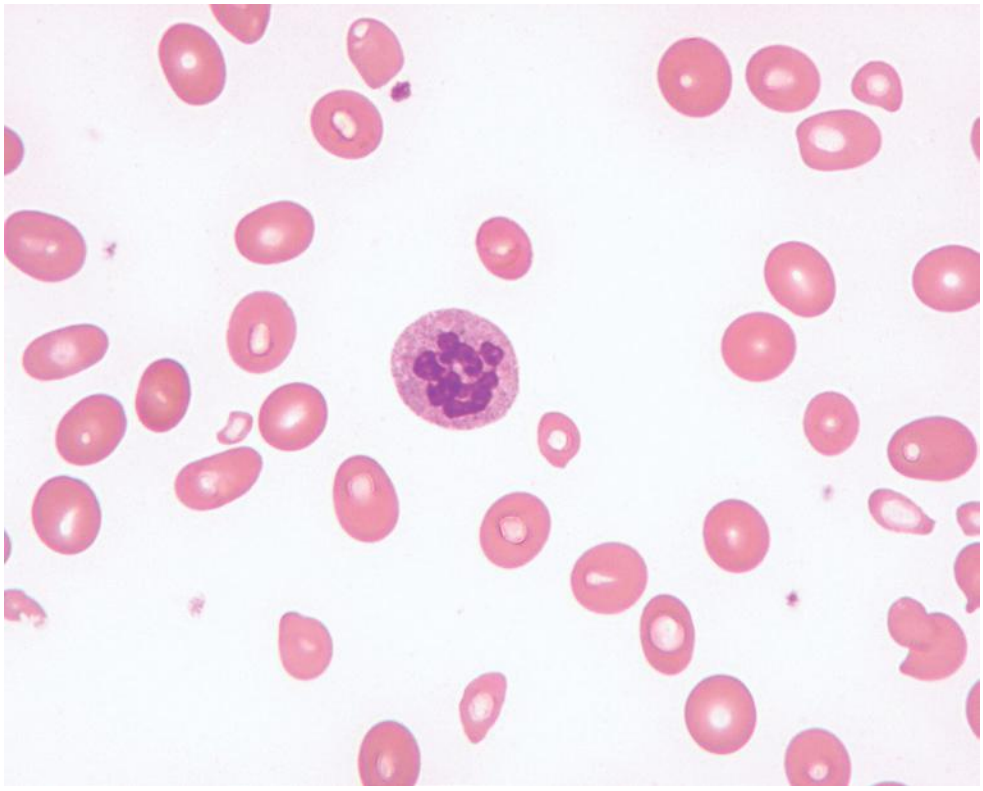


FIG. 1.4

Megaloblastic anemia, peripheral blood findings. A hypersegmented neutrophil in the peripheral blood of a patient with megaloblastic anemia. Neutrophils are generally considered hypersegmented when they contain six or more lobes.

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MEGALOBLASTIC ANEMIA—PATHOLOGIC FEATURES

Peripheral Blood

- Anemia, often pancytopenia
- Prominent anisocytosis
- Poikilocytosis
  - Macroovalocytes
  - Teardrop cells
  - Fragments
- Lack of polychromasia
- Hypersegmented neutrophils
- Howell-Jolly bodies, nucleated red cells, basophilic stippling

Bone Marrow

- Hypercellular marrow with trilineage expansion
- Left-shifted hematopoietic precursors
- Asynchronous nuclear to cytoplasmic maturation
  - Megaloblastic erythroid precursors
  - Giant metamyelocytes
  - Larger than normal megakaryocytes with abnormal nuclei

Differential Diagnosis

- Other causes of megaloblastic hematopoiesis
- Drugs
- Some myelodysplastic syndromes
- Nonmegaloblastic macrocytosis
  - Reticulocytosis
  - Liver disease
  - Alcohol
  - Hypothyroidism
  - Drugs

maturation, and the most mature erythroid precursors display a prominent fenestrated nuclear appearance that contrasts with the expected complete condensation of chromatin at this stage (Fig. 1.5). Erythroid precursors with abnormally shaped, multiple, budding, or fragmented nuclei are often seen (Fig. 1.6), resembling those seen in myelodysplastic syndromes. Giant metamyelocytes and bands with abnormally open chromatin are also characteristic (Figs. 1.5 and 1.7), whereas larger than normal megakaryocytes with unattached nuclear lobes or hyperlobated nuclei reflect similar abnormalities in megakaryocytic maturation. Because of the pronounced ineffective hematopoiesis, markedly increased iron stores are seen with a Prussian blue iron stain unless there is concurrent iron deficiency. Bone marrow core biopsy specimens demonstrate a markedly hypercellular marrow with expanded and left-shifted trilineage hematopoiesis (Fig. 1.8). In particular, islands of immature erythroid precursors with finely stippled chromatin and distinct, irregular, eosinophilic nucleoli are often prominent (Fig. 1.9).

Ancillary Studies

Serum Cbl is fairly sensitive and specific for clinically overt Cbl deficiency. Causes of falsely low serum Cbl include pregnancy, use of oral contraceptives, anticonvulsant administration, human immunodeficiency virus infection, and folate deficiency. Mixed Cbl/folate deficiency complicates the

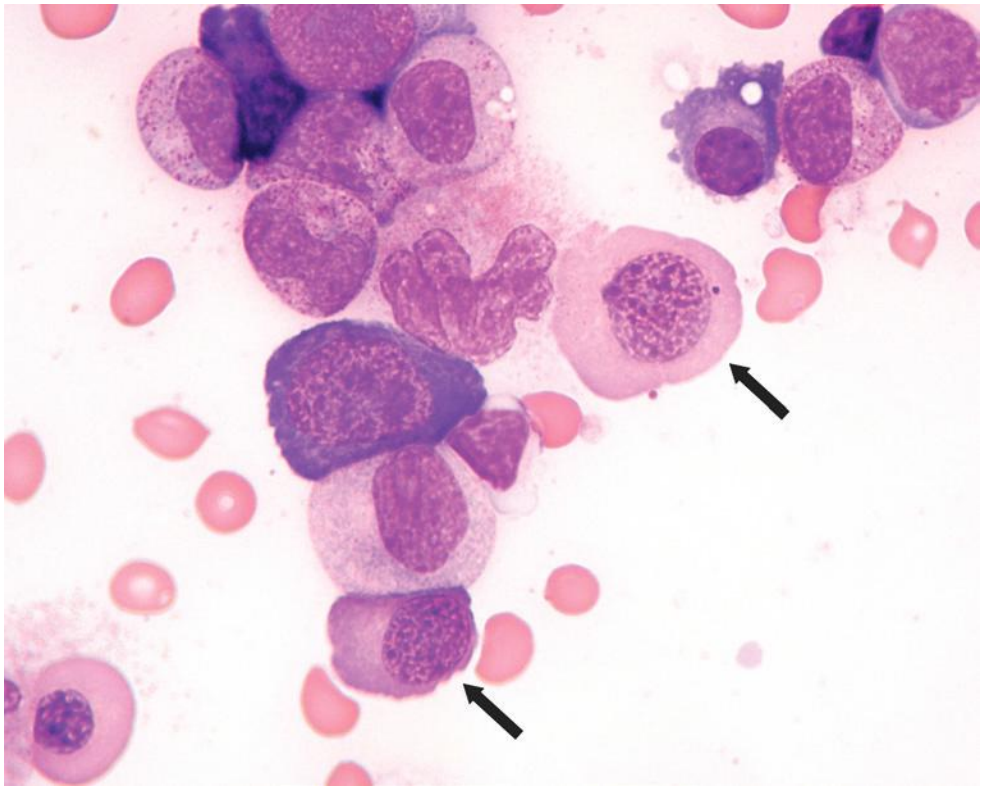


FIG. 1.5

Megaloblastic anemia, bone marrow aspirate smear findings. This marrow aspirate smear image from a patient with severe megaloblastic anemia demonstrates two late-stage erythroid precursors (based on an advanced degree of cytoplasmic hemoglobinization) that are abnormally large and have dispersed, stippled megaloblastic chromatin *(arrows)*. Also present is a giant granulocyte at approximately the metamyelocyte stage with abnormally dispersed chromatin (immediately to the left of upper arrowed megaloblast).

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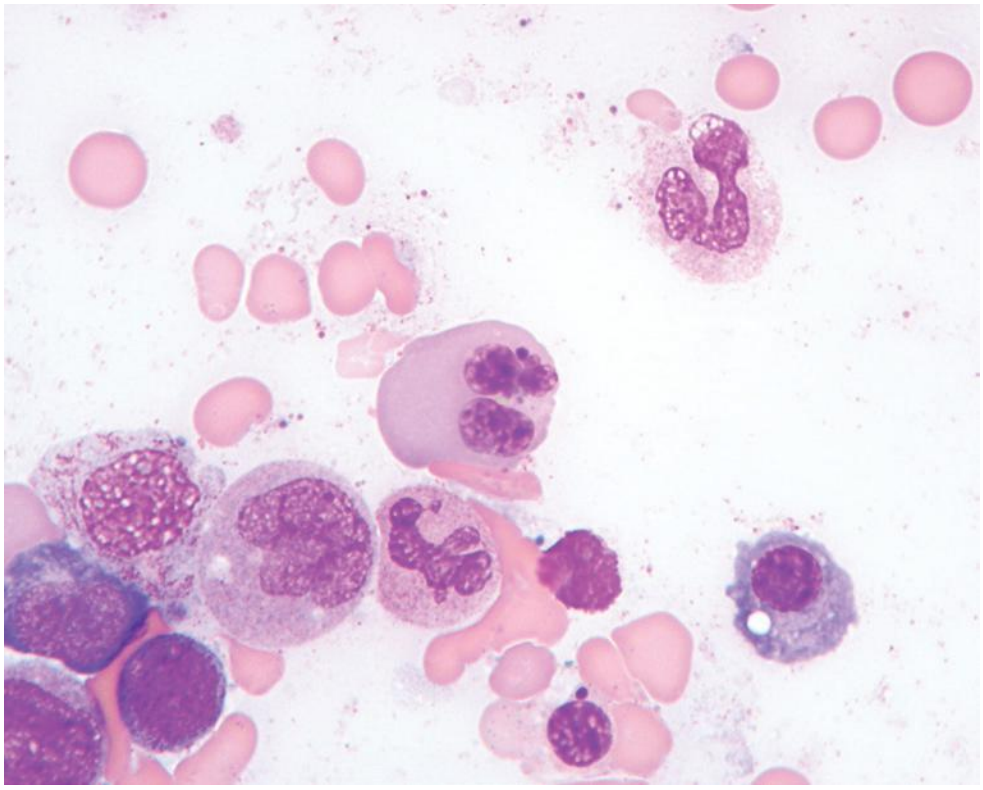


FIG. 1.6

Megaloblastic anemia, bone marrow aspirate smear findings. This image demonstrates a dysplastic-appearing orthochromic erythroid precursor with two nuclei and irregularly distributed chromatin.

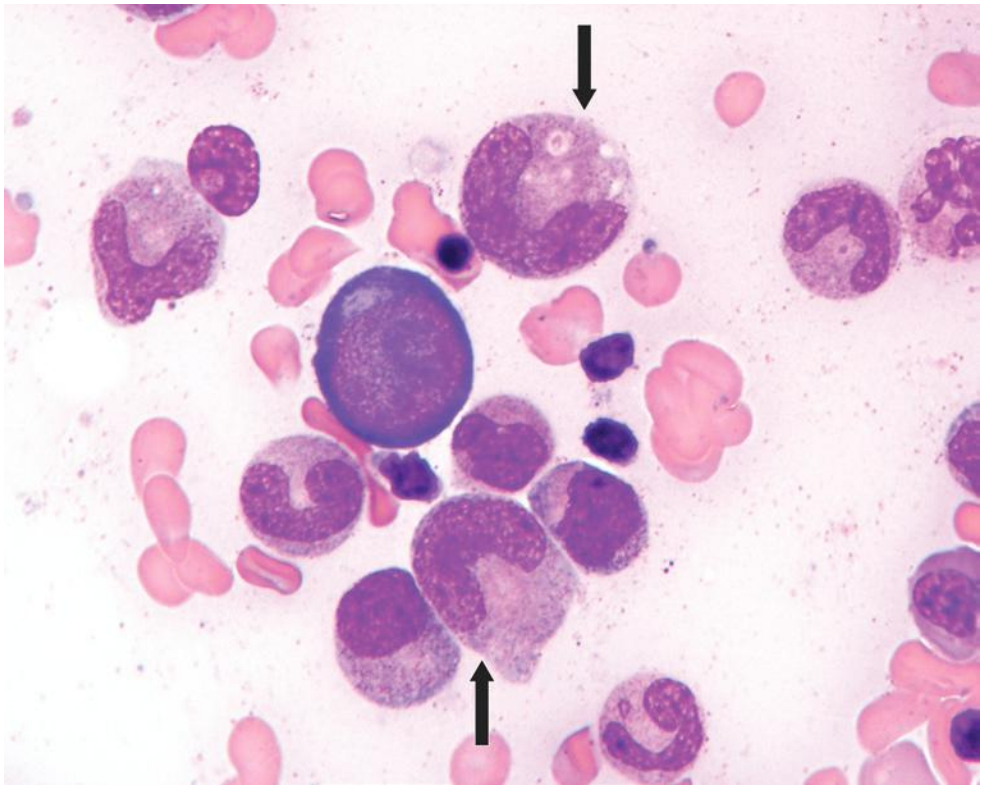


FIG. 1.7

Megaloblastic anemia, bone marrow aspirate smear findings. This marrow aspirate from the same patient as in Fig. 1.6 demonstrates two giant bands with abnormally dispersed megaloblastic chromatin. Note that these cells *(arrows)* are larger than the early basophilic normoblast in the field and other granulocytes in the field.

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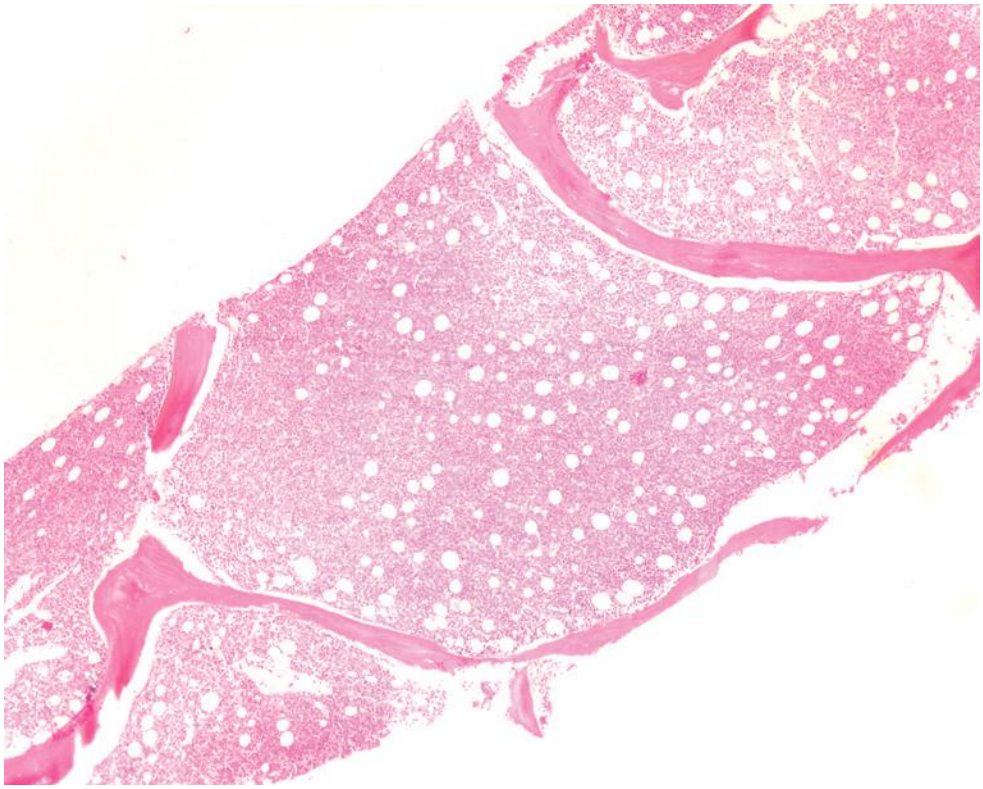


FIG. 1.8

Megaloblastic anemia, bone marrow biopsy findings. The bone marrow biopsy in megaloblastic anemia is hypercellular.

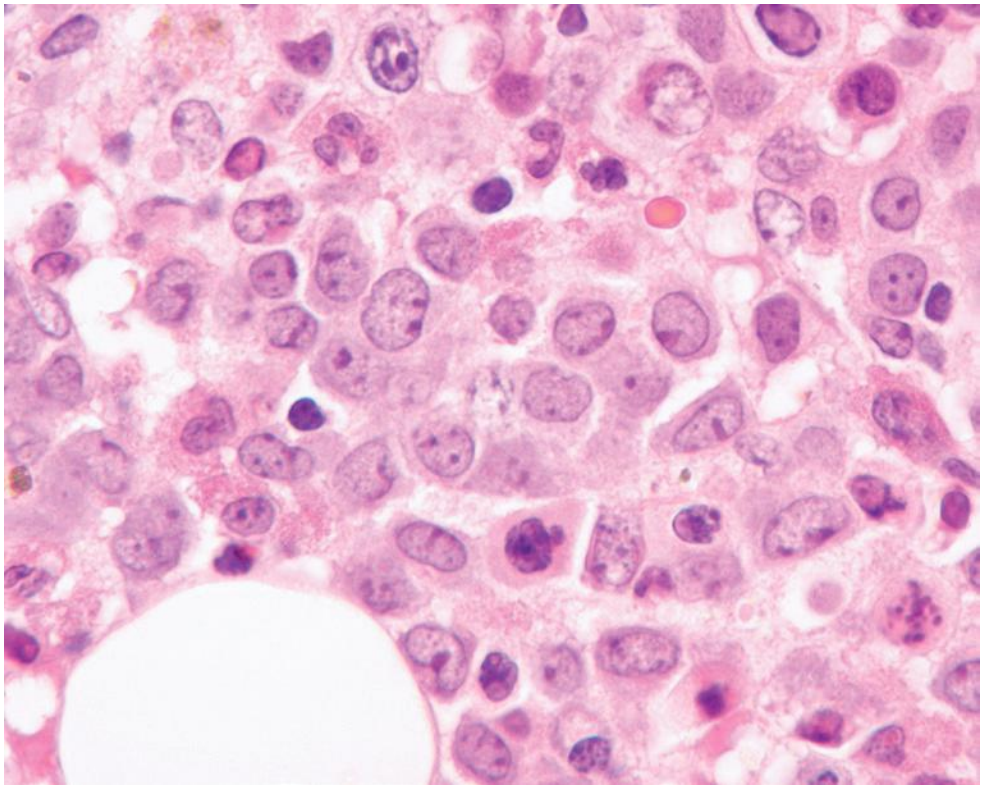


FIG. 1.9

Megaloblastic anemia, bone marrow biopsy findings. Prominent proliferations of immature erythroid precursors with fine chromatin and distinct nucleoli can be mistaken easily for acute leukemia or large cell lymphoma in biopsy sections.

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TABLE 1.1
Ancillary Tests Used to Evaluate Possible Megaloblastic Anemia

	B_{12} Deficiency	Folate Deficiency	Mixed B_{12}/Folate Deficiency	Myelodysplastic Syndrome
Serum folate	Normal/high	Low/normal	Low	Normal
Red blood cell folate	Low/normal	Low	Low	Normal
Serum cobalamin	Low/normal	Normal/low	Low	Normal/low
Methylmalonic acid	High	Normal	High	Normal
Homocysteine	High	High	High	Normal

interpretation of the laboratory workup of megaloblastic anemia (Table 1.1). Falsely normal levels of Cbl are rare with clinically overt deficiency but may be observed in association with myeloproliferative disorders, liver disease, intestinal bacterial overgrowth, and congenital TC II deficiency. Detection of antibodies to intrinsic factor helps to determine the cause of a Cbl deficiency and to confirm a diagnosis of pernicious anemia.

Folate status may be assessed by either serum or red cell folate assays. Serum folate may occasionally be misleading due to fluctuations related to short-term dietary changes. Hemolysis (in vivo and in vitro) will cause falsely elevated serum folate, whereas alcohol intake can cause transient low levels despite adequate stores. Red cell folate levels better reflect body folate stores and are less prone to short-term fluctuation. Importantly, Cbl deficiency can cause reduced red cell folate levels, but this is usually associated with normal or increased serum levels (see Table 1.1).

Measurements of serum homocysteine and methylmalonic acid are also useful in the assessment of megaloblastic anemia. Homocysteine accumulates in both Cbl and folate deficiency because of interruption of the biochemical pathway illustrated in Fig. 1.1. Unlike folate deficiency, Cbl deficiency is associated with an increase in serum methylmalonic acid because of interruption of the conversion of methylmalonyl-coenzyme A to succinyl-coenzyme A. A normal methylmalonic acid level generally rules out Cbl deficiency; however, both metabolites may be mildly elevated with renal insufficiency. Elevated serum homocysteine may also be seen with vitamin B_6 deficiency, vitamin B_2 (riboflavin) deficiency, hypothyroidism, some drugs, and genetic mutations affecting metabolism of homocysteine.

Differential Diagnosis

The major differential diagnoses of megaloblastic anemia owing to folate or Cbl deficiency are megaloblastic changes related to drug therapy and myelodysplastic syndromes. A variety of agents that interfere with DNA synthesis can cause megaloblastic changes in maturing marrow elements, including methotrexate, zidovudine, hydroxyurea, and azathioprine. Megaloblastic morphology due to drugs tends to be confined to the erythroid compartment, although this may not be the case with long-term administration. In general, an accurate drug history and biochemical tests for folate and Cbl deficiency will distinguish this cause of megaloblastosis.

Megaloblastic anemia may be confused with myelodysplastic syndromes because of the potential for dyserythropoiesis in megaloblastic anemia or the potential for megaloblastic changes in myelodysplastic syndromes. However, when megaloblastic changes are seen in the erythroid elements of myelodysplastic syndromes, they are generally accompanied by a spectrum of other abnormalities that are not a feature of megaloblastic anemia, such as neutrophil hyposegmentation or hypogranularity, hypogranular platelets, hypolobated megakaryocytes, or increased blasts. Furthermore, giant bands and metamyelocytes as seen in megaloblastic anemia are usually not a notable feature of myelodysplastic syndromes. As a general rule, a very high MCV (more than 130 fL) would favor a megaloblastic anemia due to Cbl or folate deficiency versus other causes.

In addition to other causes of megaloblastosis, nonmegaloblastic macrocytosis can also enter into the differential diagnosis of megaloblastic anemia. These diagnoses include reticulocytosis, liver disease, alcohol abuse, drug effects, and hypothyroidism. Reticulocytosis is easily discerned either with a reticulocyte count or with prominent polychromasia on blood smear examination. Other causes of nonmegaloblastic macrocytosis do not produce the degree of anisopoikilocytosis, oval macrocytes, or hypersegmented neutrophils seen in megaloblastic anemia. Liver disease, in particular, is characterized by a uniform population of round macrocytic cells and target cells.

Prognosis and Therapy

Standard therapy for Cbl deficiency, when the cause is not dietary deficiency, has traditionally been intramuscular injection of vitamin B_{12}; however, oral therapy is effective for some patients. To prevent adverse neurologic consequences because of concurrent or overlooked Cbl deficiency, Cbl status

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must be established before folate therapy is initiated. This may be difficult to do in practice because folate deficiency can cause low serum Cbl for reasons that are not well understood. If there is any doubt, Cbl supplementation should be administered along with folate therapy. Standard therapy for folate deficiency is oral supplementation. Parenteral administration of folate may be needed in cases caused by intractable malabsorption.

IRON DEFICIENCY ANEMIA

A sufficient iron supply is important for many biologic processes, but only impairment of Hb production and erythropoiesis owing to iron deficiency are addressed in this chapter. Iron serves as the ligand for binding of molecular oxygen to the porphyrin ring of heme. Anemia occurs from the inability of erythroid precursors to synthesize adequate Hb. Two stages of iron deficiency preceding anemia have been described. The first stage is iron depletion, and it is the earliest period during which other physiologic processes may be affected before an effect on erythropoiesis. Iron-deficient erythropoiesis then begins and, because of the longevity of the iron-replete red cells in circulation, continues for some time before development of overt anemia.

The risk of developing IDA depends heavily on age, diet, and regional or socioeconomic factors. The rapid utilization of iron during infancy, early childhood, and pregnancy as well as blood loss during childbirth and menstruation greatly increases the risk of IDA. Iron deficiency is widely accepted as the most common contributing cause for anemia overall. National Health and Nutrition Examination Surveys (2003 to 2006) from the United States estimated that iron deficiency was present in 14% of children 1 to 2 years old, 3.7% to 4.5% of children 3 to 5 years old, and 9% to 16% of nonpregnant females 12 to 49 years old. Inadequate dietary intake of iron is more frequent among impoverished populations. Parasitic infections, especially caused by hookworms and schistosomiasis, result in blood loss and are major contributing factors for IDA in some developing countries. In affluent societies, chronic blood loss, sometimes occult because of malignancy, is by far the most common cause of IDA. Malabsorption of iron can be due to gastrectomy, bariatric surgery, atrophic gastritis, *Helicobacter pylori* infection, and celiac disease.

IRON DEFICIENCY ANEMIA—FACT SHEET

Definition
- Anemia owing to inadequate iron supply

Incidence and Location
- Most common cause of anemia worldwide

Morbidity and Mortality
- Reduced quality of life and reduced productivity
- Probably increased maternal and fetal–neonatal morbidity and mortality
- Probably reduced cognitive development in children

Gender, Race, and Age Distribution
- Increased incidence in infants, children, women of childbearing age, and impoverished populations, particularly in underdeveloped countries
- Increased incidence in some minorities compared with white population in the United States

Clinical Features
- General signs and symptoms of chronic anemia
- Angular cheilitis, glossitis, pica, koilonychia, and esophageal webs

Prognosis and Therapy
- Oral iron supplementation
- Parenteral iron in patients with uncontrolled blood loss, intolerance to oral iron, or intestinal malabsorption

Clinical Features

Most signs and symptoms are those seen in any form of anemia, such as fatigue, dyspnea, and pallor of skin and mucous membranes. Because the anemia develops over a protracted period and compensatory mechanisms have ample time to develop, IDA is surprisingly well tolerated, even when extremely severe. Pica, koilonychia (i.e., spoon-shaped nails), and esophageal webs are rare but have a strong association with IDA. Angular cheilitis and atrophic glossitis can also be seen but are not specific. The anemia ranges from mild to profound, with Hb levels as low as 2 g/dL in the most severe cases. IDA typically is a microcytic and hypochromic anemia, reflected as a decreased MCV and mean corpuscular Hb concentration (MCHC), respectively. However, the MCV may be normal in early iron deficiency; the degree of microcytosis roughly correlates with the degree of anemia. Likewise, the MCHC may be normal in early iron deficiency when only a minority of cells is hypochromic.

Pathologic Features

The characteristic findings on the peripheral blood film are small (microcytic), under-hemoglobinized (hypochromic) RBCs with variability in size (anisocytosis) and abnormal shapes (poikilocytosis; Fig. 1.10). Elliptocytes are prominent poikilocytes in IDA; often they are long and narrow (pencil cells). Target cells, teardrop cells, very small hypochromic microcytes, and various nonspecific poikilocytes are also common. Occasional red cell fragments may be observed, but they are not prominent. Thrombocytosis is common, but platelet counts may also be normal or reduced. Variations from the typical findings occur when there has been recent

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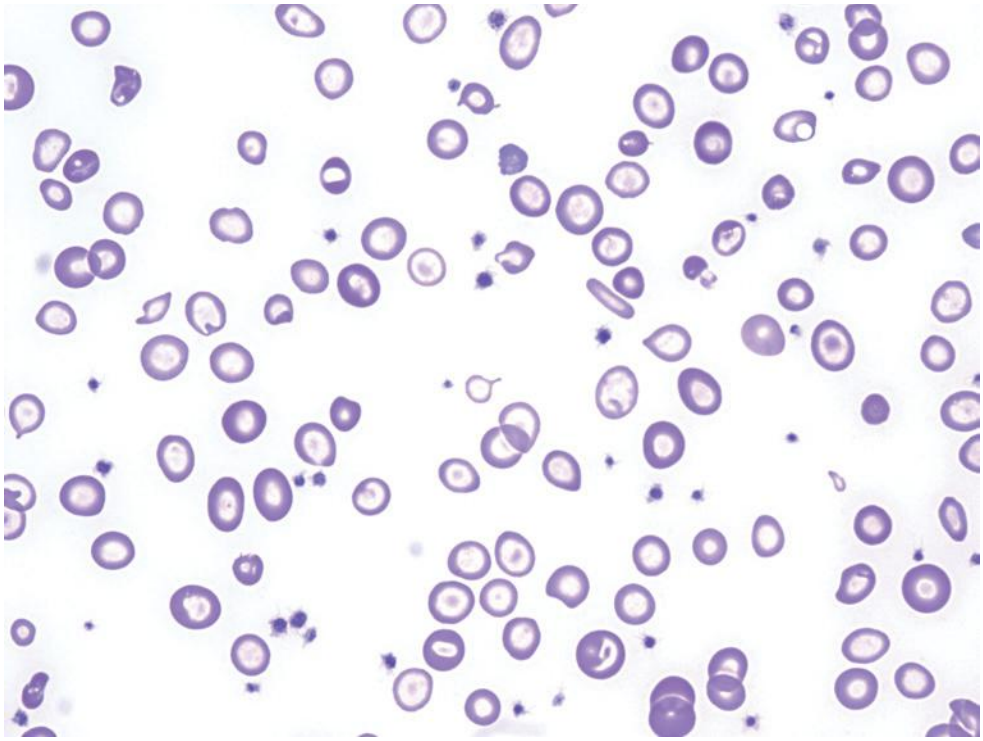


FIG. 1.10

Iron deficiency anemia, peripheral blood findings. In this smear from a patient with severe iron deficiency, there is variation in size and shape of the red cells (anisopoikilocytosis). The red cells are obviously hypochromic, with an enlarged area of central pallor and relatively little remaining hemoglobin. An elongate elliptocyte (pencil cell) is visible slightly to the right and above the center of the field. These poikilocytes are characteristic but nonspecific features of iron deficiency anemia.

IRON DEFICIENCY ANEMIA—PATHOLOGIC FEATURES

Peripheral Blood

- Hypochromic, microcytic red blood cells
- Anisocytosis
- Lack of polychromasia
- Poikilocytosis, including elliptocytes, target cells, and teardrop cells
- Thrombocytosis may be present

Bone Marrow

- Mild erythroid hyperplasia
- Absent stainable iron on a Prussian blue–stained aspirate smear

Differential Diagnosis

- Thalassemia trait or thalassemia-like hemoglobinopathy
- Anemia of chronic disease

partial repletion of iron or when additional factors, such as Cbl or folate deficiency, are also contributing to anemia.

The bone marrow in IDA may demonstrate erythroid hyperplasia, but this is generally of only a mild degree. Erythroid precursors may have scant cytoplasm with frayed cytoplasmic borders.

Ancillary Studies

Serum ferritin measurement is the most useful single laboratory test for iron deficiency. Serum ferritin below the lower limit of the reference range (about 12 mg/dL) is essentially diagnostic of iron deficiency. However, ferritin is an acute-phase reactant, and normal levels may be seen when iron deficiency coexists with infection, inflammation, or malignancy. When this occurs, the ferritin level will usually be low-normal; therefore low-normal values in an individual with an active inflammatory process support a diagnosis of iron deficiency in the appropriate setting. Ferritin levels may even be higher in patients with acute liver injury, despite iron deficiency. Measurement of serum iron alone is not useful because of diurnal fluctuations of serum iron levels and the rapid changes that may occur with dietary intake, inflammation, and blood loss. Transferrin, measured either directly or indirectly as the total iron-binding capacity, is typically increased in IDA, but this response can be blunted in the presence of hypoproteinemia. The transferrin saturation (the ratio of serum iron to total iron-binding capacity) is typically less than 15% in iron deficiency, but saturation levels in this range may also be seen in anemia of chronic disease (ACD).

Increased serum soluble transferrin receptor (sTfR) is a sensitive marker of iron deficiency, reflecting increased expression of this receptor on RBC precursors as a response to depleted tissue iron. This parameter is elevated during iron-deficient erythropoiesis before the development of overt anemia. Furthermore, inflammation does not raise sTfR levels substantially. However, conditions with an increased mass of RBC precursors such as hemolytic anemias and ineffective erythropoiesis (e.g., megaloblastic anemia) will manifest elevated sTfR; therefore this finding is not specific for iron deficiency.

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When biochemical tests are equivocal, the absence of stainable iron on a Prussian blue–stained marrow aspirate smear with adequate spicules is considered to be the gold standard for the diagnosis of IDA. An iron stain performed on a bone marrow core biopsy may also be helpful, but iron can be leached out during decalcification of the bone marrow biopsy and result in the false interpretation of absent iron stores.

In IDA, hepcidin level is generally suppressed. Hepcidin blocks iron absorption from the diet and the iron release from macrophages and liver cells. Although measurement of serum hepcidin is helpful, development of an accurate clinical assay is challenging.

Differential Diagnosis

Other common causes of hypochromic, microcytic anemia are thalassemia and ACD. Sideroblastic anemia with microcytosis is rarer. The hematologic and biochemical tests that are useful to differentiate the major causes of microcytic anemia are listed in Table 1.2. In addition, mild anemia associated with thalassemia trait or a thalassemia-like hemoglobinopathy (e.g., Hb E) characteristically exhibits a red blood cell count greater than 5×10^{12} cells/L, which is unusual for IDA. Hb electrophoresis would help establish the diagnosis of most β-thalassemias and thalassemia-like hemoglobinopathies. Patients with ACD are also at risk of developing IDA. Serum hepcidin can help identify concomitant IDA in this situation because levels are lower compared to pure ACD. When the interpretation of biochemical studies for iron status is particularly difficult, monitoring the response of Hb or the reticulocyte count to a trial of iron supplementation may be sufficient to confirm iron deficiency. An iron stain performed on a bone marrow aspirate smear can also help to resolve uncertainty about iron status.

Prognosis and Therapy

Iron supplementation with oral iron salts, most commonly ferrous sulfate, is standard therapy for uncomplicated IDA. Response to iron supplementation is usually rapid if the iron deficiency is the result of inadequate iron intake. If an appropriate response is not observed or when occult blood loss is more likely, additional evaluation to determine the cause of IDA should be considered. Parenteral iron may be needed for patients with uncontrolled blood loss, intolerance to oral iron, or malabsorption.

Because of the physiologic attempts to compensate for severe chronic anemia that has gradually developed, a rapid blood transfusion can result in hypervolemia and cardiac dilatation. Therefore red cell transfusions should be reserved for patients with severe anemia causing cardiac compromise.

SIDEROBLASTIC ANEMIA

Sideroblastic anemias (SA) are heterogeneous and characterized by anemia with ring sideroblasts in bone marrow. SA is caused by congenital or acquired defects affecting heme biosynthesis, iron-sulfur cluster biogenesis, or mitochondrial protein synthesis. Most present as isolated anemia. Rarely, SA can occur as a component of genetic syndromes with other metabolic or neuromuscular defects. Acquired SA can occur as a myeloid neoplasm (e.g., myelodysplastic syndrome with ring sideroblasts), which is frequently associated with mutations in protein constituents of the RNA spliceosome; this type will be discussed later (see Chapter 18). Acquired SA can also be a result of deregulated heme biosynthesis or mitochondrial function due to a variety of factors such as drugs, alcohol, or copper deficiency. Precipitating causes for copper deficiency include zinc toxicity, total parenteral

TABLE 1.2

Pertinent Findings and Ancillary Tests for Untreated, Uncomplicated Hypochromic, Microcytic Anemias

Laboratory Feature	Iron Deficiency Anemia	Thalassemia	Anemia of Chronic Disease
Increased anisocytosis (elevated RDW)	Present	Absent or present	Typically absent
Increased reticulocytes	Typically absent	Absent or present	Absent
Bone marrow iron stores	Absent	Normal or increased	Typically increased; sideroblasts reduced
Serum ferritin	Typically reduced	Normal or increased	Typically increased
Serum iron	Typically reduced	Normal or increased	Typically reduced
TIBC	Typically increased	Normal or reduced	Reduced or normal
Transferrin saturation	Typically decreased	Normal or increased	Normal or reduced
Serum transferrin receptor	High	Normal or high	Normal or increased

RDW, Red blood cell distribution width index; *TIBC,* total iron binding capacity.

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nutrition without copper supplementation, and malabsorption syndromes.

Clinical Features

The most common congenital form of SA is X-linked due to mutations in 5-aminolevulinate synthase (*ALAS2*) and manifests predominantly in boys who have mild to moderate microcytic anemia. Girls with the same heterozygous mutations are usually asymptomatic. Autosomal-recessive SA due to mutated *SLC25A38*, which encodes an erythroid mitochondrial transporter, usually presents in the first year of life with severe, transfusion-dependent microcytic anemia refractory to pyridoxine treatment.

Pathologic Features

The peripheral blood smear reveals anemia with dimorphic red blood cell morphology, including a distinct population of hypochromic red blood cells (Fig. 1.11). The anemia in congenital SA is usually, but not always, microcytic, and the acquired forms are often not microcytic. Ring sideroblasts in bone marrow aspirate smears are the hallmark of SA and are due to deregulated iron metabolism and mitochondrial iron overload in the erythroid precursors (Fig. 1.12). Hemosiderin-laden macrophages are increased due to ineffective erythropoiesis. A peculiar morphologic clue in bone marrow smears associated with copper deficiency is the presence of hemosiderin in plasma cells (Fig. 1.13).

SIDEROBLASTIC ANEMIA—FACT SHEET

Definition
- Anemia with ring sideroblasts in bone marrow
- Classified as congenital (X linked or autosomal inherited) or acquired (metabolic or clonal)

Incidence and Location
- Congenital forms are rare with no ethnic predilection
- Acquired forms are more common than congenital forms and occur sporadically

Morbidity and Mortality
- Major cause of death in severe cases is secondary hemochromatosis

Prevalence
- Congenital forms are very rare

Clinical Features
- General symptoms of anemia
- Rarely occurs as a part of a genetic syndrome with metabolic or neuromuscular manifestations

Prognosis and Therapy
- Congenital SA with mutated *ALAS2* may respond to pyridoxine treatment
- Severe forms are managed by transfusion and iron chelation
- Allogeneic stem cell transplant may be curative for congenital forms
- Removal of any offending agents is important in nonclonal acquired forms

Ancillary Studies

Iron studies often reflect associated iron overload. A definitive diagnosis of congenital SA can be made when specific mutations are identified. Evaluation for copper deficiency includes serum copper and ceruloplasmin.

Differential Diagnosis

The differential diagnosis for SA with microcytosis includes IDA, thalassemia, and ACD. Thus iron studies and hemoglobin electrophoresis are helpful. In adults, the acquired forms of SA are more common than congenital forms.

Prognosis and Therapy

SA with mutated *ALAS2* may respond to pyridoxine treatment. Transfusions with therapy to manage iron overload provides relief for patients with severe anemia. Hematopoietic stem cell transplantation has been successful in some patients with congenital SA. For acquired SA, the anemia is often reversible after the offending agents have been removed (e.g., alcohol, drugs) or treatment for copper deficiency has been implemented.

SIDEROBLASTIC ANEMIA—PATHOLOGIC FEATURES

Peripheral Blood
- Anemia with dimorphic red blood cell morphology

Bone Marrow
- Ring sideroblasts on Prussian blue–stained bone marrow aspirate smear

Differential Diagnosis
- Iron deficiency
- Thalassemia
- Anemia of chronic disease

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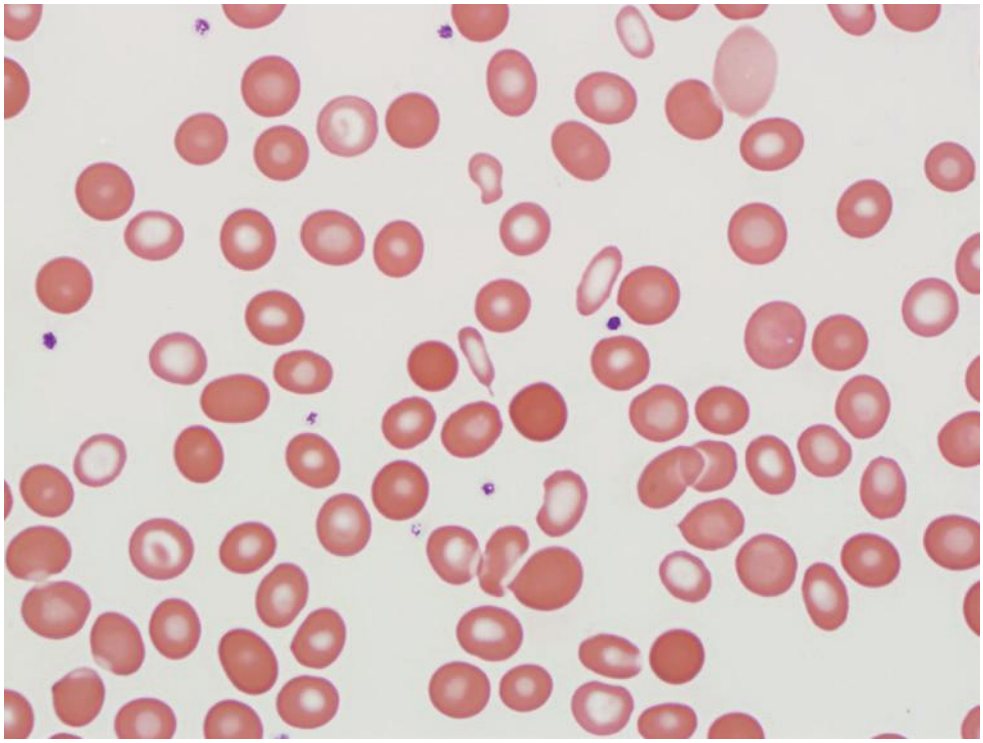


FIG. 1.11

Acquired sideroblastic anemia (myelodysplastic syndrome with ring sideroblasts), peripheral blood findings. Dimorphic red blood cells include a normocytic, normochromic population and a distinct microcytic, hypochromic subset.

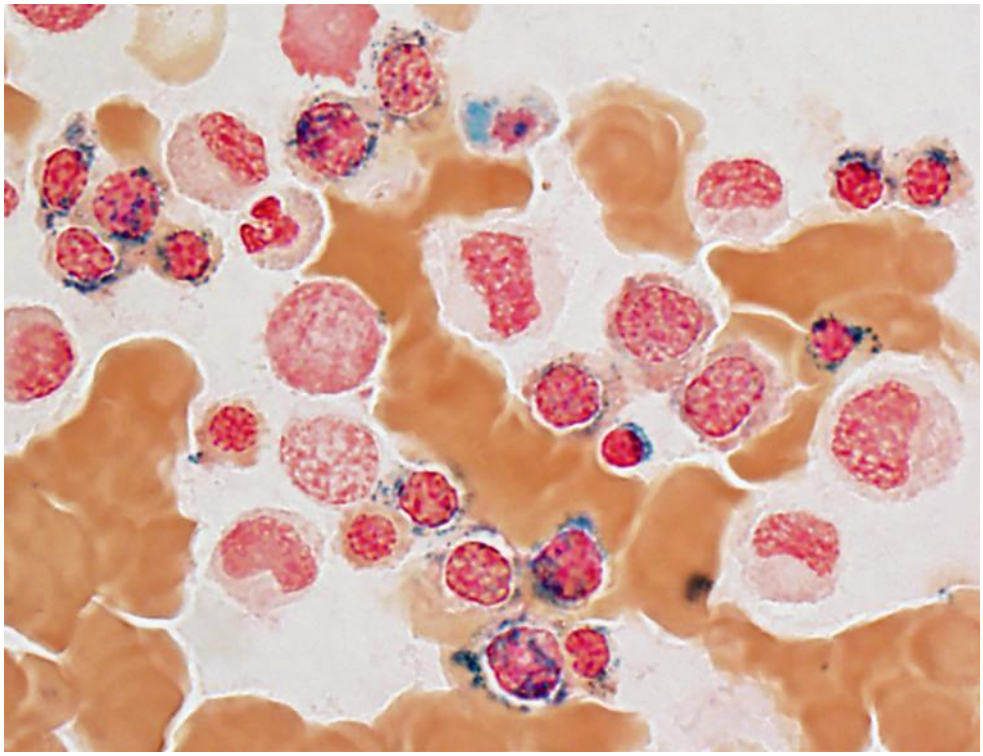


FIG. 1.12

Acquired sideroblastic anemia (myelodysplastic syndrome with ring sideroblasts), marrow aspirate smear iron stain findings. Ring sideroblasts seen in some myelodysplastic syndromes are highly associated with mutations in protein constituents of the RNA spliceosome; the underlying genetic abnormalities found in congenital sideroblastic anemia are different, but the morphologic findings are similar.

CONGENITAL DYSERYTHROPOIETIC ANEMIA

Congenital dyserythropoietic anemia (CDA) is a group of rare hereditary disorders characterized by anemia due to ineffective erythropoiesis and with distinct morphologic abnormalities of erythroblasts. Three classic subtypes (CDA I, II, III) and several minor variants have been identified. Mutations of the genes involved in most cases of CDA I and II have been identified, and more recently the genes involved in other types of CDAs have been described. CDA II is also known as hereditary erythroblastic multinuclearity with a positive acidified serum test (HEMPAS) owing to the

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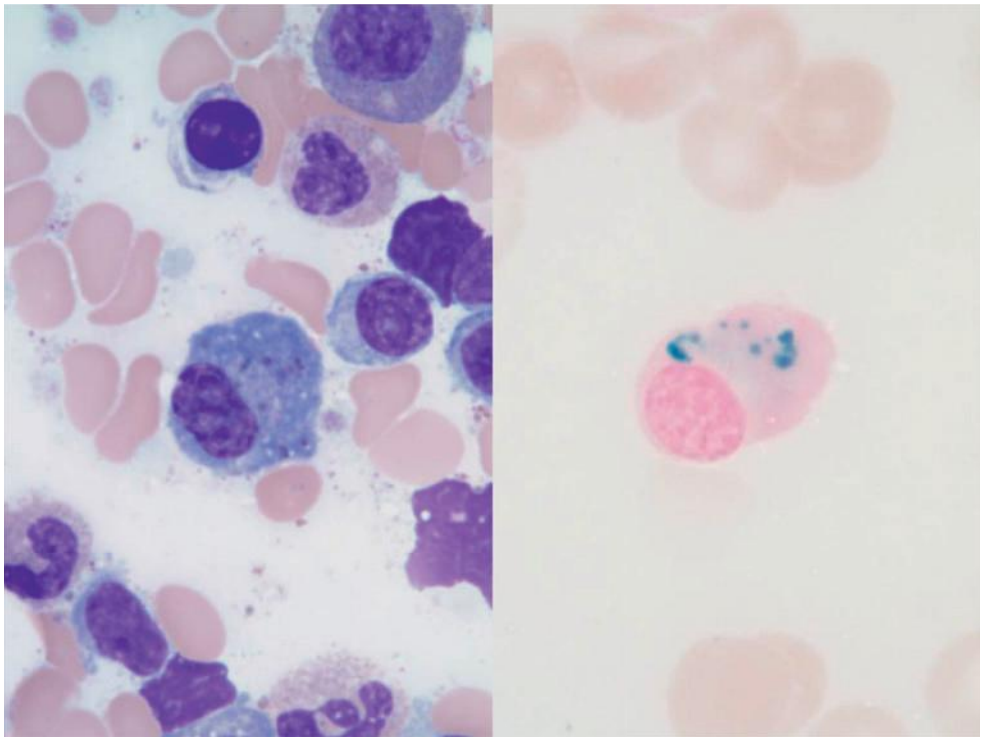


FIG. 1.13

Hemosiderin in plasma cells appears blue to greenish black with Wright-Giemsa stain *(left)* and can be confirmed with the Prussian blue stain *(right)*. This finding has been commonly associated with copper deficiency but can also be observed in some other conditions related to iron overload. *(Courtesy Dr. Karl S. Theil, Cleveland Clinic, Cleveland, OH.)*

susceptibility of the red blood cells to lysis in the acidified serum lysis test (HAM test). CDA III is rare outside of the few original families described.

Clinical Features

The clinical spectrum is highly variable. CDA I and II are autosomal recessive, whereas CDA III is autosomal dominant. CDA II is the most common, and many patients come to attention as adults due to mild or moderate normocytic anemia associated with jaundice, splenomegaly, and/or hepatomegaly. Presentation of CDA I and III is similar to CDA II, but CDA I is usually macrocytic, comes to attention at a younger age, and can be associated with skeletal abnormalities. CDA I may also present as hydrops fetalis or as severe neonatal anemia. Cholelithiasis and rarely extramedullary hematopoiesis-related mass lesions can occur with CDA. Secondary hemochromatosis from iron overload is a major complication.

Pathologic Features

The peripheral blood film reveals various poikilocytes, basophilic stippling, and, in CDA II, occasional binucleated erythroblasts. The bone marrow shows erythroid hyperplasia and dyserythropoiesis. The nonerythroid lineages are morphologically normal. A highly specific feature for CDA I on bone marrow examination is thin chromatin bridges between paired erythroblasts. CDA II is often first recognized based on binucleated and rarely multinucleated erythroblasts in more than 10% of erythroid precursors. The binucleation is often restricted to the late-stage erythroblasts (Fig. 1.14). CDA III is characterized by giant, multinucleated erythroblasts with up to 12 nuclei.

CONGENITAL DYSERYTHROPOETIC ANEMIA—FACT SHEET

Definition
- Anemia characterized by multinucleated erythroid precursors in the marrow and ineffective erythropoiesis

Incidence
- Rare

Morbidity and Mortality
- Rare severe forms may present as hydrops fetalis and stillbirth

Prevalence
- Sporadic cases in a variety of ethnic groups

Clinical Features
- Variable anemia, splenomegaly, hepatomegaly, intermittent jaundice, and cholelithiasis
- CDA I may present with severe neonatal anemia and can be associated with dysmorphic features

Prognosis and Therapy
- Mild to moderate forms have a good prognosis
- Therapy is mainly transfusion and iron chelation
- Allogeneic stem cell transplant is potentially curative

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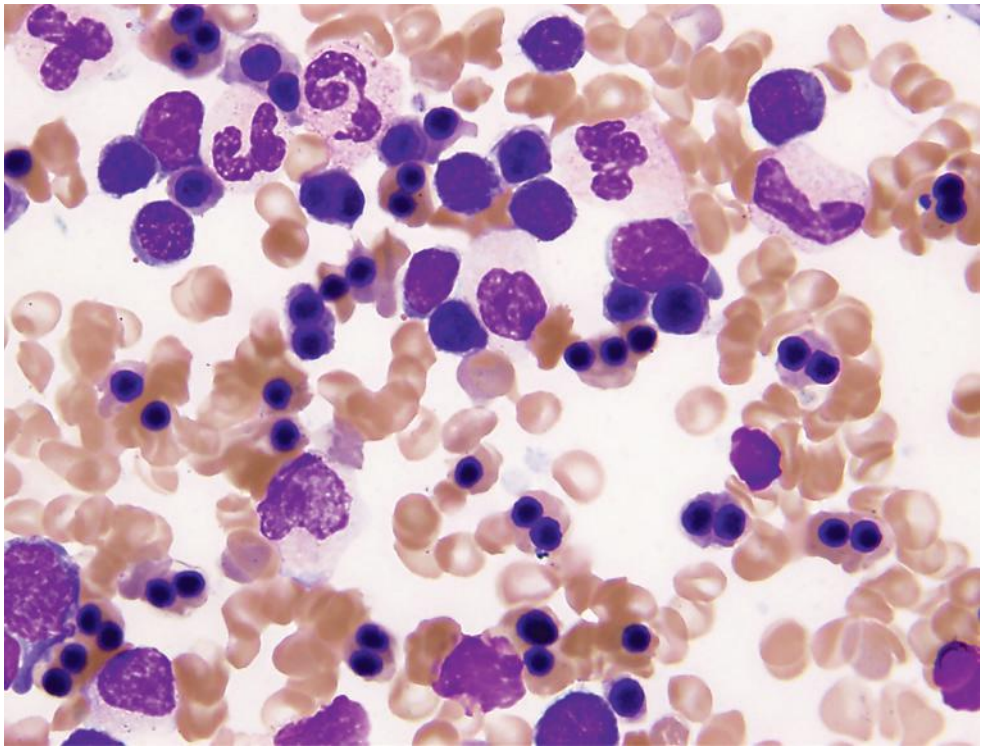


FIG. 1.14
Congenital dyserythropoietic anemia type II, bone marrow aspirate smear findings. Binucleation of the erythroid precursors is characteristic and is often restricted to the late polychromatophilic or orthochromatophilic erythroblasts.

TABLE 1.3
Congenital Dyserythropoetic Anemia

Types	Inheritance Pattern	Key Bone Marrow Morphology	Mutated Gene
I	Autosomal recessive	Chromatin bridges between erythroblasts	*CDAN1*, *C15ORF41*
II	Autosomal recessive	Binucleated and multinucleated late-stage erythroblasts	*SEC23B*
III Familial	Autosomal dominant	Giant multinucleated erythroblasts	*KIF23*
III Sporadic	Variable	Giant multinucleated erythroblasts	Unknown
Variants	Variable	Variable	*KLF1*, *GATA1*, unknown

Ancillary Studies

The diagnosis of CDA is based on long-standing hemolytic anemia with characteristic bone marrow findings and lack of an appropriate reticulocyte response. In the past, laboratory tests to confirm the diagnosis included a positive HAM test for CDA II, sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) to identify hypoglycosylated membrane proteins in CDA II (e.g., band 3 is narrowed and shows faster migration), and electron microcopy for ultrastructural abnormalities distinct in CDA I, II, and III (e.g., "Swiss cheese–like" heterochromatin in CDA I and "double plasma membrane" in CDA II). When the clinical picture, peripheral blood, and bone marrow findings are compatible with a diagnosis of CDA, sequencing for known mutations can confirm the diagnosis in many cases and exclude other rare inherited anemias. A summary of some key features and mutated genes recognized so far for CDA is shown in Table 1.3. The interested reader may consult the reference list for more information.

Differential Diagnosis

Clinically, CDA may be confused with thalassemia or hemolytic anemia. However, a relatively low reticulocyte count incommensurate to the degree of anemia and the unique bone marrow findings will lead to the correct diagnosis. CDA II is often misdiagnosed as hereditary spherocytosis (HS) due to hemolysis-related symptoms and occasional spherocytes in the peripheral blood. However, more numerous spherocytes and a greater reticulocyte response are expected for HS. The differential diagnosis of dyserythropoiesis includes many conditions such as B_{12}/folate deficiency, exposure to heavy

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metals, copper deficiency, chemotherapy, medication effect, and myelodysplastic syndromes. However, the morphologic abnormalities predominant in the classic types of CDAs are distinct and not the main feature in any of these conditions.

Therapy

Treatment depends on the type of CDA and the degree of anemia. Blood transfusions with therapy to prevent iron overload may be required for patients with more severe anemia. Interferon gamma may alleviate the symptoms of CDA I. Hematopoietic stem cell transplantation can be curative.

DECREASED EFFECTIVE ERYTHROPOIESIS

This group of anemias results from a decreased pool of erythroid stem cells available for maturation and proliferation or interference or blocking of the proliferation of an otherwise adequate stem cell pool, or both. Disorders belonging to this group include marrow failure syndromes, including aplastic anemia and pure red cell aplasia (discussed in Chapter 5), marrow replacement processes, anemia of chronic kidney disease (ACKD) and ACD. Only the last two are discussed in this chapter.

ANEMIA OF CHRONIC KIDNEY DISEASE

ACKD is primarily caused by underproduction of erythropoietin. Toxic metabolites related to uremia also likely contribute to ACKD by suppressing erythropoiesis, reducing red cell survival, and increasing the risk of blood loss due to platelet dysfunction.

CONGENITAL DYSERYTHROPOETIC ANEMIA—PATHOLOGIC FEATURES

Peripheral Blood
- Variable anemia with or without macrocytosis
- Anisopoikylocytosis

Bone Marrow
- Erythroid hyperplasia
- Thin chromatin bridges between paired erythroblasts in CDA I
- Binucleated and rarely multinucleated erythroblasts in CDA II
- Giant multinucleated erythroblasts in CDA III

Differential Diagnosis
- Thalassemia
- Hemolytic anemia (esp. hereditary spherocytosis)
- Other causes for dyserythropoiesis (Cbl/folate deficiency, heavy metals, copper deficiency, chemotherapy, medication effect, myelodysplastic syndromes)

There is variation in the level of renal function for which anemia develops, but the prevalence and severity of anemia increase as renal function decreases. Evaluation of National Health and Nutrition Examination Surveys (2007–2010) estimates that about 14% of the U.S. population has chronic kidney disease (CKD). Anemia has been estimated to be present in 15.4% of people with CKD, and the prevalence increases with stage of CKD. The prevalence of anemia is also higher and more severe among patients with diabetes, manifesting at earlier stages of CKD.

Clinical Features

Progressive uremia leads to plasma expansion and hemodilution; therefore the Hb level immediately before hemodialysis is lower than that immediately afterward. Cardiovascular and renal attempts to compensate for anemia contribute to the development of left ventricular hypertrophy and can lead to congestive heart failure. Treatment with erythropoiesis-stimulating agents (ESAs), such as epoetin alfa or darbepoetin alfa, can improve the quality of life of these patients. However, the response to these therapies is limited if either absolute or functional iron deficiency develops. Functional iron deficiency is due to depletion of the iron pool immediately available for erythropoiesis. Mobilization of iron stores becomes rate limiting and is inadequate to meet the accelerated demand for iron during aggressive ESA therapy.

ANEMIA OF CHRONIC KIDNEY DISEASE—FACT SHEET

Definition
- Anemia of variable degree primarily due to underproduction of erythropoietin

Prevalence
- The prevalence and severity of anemia increase as renal function decreases
- The prevalence and severity are increased in patients with diabetes
- Prevalence varies with age, gender, and race or ethnicity
- Higher prevalence of anemia among black versus white persons who have comparable levels of renal insufficiency

Morbidity and Mortality
- Associated with reduced quality of life, reduced cognitive function, left ventricular hypertrophy, and increased cardiac morbidity and mortality

Clinical Features
- General symptoms of chronic anemia

Prognosis and Therapy
- Optimal dialysis and nutrition
- Erythropoiesis-stimulating agent therapy and iron supplementation

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Pathologic Features

In ACKD, the peripheral blood film shows a normochromic, normocytic anemia with minimal polychromasia and minimal anisocytosis. Occasional echinocytes (burr cells) and other poikilocytes may be observed, such as acanthocytes and rare fragments. There is no increase in polychromasia.

ANEMIA OF CHRONIC KIDNEY DISEASE—PATHOLOGIC FEATURES

Peripheral Blood
- Normocytic, normochromic anemia
- Minimal polychromasia
- Occasional poikilocytes, including echinocytes, rare acanthocytes, and fragments

Bone Marrow
- Normocellular to slightly hypocellular marrow without an increase in erythroid precursors
- Bony changes related to renal osteodystrophy may be observed

Differential Diagnosis
- Iron deficiency anemia
- Megaloblastic anemia
- Anemia of chronic disease
- Hypothyroidism
- Secondary hyperparathyroidism
- Aluminum toxicity
- Pure red cell aplasia

The bone marrow is usually normocellular or slightly hypocellular; in the absence of ESA therapy, it contains normal to slightly reduced erythroid precursors. The amount of sideroblastic iron and storage iron will depend on overall iron status. Morphologic features of renal osteodystrophy may be noted on a bone marrow core biopsy specimen, especially in association with end-stage renal disease and in patients who have been treated with long-term dialysis. Renal osteodystrophy is a spectrum of abnormalities of bone turnover that variably exhibits thinning or thickening of bony trabeculae, excess osteoid without mineralization (osteomalacia), stromal fibrosis, and osteitis fibrosa cystica (Fig. 1.15).

Ancillary Studies

Plasma erythropoietin concentration and the reticulocyte count are inappropriately low for the degree of anemia in ACKD. An increase in reticulocytes is a helpful indicator of a response to therapy. However, measuring plasma erythropoietin concentration is not usually recommended.

Detection of concomitant iron deficiency may be compromised by the fact that patients with CKD may have associated inflammation; therefore ferritin levels may be difficult to interpret. Even more challenging is the detection of the functional iron deficiency that may compromise the effectiveness of ESA therapy. The small numbers of hypochromic RBCs produced in this circumstance will not be detected reliably by the MCV or MCHC. High-throughput hematology analyses provide additional parameters of hypochromia, which vary based on the manufacturer but which

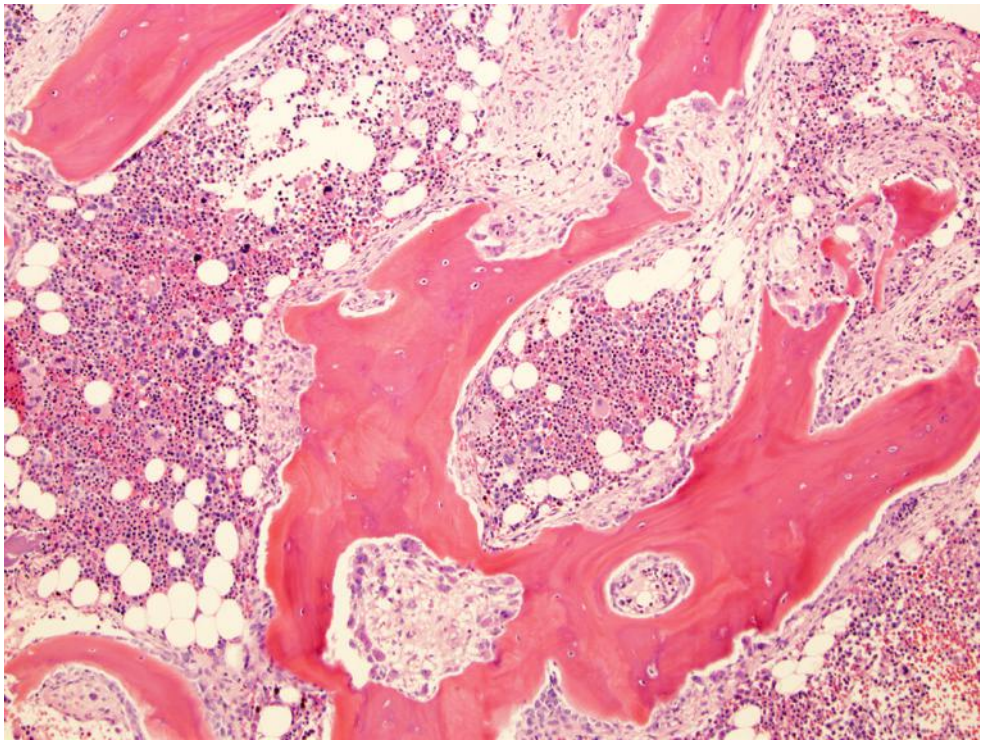


FIG. 1.15

Renal osteodystrophy, bone marrow biopsy findings. The bone in this marrow biopsy specimen from a patient with renal osteodystrophy demonstrates an irregular erosive lesion with fibrosis, prominent osteoblastic rimming, and osteoid deposition. *(Courtesy Dr. Yi-Hua Chen, Northwestern University, Chicago, IL.)*

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can help identify functional iron deficiency (e.g., percentage of hypochromic red cells, reticulocyte hemoglobin content, and low hemoglobin density).

Differential Diagnosis

Nutritional deficiencies, infections, and other causes of inflammation commonly have a role in the development of anemia in patients with CKD. In addition to malnutrition, blood loss associated with hemodialysis can contribute to iron deficiency. The diagnosis of iron deficiency in the setting of CKD is challenging due to poor predictive ability of serum iron indices. Recent guidelines (Kidney Disease: Improving Global Outcomes) recommend a course of iron therapy in patients with CKD and anemia if the transferrin saturation is less than 30% and the serum ferritin level is less than 500 mg/L. Patients receiving dialysis are also at increased risk of megaloblastic anemia, because Cbl and folate are water soluble and dialyzable.

Elevated markers of inflammation, such as C-reactive protein or erythrocyte sedimentation rate, indicate that chronic infection or inflammation may be contributing factors for anemia. Patients with high levels of inflammatory cytokines are more resistant to ESA therapy.

Hypothyroidism and secondary hyperparathyroidism can also contribute to anemia and resistance to ESA therapy among patients with CKD. Similar to ACKD, anemia related to hypothyroidism manifests with an inappropriately low reticulocyte response and is usually normocytic and normochromic. Aluminum toxicity associated with dialysis is another occasional cause of anemia in patients with renal failure; when chronic, this anemia becomes hypochromic and microcytic. In addition, a sudden lack of response to erythropoietin therapy could herald pure red cell aplasia caused by the development of antierythropoietin antibodies; however, this is rare and mostly related to a formulation that had been used outside of the United States.

Prognosis and Therapy

Appropriate use of ESAs and supplemental iron can improve the quality of life of patients with chronic kidney disease and reduce dependence on transfusions. A poor response to this therapy should prompt an evaluation to exclude other causes for anemia.

An excessive Hb target level when using this therapy appears to be associated with increased cardiovascular complications, and thus the optimal Hb target values for various groups of patients with ACKD is under investigation. The U.S. Kidney Disease Outcomes Quality Initiative, the U.S. Food and Drug Administration, and the National Institute for Health and Clinical Excellence (Royal College of Physicians, London) offer therapeutic recommendations.

ANEMIA OF CHRONIC DISEASE (ANEMIA OF INFLAMMATION)

Anemia of chronic disease (ACD), also known as *anemia of inflammation,* is a mild to moderate anemia that is associated with many different underlying disorders including inflammatory disorders, infection, and malignancy. ACD is characterized by reduced transport of iron to erythroid precursors, reduced erythropoiesis, ineffective erythropoiesis, and normal or increased iron stores. The underlying disease has usually existed more than 1 or 2 months before development of ACD, but anemia can also develop rapidly during critical illness, largely because of the same mechanisms.

Increased levels of the inflammatory cytokine interleukin-6 upregulate hepatic production of hepcidin, the major iron regulatory hormone, that binds to the iron exporter ferroportin on macrophages and enterocytes. Subsequent internalization and lysosomal degradation of ferroportin result in reduced release of iron into plasma. Other inflammatory cytokines (e.g., tumor necrosis factor α, interleukin-1, and the interferons) also likely contribute to the development of ACD by reducing mature red cell survival, suppressing erythropoietin production, and diminishing the response of erythroid precursors to erythropoietin.

ACD is considered the most frequent cause of anemia overall in hospitalized patients, and it has been estimated to affect 2.5% of individuals 65 years and older within the community-dwelling population in the United States, according to the third National Health and Nutrition Examination Survey (1988–1994). For persons 65 years and older who were surveyed, there is a significantly higher proportion of non-Hispanic black persons among those with ACD versus those without anemia. In a 2010 Centers for Disease Control and Prevention National Center for Health Statistics (NCHS) survey, 9.6% of residents in assisted living and other residential care have anemia.

Clinical Features

The clinical symptoms of ACD are often those from the underlying disease rather than the anemia. The severity of anemia is related to the level of disease activity of the chronic inflammatory disorder or the extent of tumor burden in malignancy. However, the anemia is generally mild or moderate, with hemoglobin levels usually not lower than 9 g/dL in uncomplicated cases. The symptoms and signs of ACD have an insidious onset and are similar to mild or moderate chronic anemia due to any cause. Because patients with chronic diseases tend to adjust to the effects of anemia and, in general, a lower quality of life, the consequences of ACD may be appreciated in retrospect only after response to therapy. In patients with critical illness and acute events, such as trauma, surgery, and sepsis, increasingly severe anemia may be associated with a blunted erythropoietin (EPO) response.

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ANEMIA OF CHRONIC DISEASE (ANEMIA OF INFLAMMATION)—FACT SHEET

Definition
- Anemia due to inflammatory cytokines associated with infection, inflammatory disorders, and cancer

Incidence and Location
- Most frequent contributing cause of anemia among hospitalized patients
- Affects about 2.5% of community-dwelling population 65 years and older in the United States

Morbidity and Mortality
- The effect on morbidity and mortality has not been systematically evaluated for many chronic diseases
- Amelioration of anemia contributes to improved quality of life and reduced need for red blood cell transfusions in patients with inflammatory disorders and cancer

Gender, Race, or Age Distribution
- More common among debilitated patients and the elderly
- Possible increased risk in blacks

Clinical Features
- Signs and symptoms are the same as those for chronic anemia in general
- Consequences of anemia on quality of life can be appreciated only in retrospect after response to therapy

Prognosis and Therapy
- Treat underlying disorder
- Erythropoiesis-stimulating agents and iron supplementation may be of benefit
- Transfusions for severe anemia with hemodynamic instability

ANEMIA OF CHRONIC DISEASE (ANEMIA OF INFLAMMATION)—PATHOLOGIC FEATURES

Peripheral Blood
- Mild to moderate anemia that may be normochromic and normocytic or hypochromic and microcytic
- Lack of prominent anisocytosis or polychromasia

Bone Marrow
- Normocellular to slightly hypocellular marrow with normal to slightly reduced number of erythroid precursors
- Increased stainable iron on bone marrow aspirate smears with marked reduction or absence of sideroblasts

Differential Diagnosis
- Iron deficiency anemia
- Thalassemia minor or trait
- Anemia of chronic kidney disease
- Drug effects
- Hypothyroidism
- Marrow infiltration
- Hematopoietic stem cell defects, including myelodysplastic syndromes

Pathologic Features

Because understimulation of erythropoiesis is characteristic of ACD, the peripheral blood film shows a mild to moderate anemia without noticeable polychromasia or increased anisocytosis, similar to that seen with anemia of renal failure. There may be rouleaux formation, correlating with an elevated erythrocyte sedimentation rate. The RBCs are generally normochromic and normocytic. However, iron transport to the erythroid precursors is characteristically diminished in ACD, resulting in functionally iron-deficient conditions even in light of the already reduced stimulation of erythropoiesis associated with ACD. As a result, anemia becomes hypochromic and microcytic in approximately 20% of cases. Reticulocyte count is low, reflecting the underproduction of the red blood cells.

The bone marrow is usually normocellular but may reveal diminished erythropoiesis. Other nonspecific findings associated with inflammatory conditions may be observed, such as increased plasma cells. A Prussian blue iron stain performed on an aspirate smear with sufficient marrow spicules will demonstrate features that can confirm a diagnosis of ACD. Iron stored as hemosiderin will be normal or, more commonly, increased as a result of iron retention within macrophages due to elevated hepcidin (Fig. 1.16), whereas nucleated red cells with iron-containing granules (i.e., sideroblasts) will be absent. The lack of sideroblasts indirectly reflects diminished delivery of iron to erythroid precursors, which is typical of ACD.

Ancillary Studies

Because ferritin is a positive acute-phase reactant, serum ferritin can be normal or elevated in ACD. Reduced serum iron concentration, despite adequate or increased storage iron, is a hallmark of ACD, which consequently can result in low transferrin saturation. However, the transferrin saturation is not reduced to the same extent as seen in pure IDA, because serum transferrin concentration and total iron-binding capacity are not elevated in ACD. Soluble transferrin receptor (sTfR) has been introduced as a marker for iron depletion. The sTfR level is elevated in IDA; however, it is not an acute-phase reactant and remains normal or only slightly increased in uncomplicated ACD, consistent with understimulation of erythropoiesis. Similarly, the sTfR:ferritin ratio is increased in IDA and normal in ACD. Recent studies have suggested the central role of IL6-hepcidin pathway in ACD. Elevated hepcidin levels may aid in the diagnosis of ACD, but hepcidin assays are not yet routinely available for clinical use.

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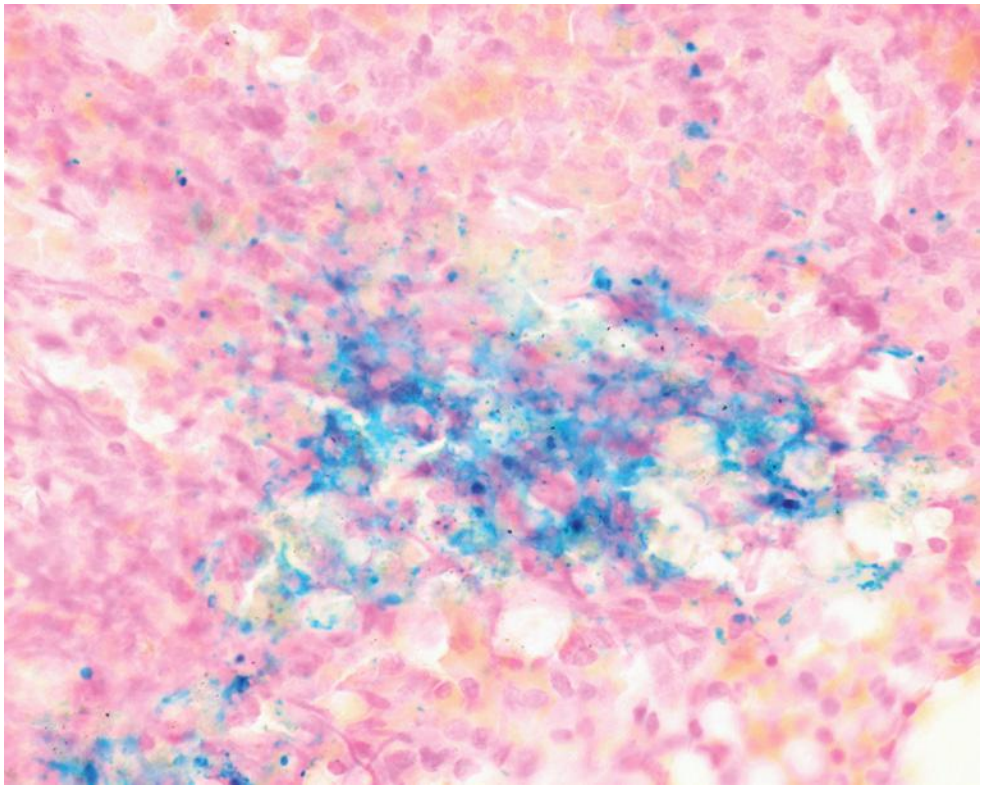


FIG. 1.16

Anemia of chronic disease, marrow iron stain findings. This Prussian blue stain of a bone marrow aspirate smear particle demonstrates increased storage iron (blue staining), characteristic of anemia of chronic disease. Close examination of this stain would reveal absence or near absence of siderotic granules in erythroid precursors.

Differential Diagnosis

Other causes for anemia associated with reduced erythropoiesis include ACKD, drug effects, hypothyroidism, marrow infiltration, myelodysplastic syndromes, other stem cell defects, and IDA. Most can be ruled out based on correlation of clinical and pathologic data (sometimes including a bone marrow examination) and appropriate laboratory testing. The distinction between ACKD and ACD requires correlation with other evidence of renal failure and inflammation.

Uncomplicated IDA is easily distinguished from ACD (see Table 1.2); however, exclusion of IDA becomes more complicated in the presence of infection, inflammation, or cancer. A low serum ferritin level signifies iron deficiency but would not rule out concurrent ACD. On the other hand, intermediate ferritin levels do not rule out IDA when there is coexisting inflammation. When serum ferritin levels are equivocal, elevated sTfR levels would support a component of IDA. A bone marrow examination with a Prussian blue stain can also help assess iron status.

Prognosis and Therapy

The best therapy for ACD is to identify and treat the underlying infection, inflammatory disease, or cancer. In some situations, the underlying disorder cannot be alleviated, and therapy with an ESA and iron supplementation, if needed, may improve a patient's quality of life. RBC transfusions are generally reserved for patients who have a poor response to therapy and for whom hemodynamic stability is compromised.

HEMOLYTIC ANEMIAS

Hemolysis is the term given to an increased rate of peripheral destruction of RBCs. When mild, the bone marrow is able to compensate, and no anemia results. When more severe, or when the ability of the marrow to compensate is compromised, anemia develops. Hemolytic anemias can be divided broadly into those caused by intrinsic red cell abnormalities, nearly all of which are inherited, and those caused by extrinsic factors, nearly all of which are acquired. An exception to this rule is paroxysmal nocturnal hemoglobinuria, an acquired intrinsic red cell disorder resulting in hemolysis. This disorder, which is closely related to aplastic anemia, is discussed in Chapter 5 in the context of bone marrow failure syndromes.

A generic feature of all forms of hemolytic anemia is a compensatory erythroid hyperplasia in the bone marrow; therefore bone marrow findings are not discussed further for individual disorders. The erythroid expansion may be accompanied by a shift toward immaturity, mild nuclear to cytoplasmic dyssynchrony and dyserythropoiesis, and increased binucleated erythroid precursors (Fig. 1.17).

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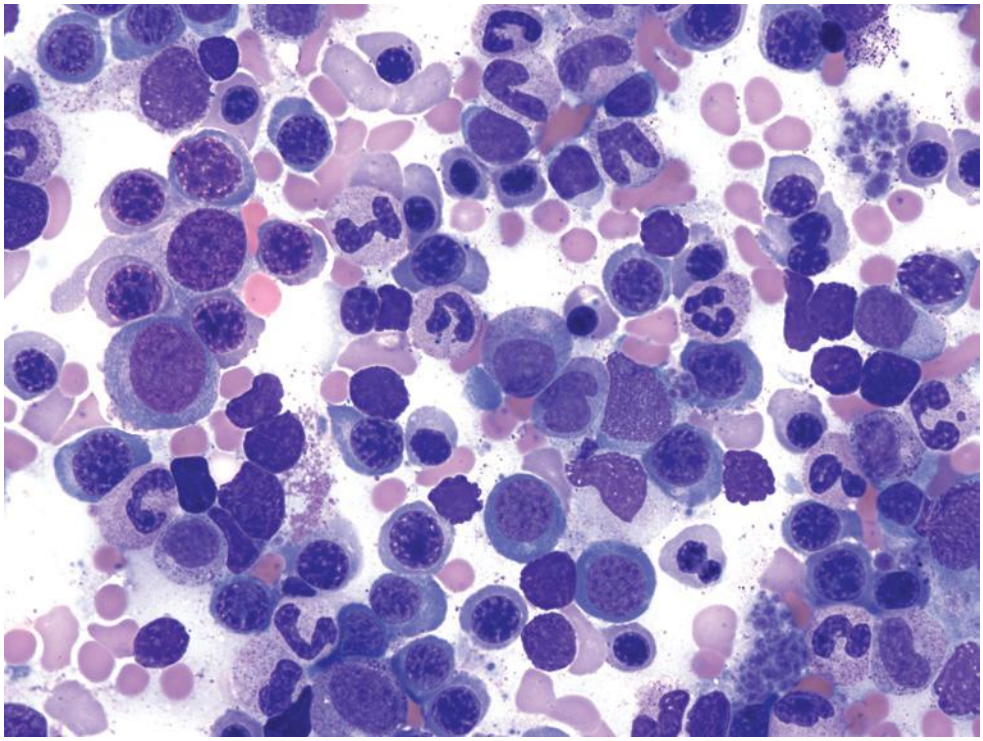


FIG. 1.17

This bone marrow aspirate smear from a patient with warm autoimmune hemolytic anemia demonstrates erythroid hyperplasia, shift toward immaturity, mild nuclear:cytoplasmic dyssynchrony, increased binucleation, and mild terminal dyserythropoiesis in the form of nuclear budding. Such changes are common to states in which there is increased red blood cell production owing to hemolysis or bleeding.

INTRINSIC RED CELL DEFECTS

HEMOGLOBIN DISORDERS

Structural

Sickle Cell Disease

Sickle cell disease (SCD) is a disorder of chronic hemolytic anemia and microvascular occlusion involving the Hb S mutation in the β-globin gene on chromosome 11. A single base change results in a substitution of valine for glutamic acid at the sixth position of the β-globin chain, an alteration that causes abnormal Hb polymerization at low oxygen tensions. This abnormal polymerization produces rigid, deformed RBCs, resulting in chronic hemolysis and vasoocclusive phenomena. SCD most commonly results from homozygous Hb S mutations (SS disease) but also is a consequence of compound heterozygosity for Hb S and Hb C (SC disease), β-thalassemia (S/β-thalassemia), or rarely other β-chain variants such as Hb D, Hb O-Arab, and Hb C-Harlem.

Clinical Features

SCD ranges from an asymptomatic disorder to a severe, debilitating disease. It occurs most frequently in patients of African descent. Of the newborns in the United States with SCD, 60% have SS disease, 30% have SC disease, and 10% have S/β-thalassemia. The disease occurs at a lower frequency in individuals with Mediterranean, Middle Eastern, or Indian ancestry and in Hispanic-Americans. Infants are clinically unaffected until 4 to 6 months of age when Hb F production is largely replaced by Hb S production. Clinical manifestations result from both the chronic hemolysis and the microvascular occlusion.

Baseline Hb levels in SS disease range from 6 to 10 g/dL (average, 8 g/dL). The clinical consequences of the hemolysis are similar to those in other types of chronic hemolytic anemias: fatigue, exercise intolerance, jaundice, gallstones, and leg ulcerations; hemolysis is suspected to have a role in the development of progressive vasculopathy, including pulmonary hypertension. Aplastic crisis resulting from parvovirus B19 infection is a potentially devastating complication because of the markedly decreased RBC life span. Clinical manifestations resulting from vasoocclusion are protean. A uniform finding in SS disease is repeated splenic infarction resulting in functional asplenia by adulthood, predisposing patients to infectious complications. Patients also almost always have recurrent pain crises due to vasoocclusion of vascular beds in deep muscle, periosteum, and bone marrow. Other manifestations include acute chest syndrome, stroke, renal damage, priapism, sudden deafness, aseptic necrosis of the femoral head, retinal pathology, and acute splenic sequestration. The last manifestation represents acute, massive pooling of blood in the spleen of toddlers or infants and can be fatal.

SC disease is a milder disorder than SS disease. Although nearly all the complications of SS disease may be seen, they are less frequent and less severe in SC disease. In addition, patients do not demonstrate autosplenectomy, but instead often have splenomegaly. S/β°-thalassemia (in which there is no normal β-chain production from the thalassemic allele) may be a slightly milder disorder than SS disease. S/β°-thalassemia can differ from SS disease in that there may be

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SICKLE CELL DISEASE—FACT SHEET

Definition
- Disorder caused by abnormal polymerization of deoxygenated hemoglobin S, resulting in chronic hemolysis and microvascular occlusion
- Mutation results in valine to glutamic acid substitution at amino acid 6 of β globin

Incidence and Location
- SCD affects 75,000 to 100,000 people in the United States
- SCD occurs among about 1 of every 365 Black or African-American births and 1 of every 16,300 Hispanic-American births in the United States

Mortality
- Deaths related to severe forms of SCD (SS disease and S/β^0-thalassemia) in the United States have decreased during early childhood but still remain significantly higher compared to children overall adjusted for age and race/ethnicity
- The average age of death in the United States is about 40 years for patients with SCD, but longer survival is expected in patients with less severe forms (SC disease and S/β^+-thalassemia)

Gender, Race, or Age Distribution
- Equal gender incidence
- Most common in the United States among African-Americans, followed by Hispanic-Americans
- Of newborns, 60% have SS disease, 30% SC disease, and 10% S/β-thalassemia, but proportions change in the aging population due to the increased mortality for patients with SS disease and S/β^0-thalassemia

Clinical Features
- Widely variable, including recurrent painful crises, chronic hemolytic anemia, gallstones, leg ulcers, stunted growth, bony abnormalities, susceptibility to infection, acute chest syndrome, stroke

Prognosis and Therapy
- Symptomatic therapy for crises
- Transfusion for acute exacerbations of anemia and in preparation for surgery
- Regular transfusion for severely affected patients
- Hydroxyurea therapy to increase levels of Hb F
- Good prognostic features include higher levels of Hb F, fewer pain crises, low frequency of acute chest syndrome, higher hemoglobin levels, lower white blood cell counts, better renal function, lack of cerebrovascular events, and lack of pulmonary hypertension

less severe hemolysis and splenomegaly. S/β^+-thalassemia (in which some normal β-globin chains are produced) is a milder disease and, in fact, is asymptomatic in many cases.

Pathologic Features

The peripheral blood smear in patients with SS disease demonstrates anemia with prominent anisopoikilocytosis

SICKLE CELL DISEASE—PATHOLOGIC FEATURES

Microscopic Findings
- SS disease
  - Sickled cells and variants (boat-shaped and holly leaf–shaped)
  - Occasional blister cells and spherocytes
  - Evidence of hyposplenism in adults (target cells, Howell-Jolly bodies, Pappenheimer bodies, nucleated red blood cells)
  - Mild leukocytosis
- SC disease
  - Rare sickled cells
  - Many target cells
  - Malformed hemoglobin C crystals
  - Bizarre red blood cell forms, including cells with spikes, hooks, and irregular condensation of hemoglobin
- S/β-thalassemia
  - Similar to SS disease but fewer sickled cells, more target cells, absence of hyposplenic features

Differential Diagnosis
- Other forms of inherited hemolytic anemia

(Fig. 1.18). Most prominently, one sees sickled cells (drepanocytes). Classic sickled cells are elongate, tapered cells with pointed ends and no central pallor (Fig. 1.19). Some, however, are broader with more blunted ends (see Figs. 1.18 and 1.19). Variant forms, such as boat-shaped (Fig. 1.20) or holly leaf–shaped cells, are also seen. In addition, a small population of blister cells is usually seen (Fig. 1.21). In these cells, the Hb is pushed to one side with a thin strip of membrane remaining on the other side. The number of sickled cells on blood smears is highly variable and cannot be used as an indication for an acute sickle cell event. A small number of spherocytes are generally seen, and manifestations of hyposplenism are nearly always evident in adult patients, including Howell-Jolly bodies (see Fig. 1.19), Pappenheimer bodies, target cells (see Fig. 1.18), and circulating nucleated red blood cells. The white blood cell count is often modestly increased, even in the absence of acute complications.

In SC disease, classic sickled cells are rare or absent. Generally there are prominent target cells and scattered Hb C crystals (Figs. 1.22 and 1.23). The C crystals tend to be misshapen, bent, or abortive appearing and tend to produce RBCs with odd spikes and hooks (Fig. 1.24). Occasionally there are classic Hb C crystals (see discussion under Hemoglobin CC Disease). Some cells show irregularly concentrated hemoglobin. S/β-thalassemia differs from SS disease in that there is microcytosis, fewer sickled cells, and more target cells.

Ancillary Studies

Hb variant analysis (e.g., electrophoresis, capillary zone electrophoresis, and high-pressure liquid chromatography [HPLC]) in SS disease will all demonstrate a predominance of Hb S, variable Hb F (1% to 20%), and no Hb A. SC disease

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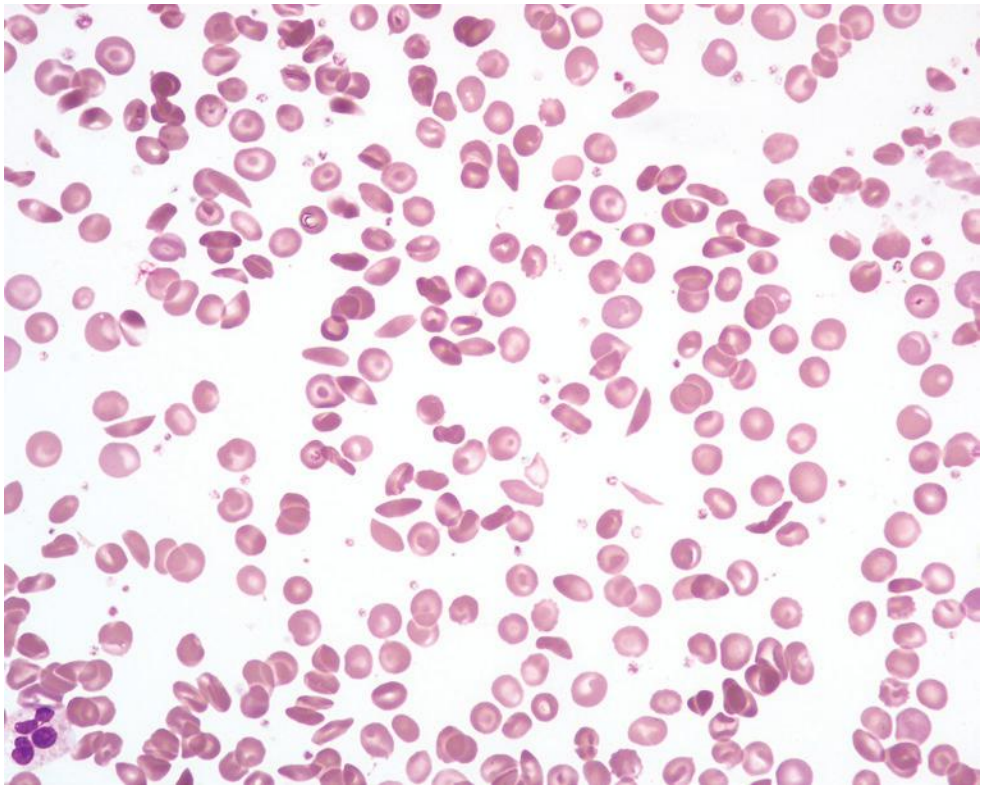


FIG. 1.18

Hemoglobin SS disease, peripheral blood findings. This blood smear from a patient with hemoglobin SS disease demonstrates prominent anisopoikilocytosis. The most prominent poikilocytes are elongate, sickled cells (drepanocytes), and cells with a droplet of hemoglobin within the area of central pallor (target cells or dacrocytes).

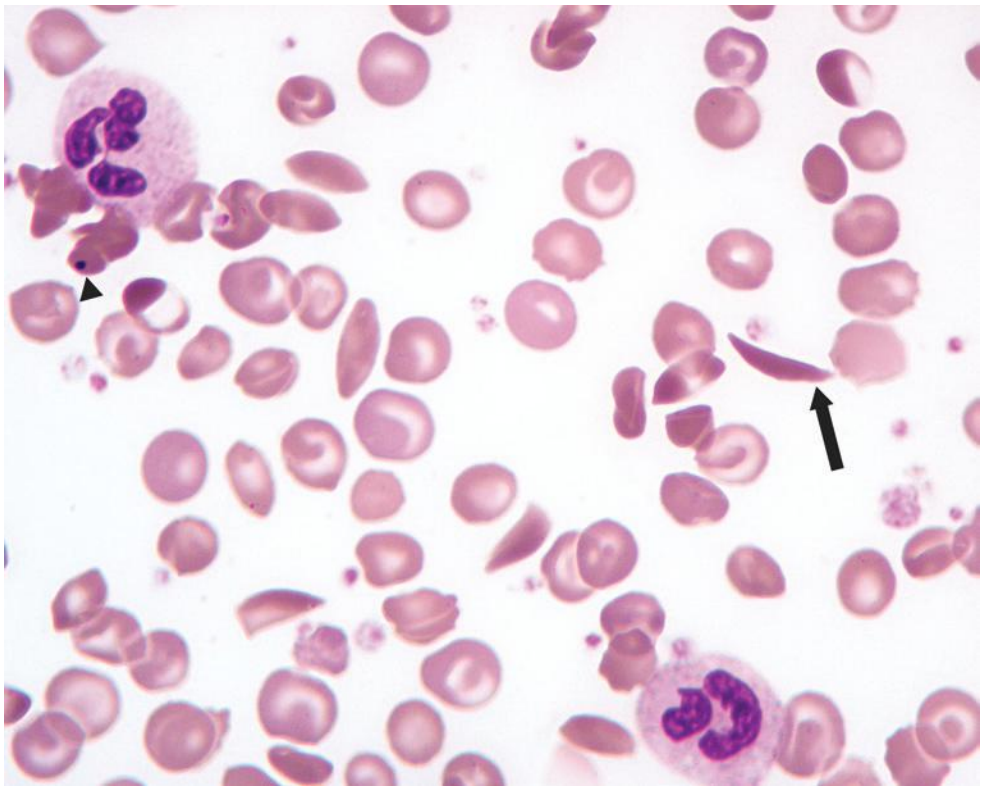


FIG. 1.19

Hemoglobin SS disease, peripheral blood findings. This high-power image of hemoglobin SS disease demonstrates a classic, slender sickled cell *(arrow)* with a concave–convex appearance, lack of central pallor, and pointed ends. Variant, broader sickled cells are also evident. Note also the Howell-Jolly body *(arrowhead)*.

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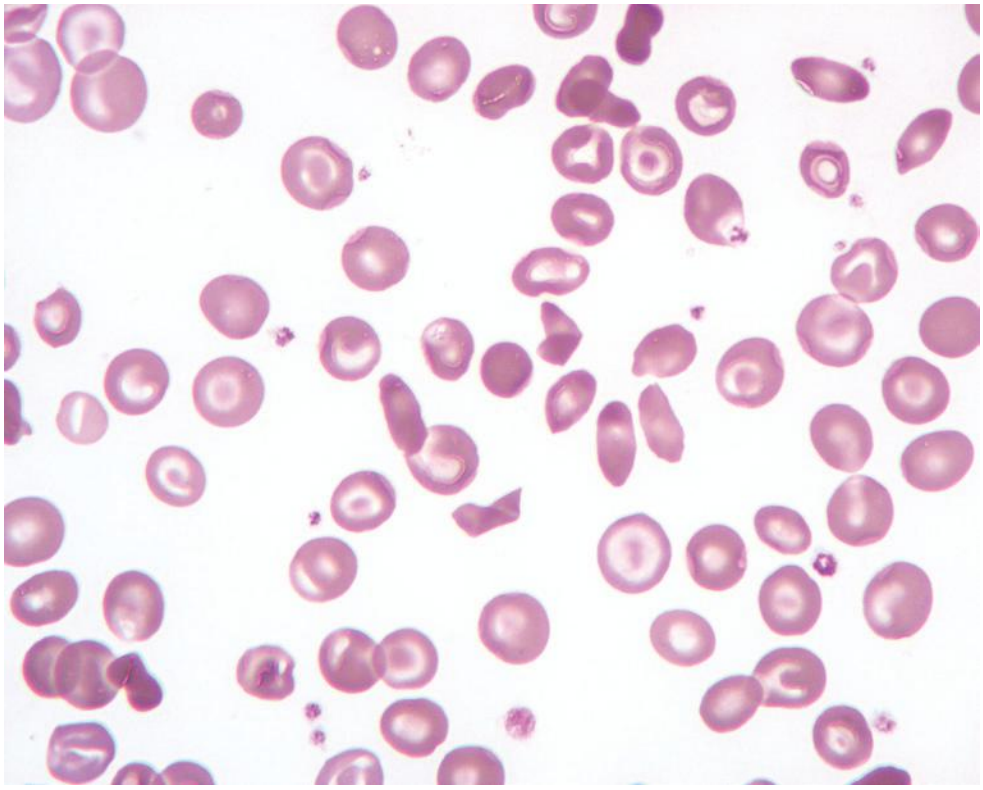


FIG. 1.20

Hemoglobin SS disease, peripheral blood findings. This image from a patient with hemoglobin SS disease features two variant sickled cells with a folded, boat-shaped appearance. Broad sickled cells are also present.

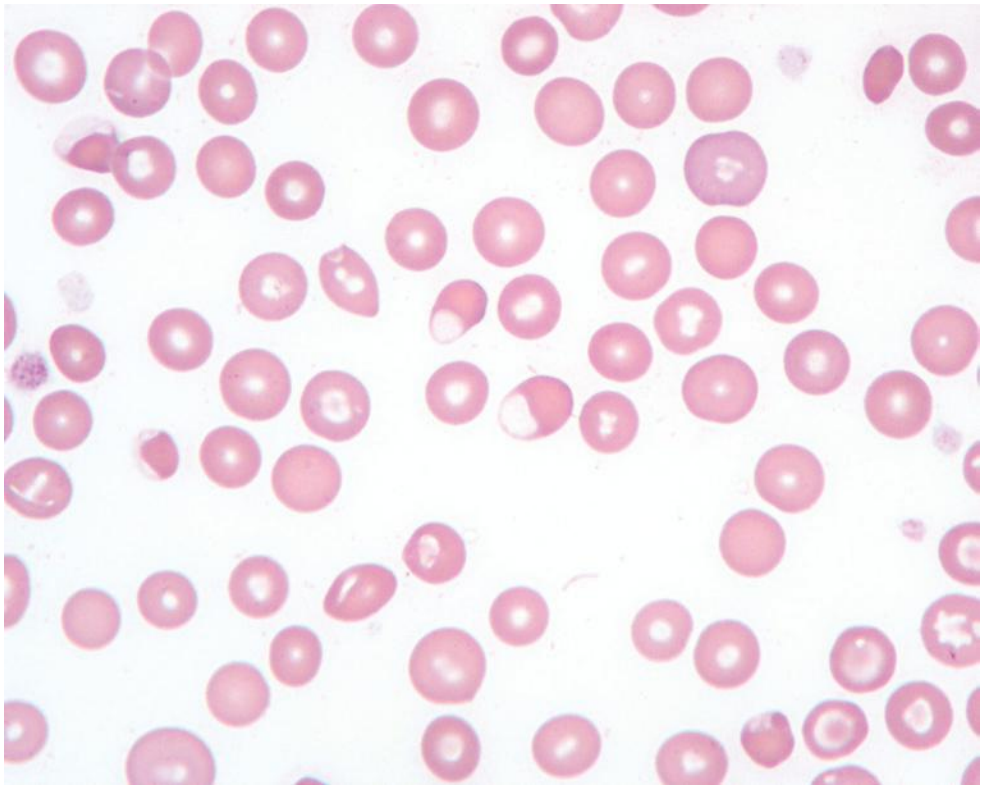


FIG. 1.21

Hemoglobin SS, peripheral blood findings. Blister cells are usually present in small numbers in smears from patients with hemoglobin SS disease. The hemoglobin mass is pushed to one side in these cells, with only a thin rim of membrane remaining on the other side.

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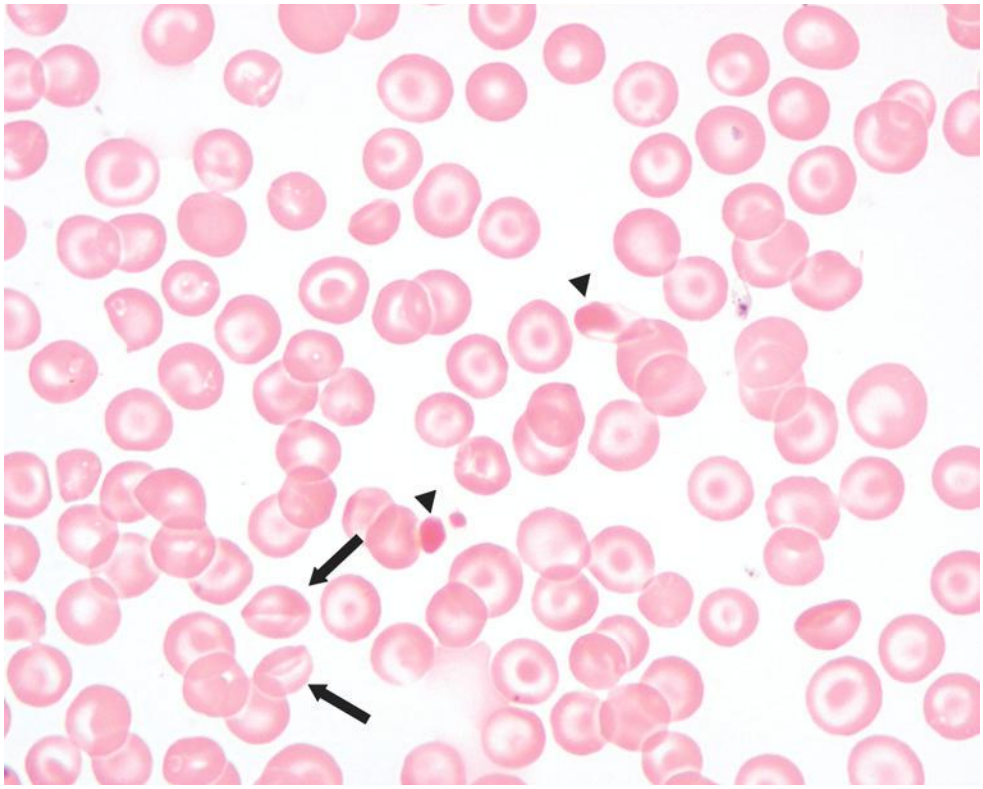


FIG. 1.22

Hemoglobin SC disease, peripheral blood findings. This smear from a patient with hemoglobin SC disease demonstrates prominent target cells, folded cells *(arrows),* and poorly formed Hb C crystals *(arrowheads).*

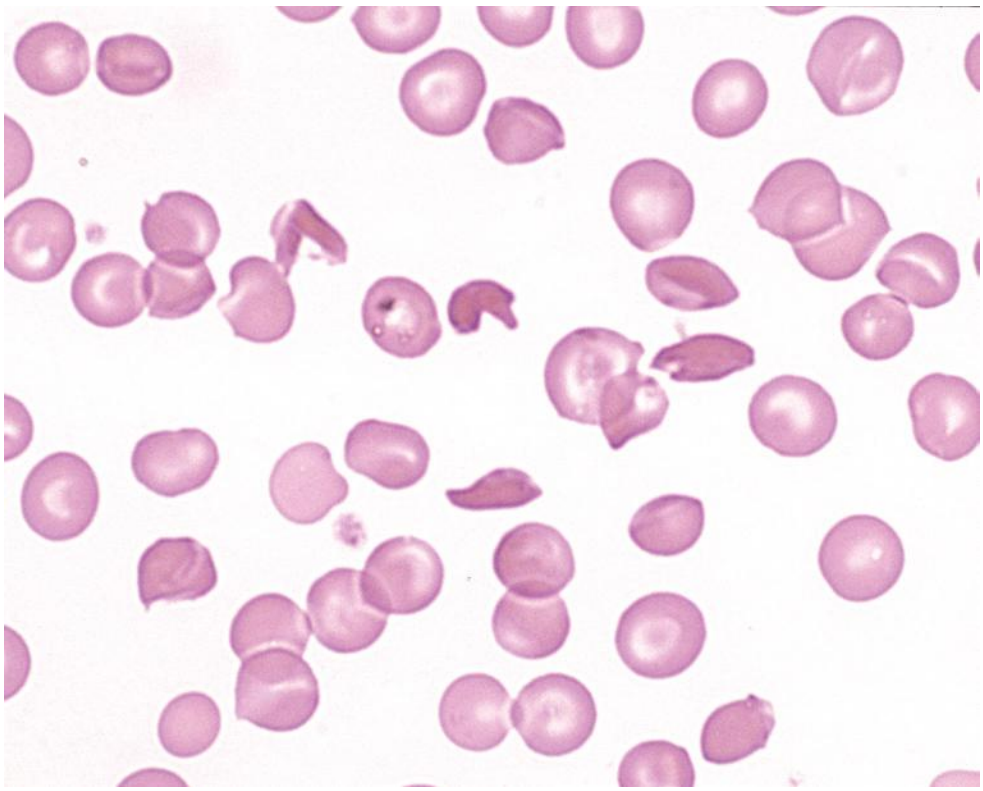


FIG. 1.23

Hemoglobin SC, peripheral blood findings. Crystals in hemoglobin SC disease may be irregular, somewhat amorphous, or bent.

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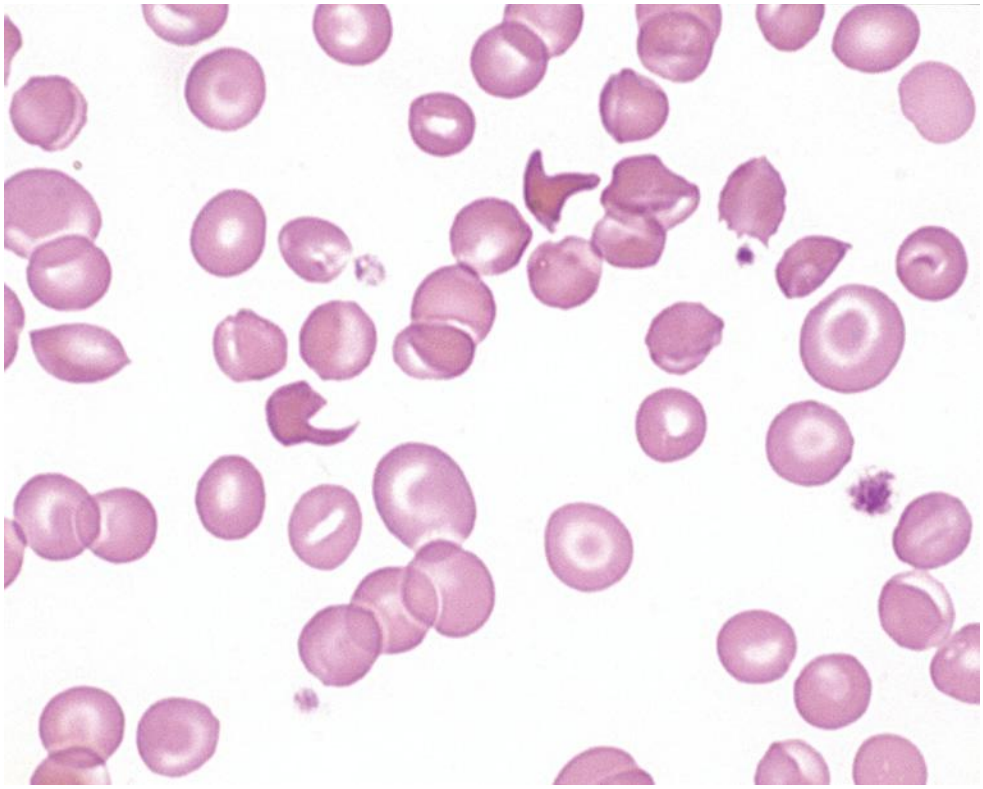


FIG. 1.24
Hemoglobin SC disease, peripheral blood findings. Red blood cells in hemoglobin SC disease may show peculiar spikelike or hooklike projections.

results in approximately equal amounts of Hb S and Hb C. S/β^0-thalassemia appears essentially identical to SS disease electrophoretically. S/β^+-thalassemia is characterized by a mixture of Hb S and Hb A, with more Hb S (in contrast to sickle trait, in which there is more Hb A than Hb S). All forms of SCD, as well as sickle trait, result in a positive sickle solubility test; therefore this procedure will not discriminate among these disorders. The Hb S mutation can also be detected by molecular methods.

Differential Diagnosis

The differential diagnosis of SCD includes other forms of chronic hemolytic anemia. However, only SCD is associated with sickled RBCs and vasoocclusive phenomena. Hb SC disease can be confused with Hb CC disease because of the presence of Hb C crystals. However, the crystals in CC disease are straight, in contrast to the irregular, bent, or malformed crystals of SC disease. Rare cases of SS disease will have few sickled cells but frequent blister cells. Such cases may be confused with oxidant hemolysis. Hemoglobinopathy studies will be definitive.

Prognosis and Therapy

Penicillin prophylaxis, immunization (including pneumococcal vaccination), and disease education have decreased the mortality of SCD during early childhood. However, mortality remains significantly higher among children who have more severe forms of disease (i.e., SS disease and S/β^0-thalassemia) compared to children overall matched for age and race/ethnicity. The average age of death in the United States is about 40 years for patients with severe forms of SCD. A variety of prognostic factors for SS disease have been identified in a number of studies. One important factor is the level of Hb F. High levels of Hb F inhibit the polymerization of Hb S, ameliorate the clinical symptoms, and prolong the life span. Other good prognostic factors that have been identified include lower frequencies of pain crises and acute chest syndrome, higher hemoglobin levels, lower baseline white blood cell counts, better renal function, lack of cerebrovascular events, and lack of pulmonary hypertension.

Patients with SC disease have significantly better life expectancy than those with SS disease, with median ages at death of 60 years for men and 68 for women. Patients with S/β-thalassemia have variable prognoses depending on the severity of the clinical phenotype.

Therapy depends on the severity of clinical manifestations. Acute vasoocclusive events, such as pain crises, acute chest syndrome, and infection, may be treated conservatively or, when severe, may require exchange transfusion. Patients generally receive prophylactic transfusion before surgery, and patients with particularly severe disease manifestations can be treated with regular transfusions. Hydroxyurea is used in patients with SS disease to elevate levels of Hb F within RBCs and thus diminish the severity and frequency of complications. Prolonged use of this agent has been shown

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to improve morbidity and mortality in SCD. The only proven curative option for SCD is allogeneic stem cell transplantation from an HLA antigen-matched sibling donor. The use of alternative donors and gene therapies is being investigated in clinical trials.

Hemoglobin CC Disease

Hb CC disease is due to homozygous inheritance of an abnormal β-globin chain, and, as in the S mutation, a single base change results in an amino acid substitution at the sixth position of the β-globin molecule. However, rather than a valine, the substituted amino acid is a lysine. Hb CC RBCs have abnormal membrane ion transport, causing them to be dehydrated. This dehydration is thought to be the primary cause of hemolysis in this disorder.

Clinical Features

Hb CC disease is a relatively innocuous clinical syndrome consisting of mild chronic hemolytic anemia. Hb CC is most prevalent in individuals of African descent. Infants are well at birth and develop hemolysis only after Hb F production is replaced by Hb C. Patients generally have Hb levels in the range of 9 to 12 g/dL with microcytosis. Clinical symptoms are largely absent, and most adults have splenomegaly that is generally asymptomatic.

Pathologic Features

Blood smears are notable for numerous target cells that are relatively uniform in appearance (Fig. 1.25). Minor subsets of folded cells and spherocytes may be seen. Hb C crystals are present in varying numbers in most patients, although

HEMOGLOBIN CC DISEASE—FACT SHEET

Definition
- Disorder caused by homozygous inheritance of the hemoglobin C mutation of the β-globin gene, resulting in red blood cell dehydration and mild chronic hemolysis

Morbidity and Mortality
- Mild anemia, occasional symptoms referable to splenomegaly
- No increased mortality

Gender, Race, or Age Distribution
- Equal gender incidence
- Common in individuals of African descent

Clinical Features
- Mild hemolytic anemia
- Splenomegaly, usually asymptomatic

Prognosis and Therapy
- No therapy required

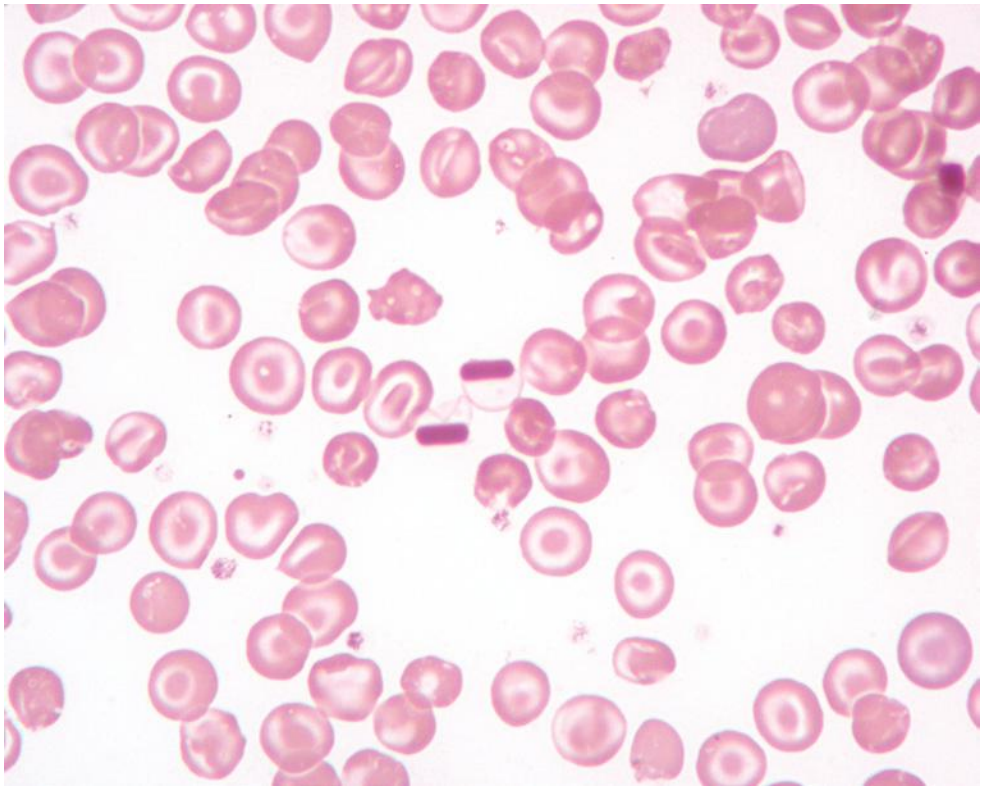


FIG. 1.25

Hemoglobin (Hb) CC disease, peripheral blood findings. This image from a patient with Hb CC disease demonstrates two classic Hb C crystals. These elongate, uniformly dense structures are present within a ghostlike, residual red blood cell membrane. Hb C crystals are diagnostic of the presence of Hb C, although they do not strictly distinguish between CC and SC diseases. Many target cells and a few folded cells are also present.

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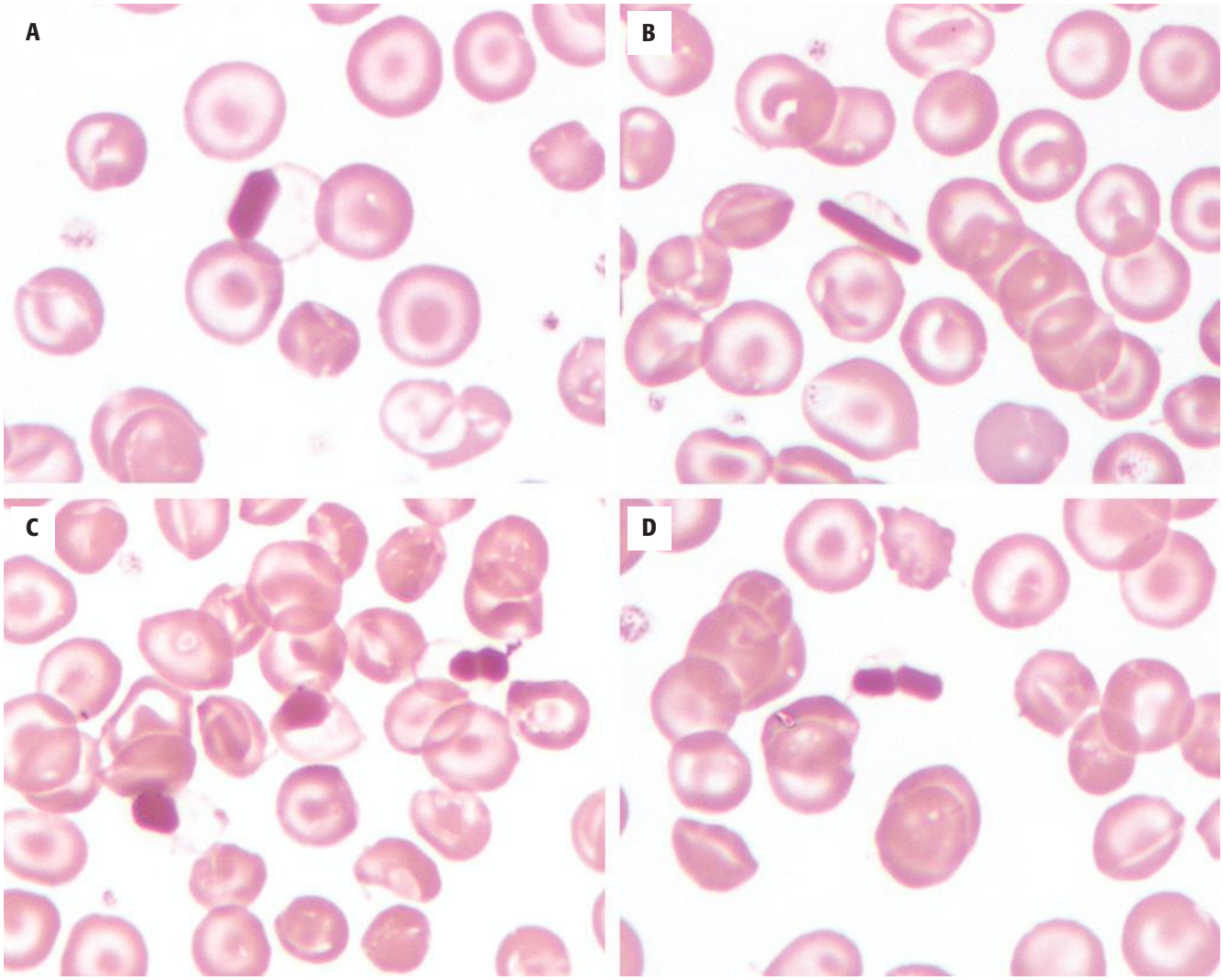


FIG. 1.26

Hemoglobin (Hb) CC disease, peripheral blood findings. This composite image shows multiple C crystals in a patient with Hb CC disease. A, The polyhedral crystalline nature of these structures is evident in this Hb C crystal. B, A long, slender crystal. C, Several blunt crystals and a double crystal. D, Two well-formed crystals within a single cell.

HEMOLOBIN CC DISEASE—PATHOLOGIC FEATURES

Microscopic Findings

- Numerous uniform target cells
- Scattered folded cells and spherocytes
- Hemoglobin C crystals

Differential Diagnosis

- Thalassemia minor
- SC disease
- C/β-thalassemia

occasionally they are rare or absent. Hb C crystals are classically elongate, straight, hexagonal crystals and are most often within residual RBC membranes (Figs. 1.25 and 1.26). However, they may be shorter and blunter and occasionally are multiple within an individual cell (see Fig. 1.26).

Ancillary Studies

Hb variant analysis in Hb CC disease will each demonstrate nearly all Hb C with small amounts of Hb F and Hb A_2. Hb C is a relatively slow Hb variant on conventional alkaline electrophoresis, migrating in the same position as Hb A_2, Hb E, and Hb O.

Differential Diagnosis

Because of the microcytosis and target cell formation, Hb CC disease can be mistaken for mild forms of thalassemia. However, target cells are much more numerous in Hb CC disease than in mild thalassemia, and the presence of C crystals will establish the diagnosis. SC disease can also be confused with Hb CC disease. However, the crystals in SC disease are

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usually not straight or well formed, and the irregular spiked or hooked cells of SC disease are not a feature of CC disease. A more pronounced microcytosis than expected and an elevated HbA_2 would support a diagnosis of C/β-thalassemia. A variable amount of Hb A would be detected in patients with C/β^+-thalassemia. Family studies or β-globin DNA analysis may be needed to definitively distinguish between Hb CC from C/β^0-thalassemia.

Prognosis and Therapy

CC disease is a clinically benign disorder with a normal life expectancy. However, patients have an increased risk of gallstones due to chronic hemolysis. Genetic counseling is warranted since there is a risk of having a child with SC disease if the other parent carries Hb S.

Thalassemias

The thalassemias are a group of disorders characterized by decreased production of either α- or β-globin chains. The diseases are designated according to which chain is underproduced, that is, β-thalassemias involve decreased β-chain production and α-thalassemia decreased α-chain production. In pure thalassemic syndromes, the globin chains that are produced are structurally normal, distinguishing them from the structural Hb disorders discussed previously. However, several structural Hb abnormalities may produce syndromes closely mimicking the thalassemias, because of underproduction of structurally abnormal globin chains. The most common of these is Hb E, a β-chain variant.

The decreased Hb production in the thalassemias results in hypochromic, microcytic RBCs. In addition, the decreased globin chain synthesis results in an excess of the chain that is produced in normal amounts. These excess chains precipitate in the more severe forms of these diseases and cause intramedullary death of RBC precursors (especially in β-thalassemia) and decreased RBC lifespan. The clinically significant thalassemias can be considered to be primarily hemolytic anemias.

Clinical Features

The thalassemias are extremely heterogeneous, both genetically and clinically. The vast majority of the heterozygous forms of β-thalassemia, two-gene deletion forms of α-thalassemia either in *cis* (-α/-α, "homozygous α^+-thalassemia") or in *trans* (–/αα, "heterozygous α^0-thalassemia"), and heterozygous and homozygous Hb E are clinically innocuous, manifesting as RBC microcytosis with mild or no anemia. One-gene deletion α-thalassemia (-α/αα, "heterozygous α^+-thalassemia") is clinically and hematologically silent. However, the homozygous or compound heterozygous β-thalassemias, the three- and four-gene deletion α-thalassemias (-α/– or –/–), and the compound heterozygous state for Hb E and β-thalassemia range from moderate to profound hemolytic anemias. The thalassemias show a wide geographic distribution, with higher frequency in Mediterranean countries, the Middle East, parts of India and Pakistan, Southeast Asia, and Africa. With large-scale population movements, thalassemias are becoming more common in other parts of the world, including northern Europe and North America.

THALASSEMIAS—FACT SHEET

Definition

- A group of hypochromic, microcytic anemias characterized by underproduction of normal α- or β-globin chains

Morbidity and Mortality

- α-Thalassemia
  - Hemoglobin (Hb) Bart's hydrops fetalis: infants stillborn or die within first hours of life
  - Hb H disease: mild morbidity, probably near-normal or normal life expectancy
  - One- and two-gene deletion α-thalassemia: no increased morbidity or mortality
- β-Thalassemia
  - Major: severe, chronic complications; death in childhood without long-term transfusion; death in second or third decade if regular transfusion without iron chelation
  - Intermedia: highly variable, ranging from similar to β-thalassemia major to no increased morbidity and mortality
  - Minor: no increased morbidity or mortality

Gender, Race, or Age Distribution

- Equal gender incidence
- Wide geographic distribution, with higher frequency in tropical and subtropical regions (Mediterranean, Middle East, India and Pakistan, Southeast Asia, Africa); becoming more common in other parts of the world

Clinical Features

- α-Thalassemia
  - Hb Bart's hydrops fetalis: severe anemia, generalized edema, organomegaly, heart failure
  - Hb H disease: jaundice, splenomegaly, gallstones, rare leg ulcers
  - Two-gene deletion α-thalassemia: mild microcytic anemia
- β-Thalassemia
  - Major: profound hemolytic anemia with stunted growth, wasting, fever, hyperuricemia, infections, organomegaly, frontal bossing, spontaneous fractures, and other bony abnormalities; if transfused, endocrine, cardiac, and liver dysfunction due to iron deposition
  - Intermedia: highly variable, ranging from similar to β-thalassemia major to asymptomatic
  - Minor: mild microcytic anemia

Prognosis and Therapy

- Lifelong transfusion-chelation regimen or hematopoietic stem cell transplantation for severe thalassemias
- Supportive care of chronic hemolytic complications
- Prognosis in severe thalassemias dependent on adequacy of transfusion-chelation regimen

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The most severe form of thalassemia is four-gene deletion α-thalassemia (–/–) "Hb Bart's hydrops fetalis." The total absence of α-chain production results in a profound defect in Hb production. Hb levels average 6.2 g/dL in affected fetuses; however, this value is deceptive because the majority of this is Hb Bart's hydrops fetalis (γ-tetramers), which is ineffective for delivering oxygen to the tissues. Only the small amounts of embryonic Hb present are effective at delivering oxygen, and these infants suffer profound tissue hypoxia. As with other forms of severe intrauterine anemia, this condition results in the syndrome of hydrops fetalis with high-output cardiac failure, generalized edema, and organomegaly.

β-Thalassemia major (Cooley anemia) results from a complete absence or profound decrease in β-chain production from both β-globin alleles. Infants are well at birth but develop severe anemia over the first few months of life as γ-chain production needs to be replaced by β-chain production. By definition, the anemia is transfusion dependent, with steady-state Hb levels of 2 to 3 g/dL. If transfusion is not performed, children develop a wide spectrum of clinical manifestations caused by the severe hemolytic anemia itself and the body's attempts to compensate. These manifestations include stunted growth, wasting, fever, hyperuricemia, infections, organomegaly, frontal bossing, spontaneous fractures, and other bony abnormalities. Patients with β-thalassemia major who receive an adequate transfusion program have essentially normal early development, but ultimately manifest complications resulting from iron overload. These manifestations include endocrine problems, such as absent menarche and pubertal growth spurt, diabetes mellitus, and adrenal insufficiency. Cardiac dysfunction resulting from iron overload ultimately is the most prominent cause of morbidity and mortality in these patients.

Moderately severe forms of thalassemia include β-thalassemia intermedia and Hb H disease. β-Thalassemia intermedia covers a wide spectrum, ranging from disorders nearly as severe as β-thalassemia major (but lacking transfusion dependence) to mild disorders with few clinical manifestations. The complications depend on the severity of the hemolysis and are largely those seen in other forms of hemolytic anemia, including jaundice, organomegaly, gallstones, susceptibility to infections, and leg ulcers. Hb H disease, usually a result of three α-gene deletions (-α/–), is a moderate hemolytic anemia with Hb levels in the range of 7 to 10 g/dL. Hb E/β-thalassemia generally manifests as β-thalassemia intermedia.

Radiologic Features

β-Thalassemia major patients who have not received transfusions manifest characteristic radiologic abnormalities, including radiating striations in the subperiosteal bone of the skull (producing a "hair on end" appearance), cortical thinning, rarefaction of long bones and small bones of the hands and feet, and pathologic fractures.

THALASSEMIAS—PATHOLOGIC FEATURES

Microscopic Findings

- Severe β-thalassemia and hemoglobin (Hb) Bart's hydrops fetalis
  - Pronounced, bizarre anisopoikilocytosis
  - Target cells, teardrop cells, fragments
  - Hypochromia
  - Prominent erythroblastosis
- Hb H disease
  - Moderate anisopoikilocytosis
  - Target cells
  - Hypochromia
  - Numerous Hb H inclusions with brilliant cresyl blue staining

Differential Diagnosis

- Hb Bart's hydrops fetalis: other causes of fetal anemia or hydrops (e.g., Rhesus disease, parvovirus B19, fetomaternal hemorrhage)
- Hb H disease: iron deficiency, Hb CC disease
- β-Thalassemia minor and two-gene–deletion α-thalassemia: IDA, ACD

Pathologic Features

The pathologic features of the thalassemias are as variable as the clinical findings. The peripheral blood smears in Hb Bart's hydrops fetalis and severe forms of β-thalassemia are similar, characterized by prominent erythroblastosis and pronounced anisopoikilocytosis, including prominent hypochromia and target cells, as well as fragments, teardrop cells, and other bizarre poikilocytes (Fig. 1.27). Whereas the MCV is low in severe β-thalassemia, it tends to be normal or high in Hb Bart's hydrops fetalis, likely due in part to the prominent reticulocytosis in this disorder.

The blood smear in Hb H disease is characterized by hypochromia, microcytosis, moderate anisopoikilocytosis dominated by target cells, and moderate polychromasia (Fig. 1.28). Erythroblastosis is not a feature of this disorder.

β-Thalassemia minor and two-gene deletion α-thalassemia are characterized by a fairly uniform population of microcytic RBCs with occasional target cells (Fig. 1.29). Coarse basophilic stippling is a classic (although fairly insensitive) feature of β-thalassemia minor, but it is not a feature of mild α-thalassemia.

Ancillary Studies

Hemoglobin Analysis

Because of the complete absence of α-chain production in Hb Bart's hydrops fetalis, no Hb A, Hb F, or Hb A_2 will be detected by Hb variant analysis (e.g., electrophoresis, capillary zone electrophoresis, or HPLC). Instead, most of the Hb present will be Hb Bart's, which represents γ-chain

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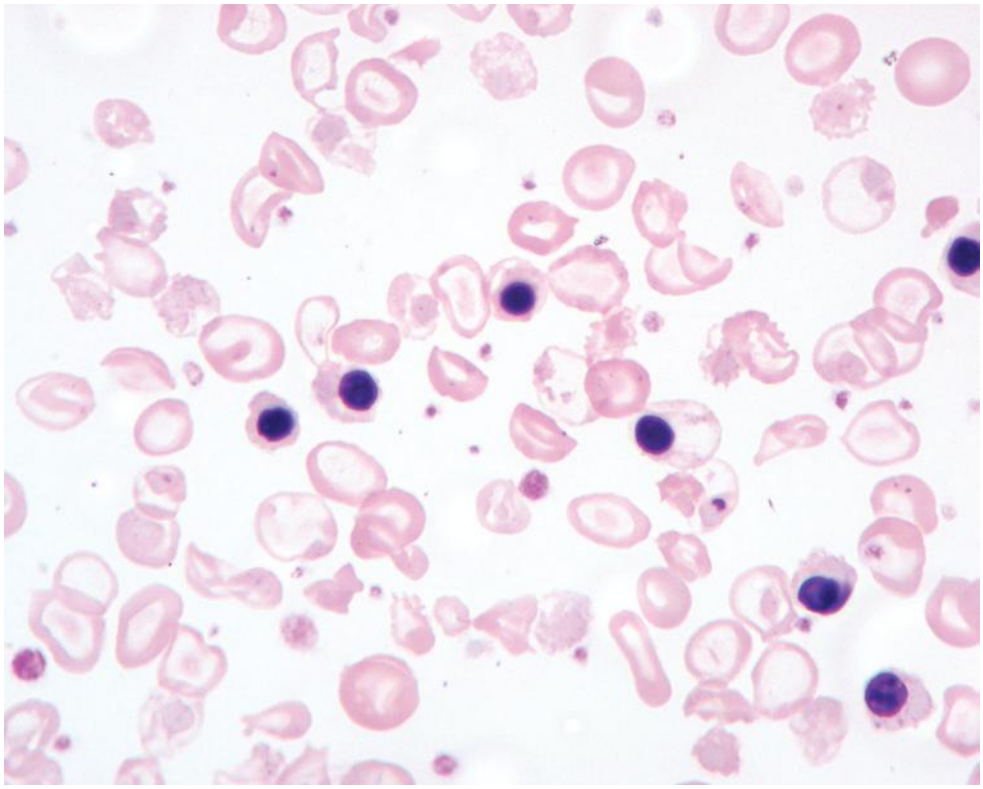


FIG. 1.27

Severe β-thalassemia, peripheral blood findings. This smear from a patient with severe β-thalassemia demonstrates erythroblastosis, prominent hypochromia, target cells, and other bizarre poikilocytes.

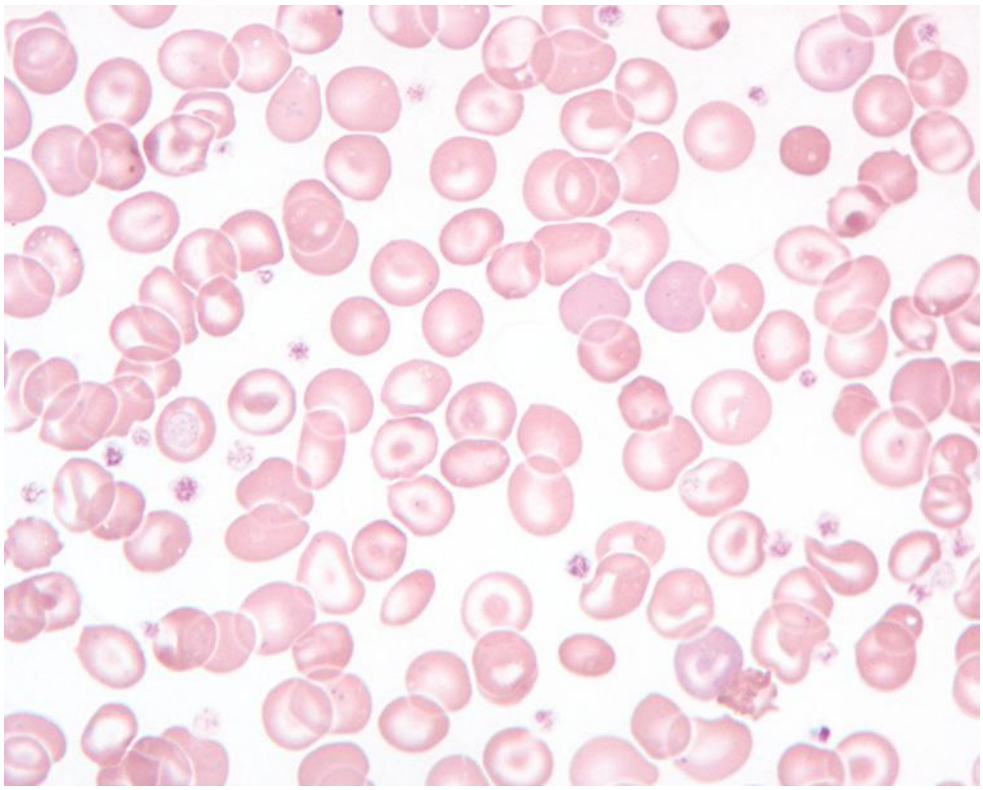


FIG. 1.28

Hemoglobin H disease, peripheral blood findings. Compared to severe β-thalassemias, the blood smear findings in hemoglobin H disease are relatively bland, with hypochromia and target cells.

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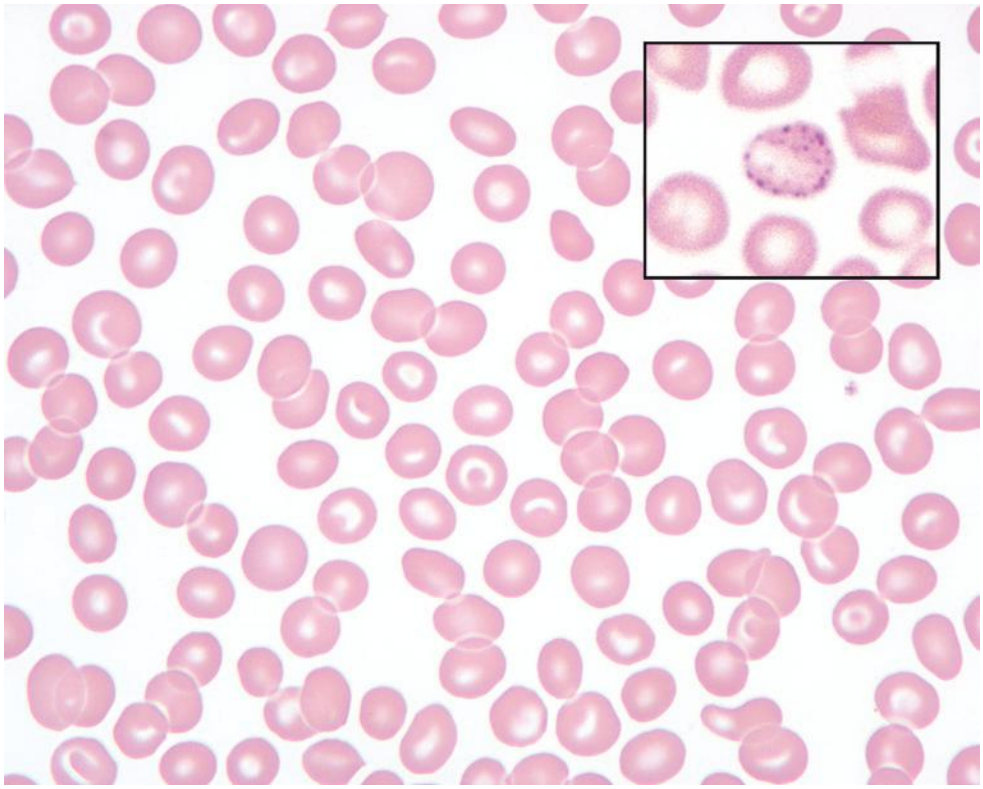


FIG. 1.29

β-Thalassemia minor, peripheral blood findings. This smear from a patient with β-thalassemia minor demonstrates a fairly uniform population of microcytic cells with scattered target cells. Coarse basophilic stippling *(inset)* may be seen in β-thalassemia minor, helping to distinguish it from iron deficiency anemia and anemia of chronic disease, in which this is an uncommon finding.

tetramers. Small amounts (5% to 20%) of the embryonic Hbs Gower-1 (ζ2ε2) and Portland (ζ2γ2) are also present.

In Hb H disease, Hb Bart's constitutes more than 25% of total Hb at birth. Hb H disease in adults is characterized by the presence of Hb H (β-chain tetramers); the fraction of Hb H varies, but it usually accounts for 8% to 10% of total hemoglobin. Hb Bart's and Hb H are rapidly migrating and eluting hemoglobins (i.e., fast Hbs). Hb H disease can also be due to deletion of both α-globin genes on the same chromosome and a structurally abnormal α-chain variant that is produced at a reduced rate due to a mutated α-globin gene on the other chromosome. Hb Constant Spring is one of the most common such thalassemic α-chain variants, and, in adults with this type of Hb H disease ($\alpha^{CS}\alpha/-$), it will account for about 1.5% to 2% of hemoglobin.

Neonates with one-gene deletion α-thalassemia may or may not have a detectable level of Hb Bart's (<5%). Neonates with two-gene deletion α-thalassemia have more Hb Bart's (<10%), but it generally falls below detectable levels by 3 to 6 months of age. Adults who are heterozygous for Hb Constant Spring ($\alpha^{CS}\alpha/\alpha\alpha$) usually have 0.5% to 1% Hb Constant Spring, and homozygotes ($\alpha^{CS}\alpha/\ \alpha^{CS}\alpha$) have 2% to 11%.

β-Thalassemia major manifests as all or nearly all Hb F. Hb A may be present in small amounts, and Hb A_2 may be normal or increased. β-Thalassemia intermedia shows varying proportions of Hb A and Hb F, depending on the severity of the disease and may or may not show elevated Hb A_2. The hallmark of β-thalassemia minor is an increase in Hb A_2 (3.5% to 5.5%), but rarely Hb A_2 levels can be normal. For Hb E/β-thalassemia, Hb E and Hb F will be detected with or without a low level of Hb A depending on whether or not any normal β-chains are produced.

Other Studies

Incubation of red cells with brilliant cresyl blue in patients with Hb H disease will produce numerous pale blue inclusions in RBCs, producing a "golf ball" appearance (Fig. 1.30). Gap-polymerase chain reaction (PCR) is the molecular testing used in practice to detect the most common deletions for α-thalassemia. DNA sequencing of the globin genes can be used to detect point mutations, which account for the majority of the β-thalassemias and a minority of α-thalassemias (also see Chapter 24).

Differential Diagnosis

The clinical differential diagnosis of Hb Bart's hydrops fetalis includes other causes of hydrops fetalis associated with anemia, including Rh hemolytic disease, intrauterine parvovirus B19 infection, severe fetomaternal hemorrhage, twin-twin transfusion, and cystic hygroma. Whereas erythroblastosis will be a feature of any form of hydrops in which RBC production is

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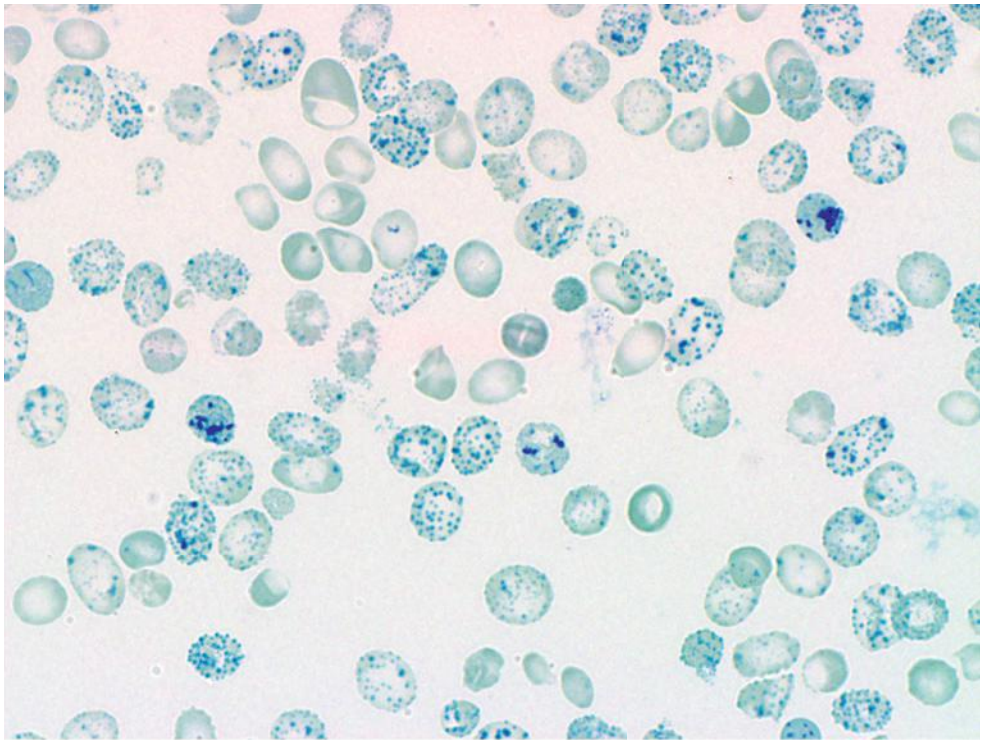


FIG. 1.30

Hemoglobin H disease, peripheral blood findings. This brilliant cresyl blue stain reveals numerous pale blue inclusions in most of the red cells, producing a golf ball–like appearance. *(Courtesy Dr. Tracy I. George, University of New Mexico, Albuquerque, NM.)*

not compromised, the morphologic changes of the RBCs in Hb Bart's hydrops fetalis will serve to distinguish it from other causes of fetal anemia. Hb variant analysis will be diagnostic. Hb H disease may potentially be confused with other forms of moderate microcytic anemia. IDA might be considered; however, target cells are much more prominent in Hb H disease, and reticulocytosis is not a feature of untreated iron deficiency. Because of the prominence of target cells, Hb CC disease may also be a consideration. However, hypochromia is not a prominent feature of Hb CC disease, and Hb C crystals will be evident. Hb variant analysis demonstrating a fast moving Hb H band will be diagnostic.

The clinical differential diagnosis of the severe β-thalassemias includes other forms of moderate to severe chronic hemolytic anemia. However, the bizarre morphologic changes in the RBCs and the degree of erythroblastosis in β-thalassemia major are essentially diagnostic. Hb analysis, which will show large amounts of Hb F without other abnormal Hb species, will confirm the diagnosis. Based on Hb variant analysis, severe β-thalassemia may be confused with some forms of hereditary persistence of fetal Hb. However, patients with hereditary persistence of fetal Hb either have normal Hb levels or polycythemia, permitting ready distinction.

The differential diagnosis of β-thalassemia minor and two-gene deletion α-thalassemia are IDA and ACD (see Table 1.2). Elevated Hb A_2 levels and normal iron studies will confirm a diagnosis of β-thalassemia minor. Two-gene deletion α-thalassemia is often a diagnosis of exclusion in adults, although the presence of small amounts of Hb Bart's may be detected in newborns. Molecular studies to identify the precise genotype for individuals with α-thalassemia are available (see Chapter 24).

Prognosis and Therapy

Without therapy, fetuses with Hb Bart's hydrops fetalis are stillborn or die shortly after birth, but they may occasionally be rescued with intrauterine transfusion followed by lifelong transfusion and iron chelation therapy. Patients with β-thalassemia major require regular transfusions and chelation therapy. Without chelation, patients with β-thalassemia major will die in the second or third decade due to iron overload. Allogeneic stem cell transplantation in patients with an available donor is potentially curative. The prognosis after transplantation depends in part on the amount of end-organ damage incurred prior to transplantation. Patients with moderately severe forms of thalassemia are not dependent on transfusion for survival but may need chelation therapy due to increased iron absorption and intermittent transfusions.

MEMBRANE DISORDERS

Hereditary Spherocytosis

Hereditary spherocytosis (HS) is one of the most common inherited hemolytic anemias, with a frequency of 1 in 5000 in the United States and 1 in 2000 in individuals of Northern European ancestry. It is a genetically and clinically

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heterogeneous disorder of the RBC membrane characterized by defective vertical interactions between the lipid bilayer and the cytoskeleton of the RBC membrane, resulting in increased vesiculation and loss of RBC membrane. The structural proteins affected by mutations in HS are ankyrin-1, band 3, β-spectrin, α-spectrin, and protein 4.2. Progressive loss of membrane results in rigid spherocytic cells that show delayed egress from the splenic cords. Prolonged exposure to the splenic cordal environment causes further membrane damage, and ultimately these cells are prematurely removed from circulation by the spleen. In three fourths of cases, HS is inherited in an autosomal-dominant manner. Of the remaining cases, approximately half are autosomal recessive and half are new mutations.

Clinical Features

HS ranges from an asymptomatic disorder to a severe, lifelong hemolytic anemia. Approximately one fourth of patients have mild, completely compensated hemolysis with no anemia. Most of the remainder have only mild to moderate anemia, with Hb levels ranging from 8 to 12 g/dL. Only a minority (5% to 10%) have more severe anemia. In symptomatic forms, anemia is typically absent at birth but develops over several weeks. A common presentation of HS is unexplained neonatal hyperbilirubinemia, which appears in the first 2 days of life and may then increase rapidly. Splenomegaly is common in patients with HS, seen in 50% of infants and 75% to 95% of older children and adults. Jaundice is seen in half of patients. Other complications include gallstones, megaloblastic anemia, and aplastic crisis owing to parvovirus B19 infection. The latter is a catastrophic, potentially fatal complication.

HEREDITARY SPHEROCYTOSIS—FACT SHEET

Definition
- Disorder caused by defective vertical interactions between the lipid bilayer and cytoskeleton of the RBC membrane, resulting in vesiculation and loss of membrane, spherocyte formation, and hemolysis

Incidence and Location
- One in 5000 in the United States

Morbidity and Mortality
- Mild morbidity owing to complications of chronic hemolysis
- Aplastic crises owing to parvovirus B19
- Megaloblastic anemia
- Slightly increased mortality, usually a result of postsplenectomy sepsis

Gender, Race, or Age Distribution
- Equal gender incidence
- More common in patients of Northern European descent (1 in 2000)

Clinical Features
- Variably severe hemolytic anemia
- Jaundice
- Splenomegaly

Prognosis and Therapy
- Supportive therapy
- Folate supplementation and splenectomy for more severe variants

Pathologic Features

The blood smear in patients with HS shows variable spherocytosis and polychromasia (Fig. 1.31). Spherocytes are round RBCs that are smaller in diameter than normal RBCs, lack central pallor, and have a denser (hyperchromic) staining quality. Thus the MCV is typically decreased, especially in more severe disease, and the MCHC can be high. Spherocytes vary in number considerably, depending on the severity of the disease. In the mildest forms of HS, spherocytes may be rare or absent. Postsplenectomy, spherocytes are still present, in addition to postsplenectomy RBC changes such as target cells, Howell-Jolly bodies, and Pappenheimer bodies. Histologic examination of the spleen typically demonstrates congestion of the splenic cords because of entrapped spherocytes as opposed to the typical sinusoidal pattern in passive congestion.

Ancillary Studies

Osmotic Fragility Test

The osmotic fragility test is the classic screening test for HS. Because of decreased surface area-to-volume ratio, spherocytes are more susceptible to osmotic lysis in hypotonic media than normal RBCs. RBCs are suspended in hypotonic saline of varying concentrations. The amount of hemolysis at each concentration is measured, and these values are plotted on a graph and compared with a normal control. A shift of the osmotic fragility curve toward lysis at higher concentrations of saline, either immediately or after 24 hours of incubation at 37° C, constitutes a positive result.

HEREDITARY SPHEROCYTOSIS—PATHOLOGIC FEATURES

Microscopic Findings
- Spherocytes
- Polychromasia

Differential Diagnosis
- Spherocytic immune hemolysis (warm autoimmune hemolytic anemia or ABO hemolytic disease)

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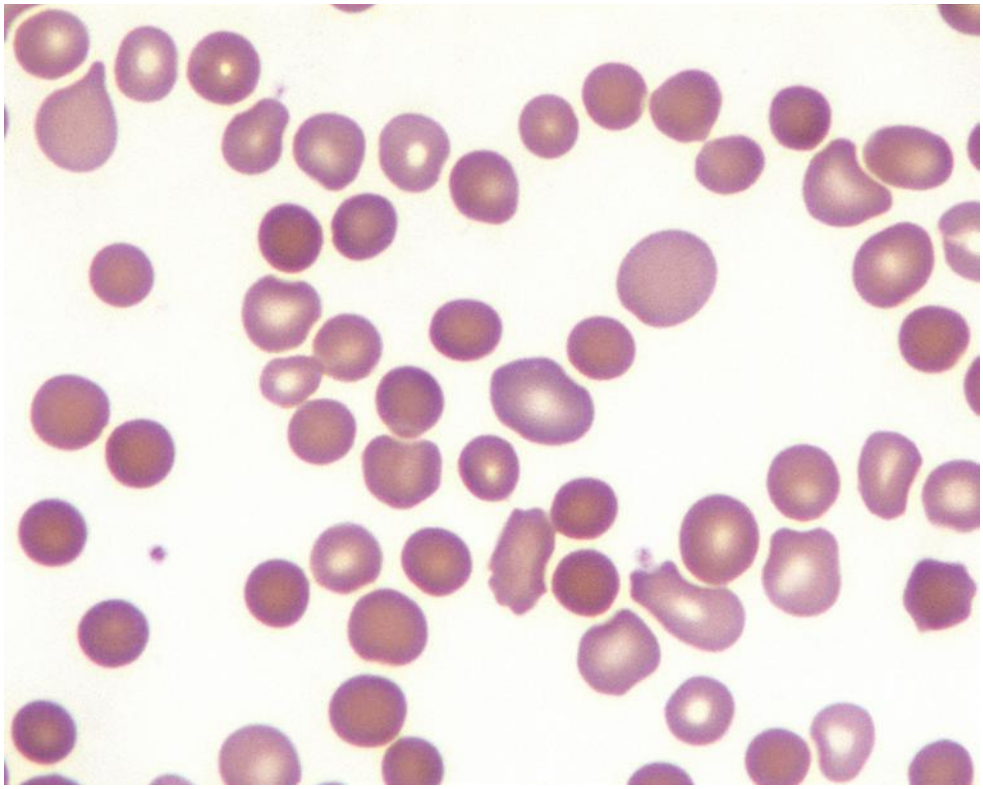


FIG. 1.31

Hereditary spherocytosis (HS), peripheral blood findings. This blood smear from a patient with HS shows spherocytes and polychromasia. Spherocytes are round cells with a dense staining quality, lack of central pallor, and reduced diameter compared to other red cells. Polychromatophilic cells represent young reticulocytes and are larger than mature red cells, have a slightly bluish staining quality, and may lack central pallor.

Other Studies

Flow cytometric analysis of red cells using the dye eosin-5′-maleimide, which binds specifically to the anion transport protein (band 3), is a more sensitive screening test than the osmotic fragility test; decreased fluorescence is seen in HS but can also be detected in congenital dyserythropoietic anemia type II, hereditary pyropoikilocytosis, and Southeast Asian ovalocytosis. For atypical cases, analysis of membrane protein composition by SDS-PAGE or DNA sequencing of cytoskeletal protein genes are definitive methods to diagnose HS.

Differential Diagnosis

The major differential diagnosis of HS is spherocytic immune hemolytic anemia due to either alloantibodies or warm autoantibodies. The blood smear findings will be essentially identical in these disorders. Furthermore, the osmotic fragility test will also be positive in spherocytic immune hemolysis. In addition to the fact that HS is a lifelong condition, demonstration of RBC antibodies with a direct antiglobulin test will usually distinguish between these disorders. Evaluating family members of the patient is also recommended because the majority of patients with HS with have affected first-degree relatives.

Spherocytes are also seen in small numbers in most other forms of hemolytic anemias. However, in these disorders, they are not the dominant abnormality and are generally seen in small numbers.

Prognosis and Therapy

HS, in most cases, requires no specific therapy other than treatment for complications, such as gallstones. Patients with more severe symptomatic disease may be considered for splenectomy, which corrects the anemia in most cases. However, splenectomized patients are at risk of postsplenectomy sepsis, which can be fatal.

Hereditary Elliptocytosis and Hereditary Pyropoikilocytosis

Hereditary elliptocytosis (HE) and hereditary pyropoikilocytosis (HPP) are related disorders caused by defects in the horizontal interactions between membrane cytoskeletal proteins, specifically the association of αβ spectrin heterodimers into heterotetramers. Like HS, these are genetically and clinically heterogeneous. The structural proteins affected by mutations in these disorders are α-spectrin, β-spectrin, and protein 4.1R. HE results from a heterozygous gene defect, whereas HPP results from homozygosity for a particular HE mutation, double heterozygosity for two different HE mutations, or double heterozygosity for an HE mutation and a low-expression α-spectrin allele. HE is usually inherited in an autosomal-dominant fashion. Patients with HPP either

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have two parents with HE or one parent with HE and one without apparent hematologic abnormalities (carriers of the low-expression α-spectrin allele). HE is common, with an incidence in the United States of at least 1 in 2000 to 4000, and it is more common in individuals of African or Mediterranean heritage. HPP is rare.

Clinical Features

The most common form of HE is a very mild, completely compensated hemolysis that does not produce anemia and is completely asymptomatic; therefore HE is most commonly discovered incidentally. More severe forms of HE occur in which the hemolysis is incompletely compensated, with Hb levels in the range of 9 to 12 g/dL. These patients commonly have jaundice, splenomegaly, and gallstones. Patients with HPP have Hb levels in the range of 4 to 8 g/dL and experience clinical complications similar to those with more severe HE. In addition, patients with the most severe forms of HPP may have clinical manifestations similar to those seen in β-thalassemia major, such as growth retardation and frontal bossing.

HEREDITARY ELLIPTOCYTOSIS AND HEREDITARY PYROPOIKILOCYTOSIS—FACT SHEET

Definition
- A group of disorders caused by defective horizontal interactions between spectrin heterodimers in the red blood cell cytoskeleton

Incidence and Location
- At least 1 in 2000 to 4000 in the United States

Morbidity and Mortality
- Most asymptomatic patients with hereditary elliptocytosis (HE) have no significant morbidity or mortality
- Complications of chronic hemolysis in severe HE and hereditary pyropoikilocytosis (HPP)

Gender, Race, or Age Distribution
- Equal gender incidence
- HE more common in patients of African or Mediterranean descent
- Most HPP occurs in black patients

Clinical Features
- Common HE
  - None
- Severe HE and HPP
  - Moderate to severe hemolytic anemia
  - Jaundice
  - Splenomegaly
  - Gallstones

Prognosis and Therapy
- No therapy required in most patients with HE
- Supportive therapy
- Splenectomy for HPP and severe HE

Pathologic Features

Blood smears in patients with HE typically demonstrate 25% to nearly 100% elliptocytes (Fig. 1.32). These cells are rod-shaped with nearly parallel sides and rounded ends. In the common form of HE, these cells are almost the only poikilocyte present. In more severe forms, there is usually a subpopulation of fragments and bizarre poikilocytes. In addition, some newborn infants present with prominent RBC fragmentation and moderate hemolysis, closely mimicking HPP; however, this evolves over time to mild HE.

HPP is characterized by striking RBC fragmentation and bizarre poikilocytosis, usually, but not always, in a background of elliptocytosis (Fig. 1.33). Because of the extreme RBC fragmentation, the MCV in HPP may be as low as 25 fL.

Ancillary Studies

Additional tests, such as osmotic gradient ektacytometry, membrane protein analysis by SDS-PAGE, and/or DNA sequencing, are available but are not usually needed for patients with characteristic red cell morphology and a family history of HE. However, they can be useful for patients with subtle morphologic findings, those with potential compound heterozygosity, or those with a de novo mutation.

HEREDITARY ELLIPTOCYTOSIS AND HEREDITARY PYROPOIKILOCYTOSIS—PATHOLOGIC FEATURES

Microscopic Findings
- Hereditary elliptocytosis (HE)
  - Typically 25% to 100% elliptocytes
  - Variable polychromasia
  - Red blood cell (RBC) fragmentation in more severe forms
- Hereditary pyropoikilocytosis (HPP)
  - Severe RBC fragmentation
  - Bizarre poikilocytosis
  - Low mean corpuscular volume
  - Variable elliptocytosis

Differential Diagnosis
- HE
  - Disorders with increased elliptocytes, including iron deficiency, megaloblastic anemia, myelofibrosis, myelodysplasia, and thalassemia
- HPP
  - Severe burns
  - Clostridial hemolysis
  - Accidental heating of specimen

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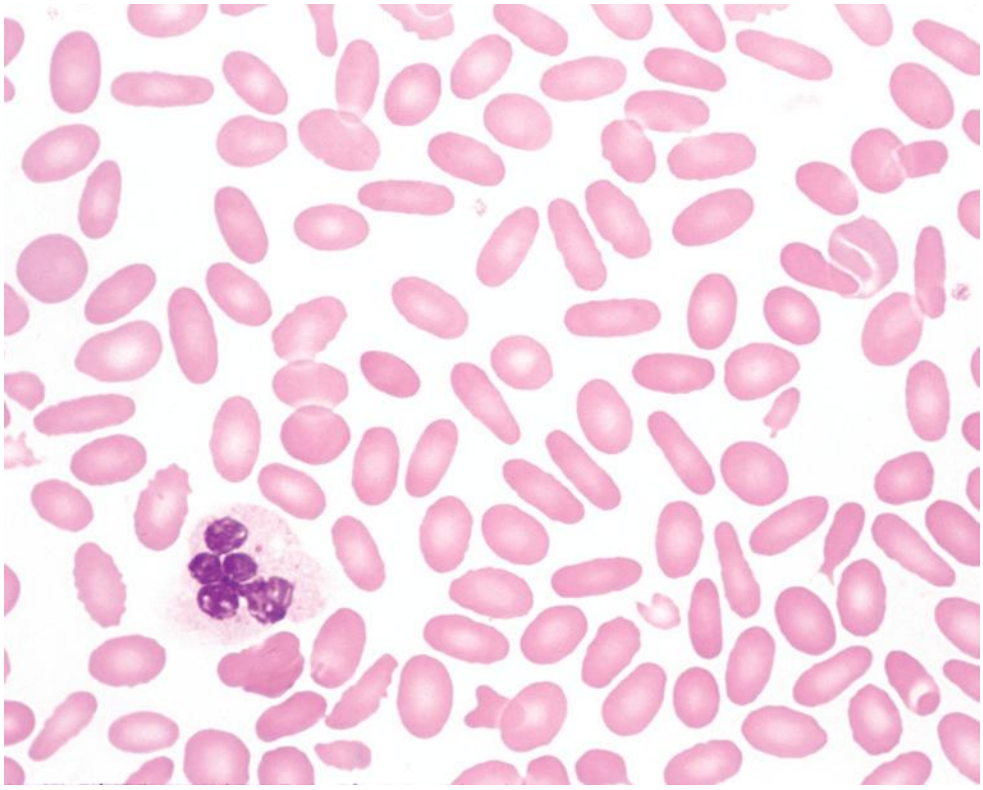


FIG. 1.32

Hereditary elliptocytosis (HE), peripheral blood findings. Patients with HE have 25% to 100% elliptocytes-elongate cells with rounded ends and nearly parallel sides. A few fragments are also present in this case.

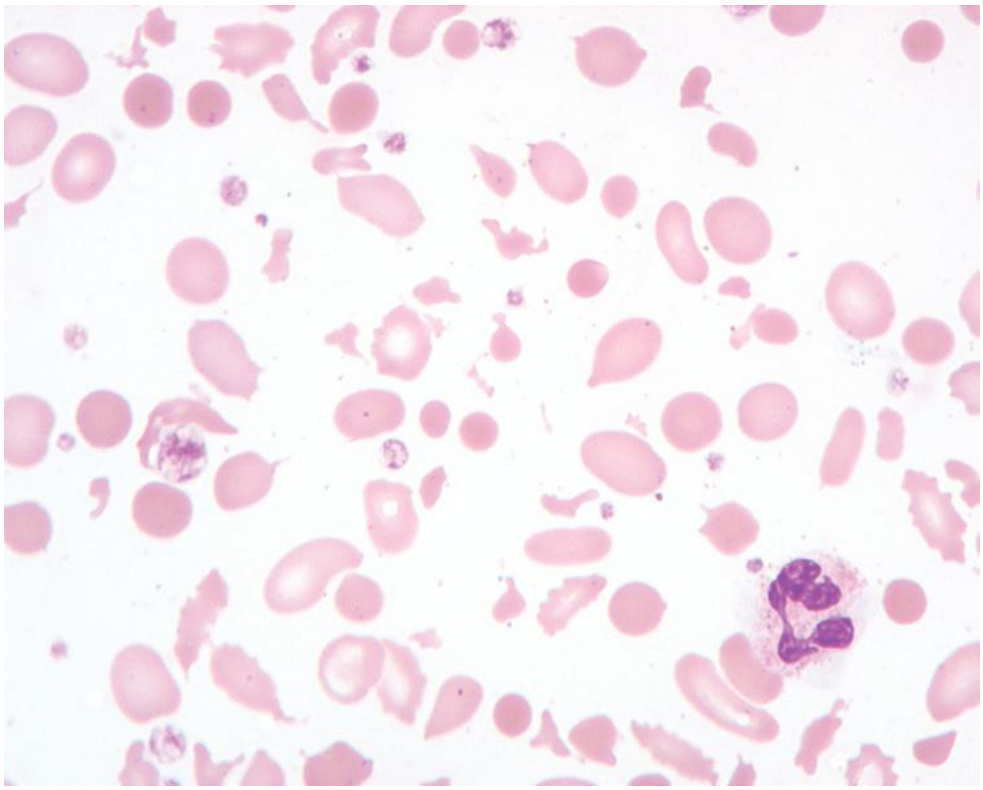


FIG. 1.33

Hereditary pyropoikilocytosis, peripheral blood findings. In this case there are fragmented red cells of varying shape and size, microspherocytes, elliptocytes, teardrop cells, and other bizarre poikilocytes.

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Differential Diagnosis

A variety of disorders may be accompanied by increased elliptocytes, such as iron deficiency, megaloblastic anemia, myelofibrosis, myelodysplasia, and thalassemia. However, the number of elliptocytes is usually less than 25% and should not exceed 35%. Other features of these disorders will usually allow their distinction from HE.

The differential diagnosis of HPP includes severe burns, hemolysis resulting from *Clostridium perfringens* sepsis, and artifactual RBC fragmentation resulting from accidental heating of the blood tube. These disorders are usually easily distinguished from HPP on a clinical basis.

Prognosis and Therapy

Most patients with HE require no specific therapy. Other than supportive care, splenectomy is the main therapy for HPP and more severe forms of HE, and it generally results in an improvement in Hb levels and a decrease in clinical symptoms, although the anemia is not typically completely eliminated.

ENZYME DISORDERS

Although deficiencies of a number of RBC enzymes in the glycolytic and hexose monophosphate pathways may result in hemolytic anemia, the only one that is discussed here is the most common one, glucose-6-phosphate dehydrogenase (G6PD) deficiency. The interested reader may refer to the references listed for other, rarer enzyme disorders.

Glucose-6-Phosphate Dehydrogenase Deficiency

G6PD deficiency is the most common RBC metabolic disorder, with at least 400 million people affected worldwide. Because the gene for G6PD is located on the X chromosome, clinical disease predominates among males. However, heterozygous females may manifest clinical disease, particularly in cases of extreme lyonization. More than 180 mutant G6PD alleles have been characterized. Commonly affected populations include Africans, Greeks, Sardinians, Sephardic and Kurdish Jews, and Southeast Asians. Up to 11% of African-American males in the United States are affected.

G6PD is an enzyme in the hexose monophosphate pathway, a biochemical pathway for metabolizing glucose that bypasses the glycolytic pathway. The hexose monophosphate shunt is necessary for the generation of NADPH, which in turn is required to regenerate reduced glutathione from its oxidized form. Reduced glutathione levels render RBCs more susceptible to oxidant stress. The severity of the deficiency of G6PD correlates well with the severity of the clinical phenotype in this disorder.

Clinical Features

The African A- form (G6PD A-) is most commonly encountered in the United States. In this form of the disease, there is no hemolysis under normal physiologic conditions. However, 2 to 4 days after exposure to an oxidant stress—most commonly in the form of drugs (e.g., primaquine, sulfones), toxins, and infections—there is an acute, primarily intravascular hemolysis manifested clinically as jaundice, pallor, dark urine, and sometimes abdominal or back pain. In patients with G6PD A–, there is a drop in Hb (3 to 4 g/dL) that resolves within 1 week, even without removal of the offending agent. The self-limited nature of the hemolytic episode results because the young RBCs in patients with G6PD A– have sufficient amounts of G6PD to withstand oxidant stress. More severe variants of G6PD deficiency, such as the Mediterranean and Southeast Asian Mahidol variants, can manifest more severe drops in Hb and also may be sensitive to less severe oxidant stresses. For example, patients with G6PD Mediterranean variant may have acute hemolytic episodes in response to fava bean ingestion (i.e., favism). Rare G6PD variants manifest as chronic, ongoing hemolysis, even in the absence of oxidant stress (i.e., chronic nonspherocytic hemolytic anemia).

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY—FACT SHEET

Definition
- An inherited RBC enzyme deficiency resulting in episodic hemolysis in response to oxidant stress

Incidence and Location
- Highly variable geographically

Morbidity and Mortality
- Clinical effects related to acute anemia
- Fatalities rare

Gender, Race, and Age Distribution
- Men most commonly affected; female heterozygotes often clinically unaffected
- Prevalent in African-Americans, Greeks, Sardinians, Sephardic and Kurdish Jews, and Southeast Asians

Clinical Features
- Neonatal jaundice
- Acute hemolytic anemia in response to oxidant drugs, toxins, infections, and fava beans

Diagnosis
- Qualitative fluorescent assay screen and quantitative enzyme assay
- G6PD gene sequencing

Prognosis and Therapy
- Transfusion support if necessary
- Avoidance of oxidant drugs

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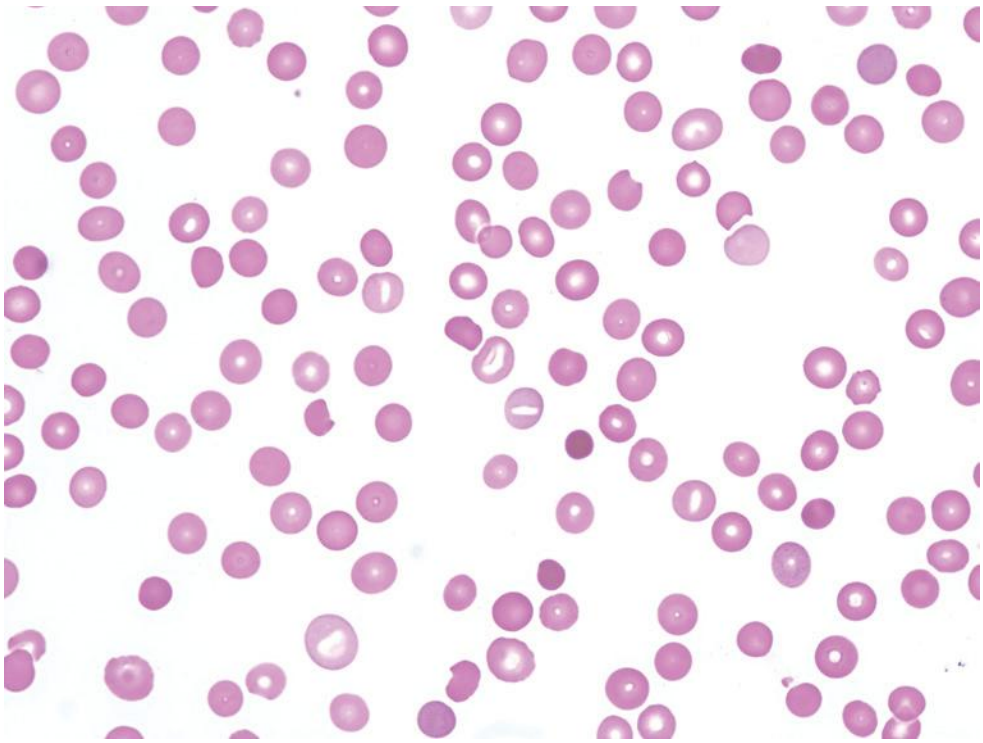


FIG. 1.34

Oxidant hemolysis, peripheral blood findings. A case of oxidant hemolysis due to glucose-6-phosphate dehydrogenase deficiency with multiple bite cells. These cells have variably sized, peripheral "bitelike" defects. Note also the presence of occasional spherocytes.

Importantly, G6PD deficiency may manifest as neonatal jaundice after exposure of an infant to oxidative stress (e.g., moth balls, antimalarials, maternal ingestion of fava beans, infection) and may lead to kernicterus with devastating consequences. Other individuals with G6PD deficiency may remain clinically asymptomatic throughout their life.

Pathologic Features

The characteristic poikilocyte seen in G6PD deficiency, and in other uncommon forms of oxidant-induced hemolysis, is the bite cell. Bite cells are characterized by peripheral defects in the RBC contour that resemble bites. These defects can be smoothly contoured or somewhat irregular, small or large, single or multiple (Figs. 1.34 and 1.35). A characteristic form seen in oxidant hemolysis is the "apple core" cell, in which two large bites are present on opposite sides of the cell (see Fig. 1.35). Bite cells are often slightly dense and appear contracted relative to normal RBCs. A second poikilocyte related to the bite cell seen in some cases is the eccentrocyte (Fig. 1.36). This cell is similar to the blister cells seen in SCD, with Hb pushed to one side and a residual thin blister. However, in contrast to the smooth contour seen in blister cells (see Fig. 1.21), the hemoglobin mass in eccentrocytes typically has an irregular edge adjacent to the vacuole. Finally, small numbers of spherocytes are usually seen as well. Polychromasia can be minimal due to the acute nature of the hemolysis and because it takes the reticulocyte response several days to develop.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY—PATHOLOGIC FEATURES

Microscopic Findings

- Bite cells, eccentrocytes, or both
- Scattered spherocytes
- Little polychromasia during acute episode

Differential Diagnosis

- Other causes of oxidant hemolysis
- Microangiopathic hemolytic anemia

Ancillary Studies

An easily performed fluorescent screening ("spot") test for G6PD deficiency is available in many clinical laboratories. However, this test gives false-negative results during or soon after acute hemolytic episodes because the older, more deficient RBCs are hemolyzed and what remains are younger cells with higher levels of G6PD. Ideally, the test should be performed 2 to 3 months after resolution of the acute hemolytic episode to avoid this problem. Since the level of G6PD activity is highly variable in female heterozygotes, the spot test will also miss some female heterozygotes. A spectrophotometric assay for G6PD available in specialized laboratories enables quantification of enzyme activity. Molecular studies are most reliable in identifying female heterozygotes and individuals after recent hemolysis. However, DNA analysis will not clarify

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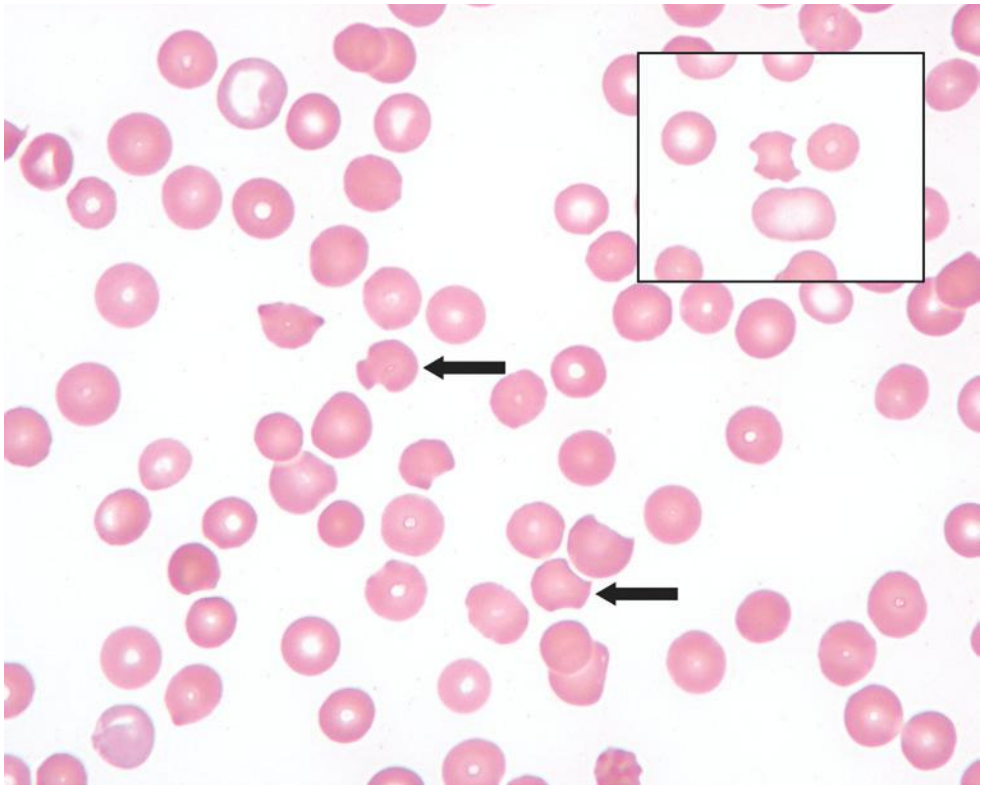


FIG. 1.35

Oxidant hemolysis, peripheral blood findings. This image, from the same patient as in Fig. 1.34, reveals two "double-bite" cells *(arrows)*. An "apple core" cell is illustrated in the inset.

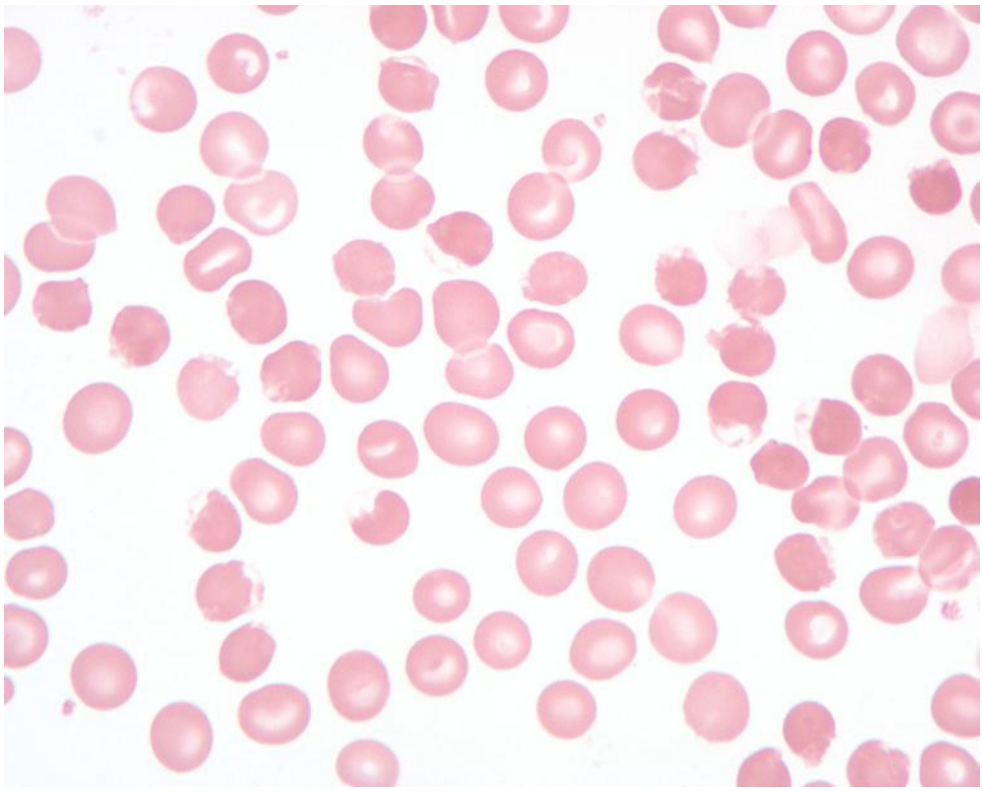


FIG. 1.36

Oxidant hemolysis, peripheral blood findings. Multiple eccentrocytes in a patient with severe oxidant hemolysis owing to glucose-6-phosphate dehydrogenase deficiency. These cells resemble the blister cells of sickle cell disease, with a peripheral defect covered by a thin residual membrane.

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whether or not an individual with a mutation has an activity level of G6PD within a deficient range.

The oxidant damage in G6PD deficiency causes denaturation of Hb within the RBCs to form Heinz bodies. These inclusions are usually not visible on Wright-stained preparations but are visualized as dark blue, peripheral masses on staining with supravital stains such as methyl violet.

Differential Diagnosis

Oxidant hemolysis resulting from other causes can produce the same morphologic features as in G6PD deficiency. These include unstable Hbs, other glutathione-related RBC enzyme deficiencies (glutathione synthetase, glutathione reductase, γ-glutamyl cysteine synthetase), and severe oxidant stress resulting from exposure to oxidant chemicals or drugs. For example, dapsone, an antibiotic commonly used for prophylaxis against *Pneumocystis jirovecii* pneumonia, frequently causes mild oxidant hemolysis, even in the absence of G6PD deficiency. Correlation with clinical history and laboratory results should serve to distinguish these causes.

Microangiopathic hemolytic anemias can also be confused with oxidant hemolysis because of similarities between bite cells (Fig. 1.37) and RBC fragments. In fact, on a cell-by-cell basis, bite cells can be impossible to distinguish from helmet cells. However, the finding of double bite cells and cells with very small defects (i.e., "nibble cells") is much more indicative of oxidant hemolysis. On the other hand, helmet cells in microangiopathic states are usually accompanied by other types of fragments (e.g., triangular fragments). Finally, thrombocytopenia is not a feature of oxidant hemolysis but will be present in common forms of microangiopathic hemolytic anemia.

Prognosis and Therapy

Screening for G6PD deficiency, especially among subgroups with a high prevalence, can help to identify neonates at risk for severe hyperbilirubinemia and individuals of any age who should not receive drugs that cause oxidative stress. The therapy for acute hemolysis resulting from G6PD deficiency is withdrawal of the offending agent or treatment of the precipitating condition. Some patients may require transfusion support.

EXTRINSIC DEFECTS

AUTOIMMUNE HEMOLYTIC ANEMIA

Autoimmune hemolytic anemia (AIHA) can largely be divided into two main forms. Warm antibody type (WAT-AIHA) and cold antibody type (CAT-AIHA) differ in their pathophysiology

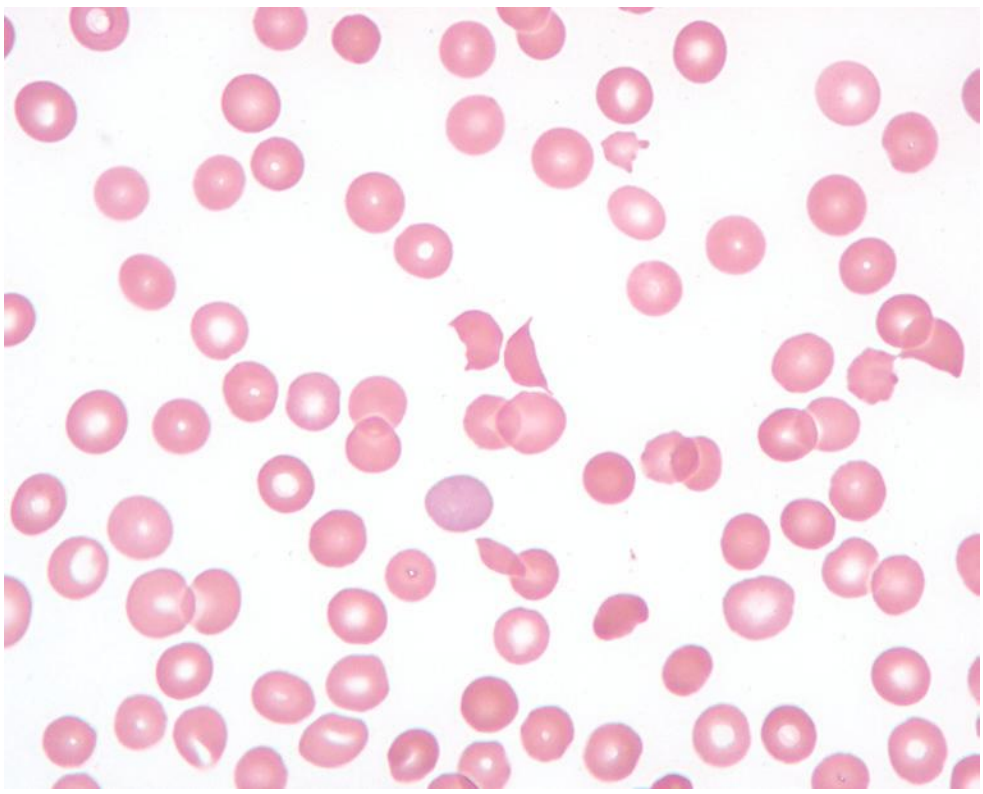


FIG. 1.37

Oxidant hemolysis, peripheral blood findings. Two cells resembling helmet cells *(center)* in a patient with oxidant hemolysis owing to glucose-6-phosphate dehydrogenase deficiency.

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and in their clinical and pathologic features. WAT-AIHA is usually caused by immunoglobulin (Ig) G antibodies that are most active at 37° C and may or may not bind complement. These antibodies sensitize RBCs, which are then removed from circulation by the spleen. CAT-AIHA is usually caused by IgM antibodies that have maximal affinity at lower temperatures. They bind complement, leading to removal of red cells in the liver (extravascular hemolysis) and intravascular RBC destruction. Mixed-type AIHA with features of both WAT- and CAT-AIHA and paroxysmal cold hemoglobinuria, a special type of CAT-AIHA associated with viral illness in children, will not be discussed. Specifics about drug-induced immune hemolytic anemia are also beyond the scope of this chapter.

Clinical Features

WAT-AIHA has an incidence of approximately 10 per 1 million. It occurs at any age and affects women twice as often as men. WAT-AIHA can be primary (idiopathic) or associated with a wide variety of underlying conditions, including autoimmune disorders, immunodeficiency syndromes, lymphoproliferative disorders, and other malignancies. The hemolysis in this disorder ranges from a compensated, subclinical increase in RBC destruction to severe hemolysis. The onset may be insidious or acute. Symptoms are those generically associated with anemia, including weakness, dizziness, and dyspnea. The severity of symptoms correlates with the severity of the anemia. Other symptoms may be seen because of associated underlying disorders. The majority of patients with WAT-AIHA have splenomegaly. Jaundice may be seen in more severe cases.

CAT-AIHA is less than half as common as WAT-AIHA. As with WAT-AIHA, cold AIHA can be primary or secondary. Primary CAT-AIHA, also known as *cold agglutinin disease* (CAD), is a chronic disorder generally seen in older patients, with a female predominance. The pathologic antibody in CAD is a product of a low-grade monoclonal IgM disorder akin to IgM monoclonal gammopathy of undetermined significance or lymphoplasmacytic lymphoma. Secondary CAT-AIHA occurs in association with overt/symptomatic lymphoid or nonlymphoid malignancies and with autoimmune disorders such as systemic lupus erythematosus. However, the most common cause of secondary CAT-AIHA, usually seen in younger patients, is infection. *Mycoplasma pneumoniae* infection is the most frequent but Epstein-Barr virus and other infections can also be the cause. The autoantibodies in infection-related CAT-AIHA are polyclonal.

Hemolysis is usually mild in chronic CAD with exacerbations upon cold exposure, but rare cases are severe with ongoing hemolysis. Acrocyanosis upon exposure to cold may be seen because of agglutination of RBCs in cool extremities. Acute CAT-AIHA caused by infection usually resolves within several weeks.

AUTOIMMUNE HEMOLYTIC ANEMIA—FACT SHEET

Definition
- Hemolytic anemia caused by pathogenic autoantibodies against red blood cell antigens

Incidence and Location
- Warm antibody–type autoimmune hemolytic anemia (WAT-AIHA): about 10 per 1,000,000
- Cold antibody type (CAT-AIHA): approximately 1 per 1,000,000

Morbidity and Mortality
- WAT-AIHA
  - Complications of chronic hemolysis
  - Life-threatening anemia in a small number of cases
- CAT-AIHA
  - Often mild hemolysis with exacerbations
  - Sometimes severe and symptomatic

Gender Distribution
- Women affected more often than men

Clinical Features
- WAT-AIHA
  - Underlying lymphoproliferative disorder in some cases
  - Chronic hemolysis of variable severity
  - Jaundice and splenomegaly in more severe cases
- CAT-AIHA
  - Primary cold agglutinin disease (CAD) is a low-grade monoclonal IgM disorder
  - Overt/symptomatic lymphoma, mycoplasma, or other infection (e.g., Epstein-Barr virus), and autoimmune disease are precipitating causes of secondary CAT-AIHA
  - Mild hemolysis with exacerbations upon exposure to cold are typical
  - Acrocyanosis

Therapy
- WAT-AIHA
  - Steroids
  - Cytotoxic agents
  - Splenectomy in severe cases
  - Rituximab
- CAT-AIHA
  - No treatment may be required
  - Rituximab ± cytotoxic agents or monoclonal anti-C5 antibody for severe CAD

Pathologic Features

The blood smear in WAT-AIHA demonstrates variable spherocytosis and polychromasia (Fig. 1.38). Occasionally, erythrophagocytosis may be seen on a peripheral blood film or bone marrow aspirate smear.

CAT-AIHA is characterized by RBC agglutination in unwarmed (and occasionally warmed) blood smears (Fig. 1.39). Agglutination of RBCs is visible as clumps of several to many RBCs.

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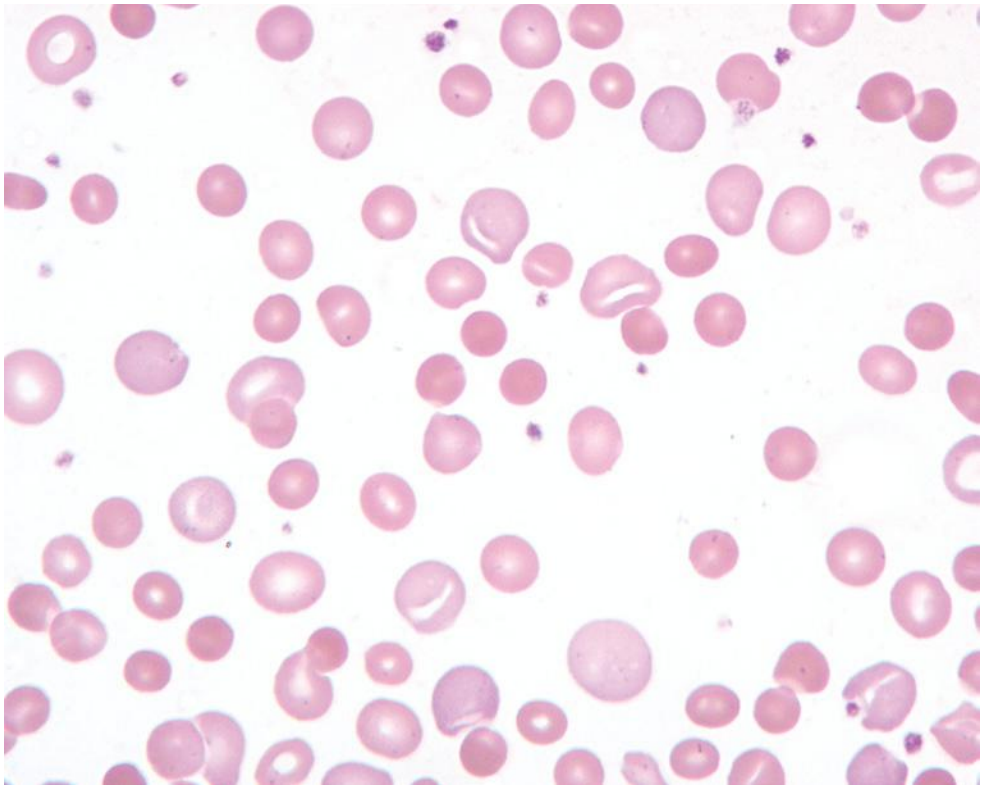


FIG. 1.38

Warm autoimmune hemolytic anemia (AIHA), peripheral blood findings. In this patient with severe warm AIHA, there are prominent spherocytes and polychromatophilic cells. Compare with hereditary spherocytosis, as seen in Fig. 1.31.

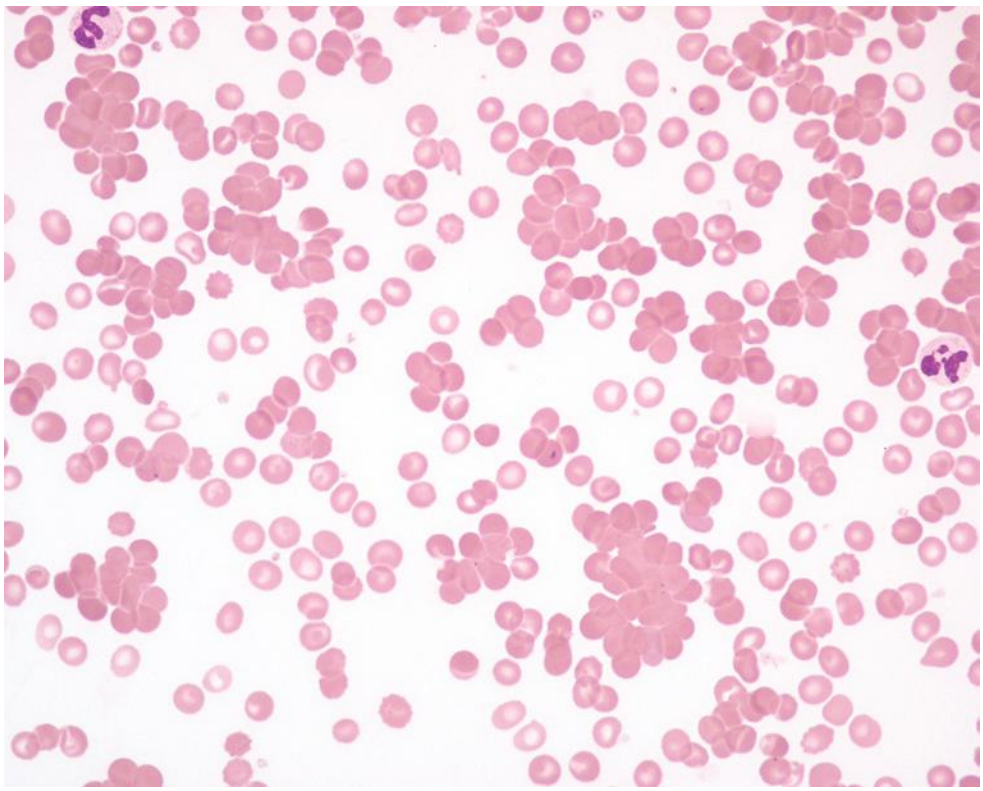


FIG. 1.39

Cold agglutinin disease, peripheral blood findings. In this patient with cold agglutinin disease, there are prominent clumps of red blood cells. These differ from the linear, "stack of coins" formations that characterize rouleaux.

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AUTOIMMUNE HEMOLYTIC ANEMIA—PATHOLOGIC FEATURES

Microscopic Findings

- Warm antibody–type autoimmune hemolytic anemia (WAT-AIHA)
  - Spherocytes
  - Polychromasia
  - Rare erythrophagocytosis
- Cold antibody type (CAT-AIHA)
  - Red blood cell agglutination (clumping)
  - Polychromasia
  - Lack of spherocytosis

Differential Diagnosis

- WAT-AIHA
  - Hereditary spherocytosis
- CAT-AIHA
  - Rouleaux
  - WAT-AIHA

Ancillary Studies

The most important ancillary study in the evaluation of AIHA is the direct antiglobulin test (DAT), which is positive with polyspecific sera (a mixture of anti-IgG and anticomplement) in the vast majority of patients. Monospecific DAT will detect IgG, complement, or both on the surface of RBCs in patients with WAT-AIHA. Complement on the RBC surface is strongly positive in CAT-AIHA, but IgG is negative; IgM is also negative since it dissociates from the RBCs at warm temperatures. Cold agglutinin titers and thermal amplitude are also important for evaluation of CAT-AIHA.

Differential Diagnosis

The differential diagnosis of WAT-AIHA is largely restricted to HS, and these two disorders are not distinguishable morphologically. A direct antiglobulin test will discriminate in the vast majority of cases. Clinical and family histories can also be helpful. Further evaluation to diagnose DAT-negative AIHA may be needed for occasional patients who have IgA antibodies, low-affinity IgG, IgG below the threshold of detection with routine testing, or rare patients with warm IgM without complement fixation.

The agglutination of CAT-AIHA is sometimes confused with rouleaux, a phenomenon associated with increased levels of plasma proteins. However, agglutination differs from rouleaux in that the RBCs are arranged in clumps rather than the single-file, "stack of coins" arrangement that is characteristic of rouleaux. Occasionally WAT-AIHA produces agglutination; the direct antiglobulin test results should distinguish this phenomenon from CAT-AIHA.

It is important to remember that RBC autoantibodies are not uncommon in individuals without AIHA. Hemolysis needs to be documented and, even when present, other possible causes should be considered. Greater strength of the DAT (i.e., higher amounts of RBC bound autoantibody) and high serum titers of autoantibody can support the diagnosis of AIHA but are not the only factors that determine whether or not the antibodies are causing hemolysis.

Prognosis and Therapy

The therapy for patients with AIHA depends on the severity of the hemolysis. In patients with completely compensated hemolysis, no therapy may be required other than folate supplementation to prevent megaloblastic anemia. Addressing the underlying cause is important for secondary AIHA.

For more severe cases of WAT-AIHA, steroids are first-line therapy. Patients with steroid-refractory disease may benefit from splenectomy or rituximab therapy. Severe refractory WAT-AIHA can be life threatening; aggressive therapy with immunosuppressive or other drugs, intravenous immunoglobulin, and/or plasma exchange may be attempted.

Avoiding cold temperatures can be helpful in mild CAT-AIHA. Rituximab is first-line therapy for more severe CAD. The addition of chemotherapy (e.g., fludarabine or bortezomib) or monoclonal anti-C5 (eculizumab) may be helpful in refractory cases. Steroids and splenectomy are usually ineffective and have little role in CAD. If necessary, packed red cell transfusions can be administered with in-line blood warmers.

MICROANGIOPATHIC HEMOLYTIC ANEMIA

Microangiopathic hemolytic anemia (MAHA) is characterized by mechanical disruption and fragmentation of RBCs because of occlusion of the microvasculature. Thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) are the most well-known syndromes that exhibit MAHA. For practical purposes, there are three distinct mechanisms that account for most cases between these two syndromes. Cases satisfying traditional criteria for TTP are known to be associated with either acquired (in most cases) or inherited (rare) deficiencies in ADAMTS13, a metalloprotease responsible for cleaving von Willebrand factor (VWF) and preventing excess platelet aggregation. The acquired form of this deficiency is due to inhibitory autoantibodies directed at ADAMTS13. ADAMTS13 deficiency in conjunction with noxious stimuli results in an increase in endothelial-anchored, unusually large VWF multimers, activation of the alternative complement pathway, platelet microthrombi, and mechanical shearing of red cells during passage. The second major group of disorders, corresponding largely to classic childhood HUS, is associated with gastrointestinal infection with Shiga toxin–producing organisms, most often the O157:H7 or O104:H4 serotypes of *Escherichia coli*. These toxins produce

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direct endothelial damage, especially in the glomeruli, with resultant small vessel thrombosis. A third group, termed *atypical HUS,* is not due to enteric infections. Instead, mutations in genes for one of several proteins that regulate complement activation are found in up to one half of the cases of *atypical HUS.* Additional details on TTP can also be found in Chapter 2.

MAHA may also be seen with disseminated intravascular coagulation (DIC) and in association with other underlying conditions, including systemic lupus erythematosus, catastrophic antiphospholipid antibody syndrome, widely metastatic carcinoma, HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome, hematopoietic stem cell transplantation, and malignant hypertension. Drugs can also cause MAHA by inducing an immune-mediated reaction and/or toxic endothelial injury.

Clinical Features

The clinical features of MAHA depend on the associated disorder. Acquired TTP due to ADAMTS13 deficiency is mainly a disorder of adults with a female predominance. The standardized annual incidence rate of TTP associated with severe ADAMTS13 deficiency is 2.17 per 1 million. It is characterized by acute or insidious onset of hemolytic anemia, thrombocytopenia, and often neurologic symptoms. There may also be variable (usually mild) renal dysfunction and fever. Shiga toxin–associated HUS is much more common in children than in adults. After a prodrome of bloody diarrhea, patients develop signs of intravascular hemolysis, variable thrombocytopenia, and renal failure, sometimes in an acute and dramatic fashion. HUS is characterized by more severe renal impairment, whereas TTP has more prominent neurologic manifestations. Atypical HUS is characterized by acute renal failure and MAHA but lacks a diarrheal prodrome. DIC is the result of systemic activation of the clotting system characterized by both tissue infarction due to occlusive microthrombi and hemorrhage due to depletion of clotting factors.

MICROANGIOPATHIC HEMOLYTIC ANEMIA—FACT SHEET

Definition
- Hemolytic anemia due to fragmentation of red blood cells caused by microvascular occlusion and mechanical disruption

Incidence and Location
- Standardized annual incidence rate of TTP with severe ADAMTS13 deficiency: 2.17 per 1,000,000
- Annual incidence of Shiga toxin–producing *E. coli* HUS among children <5 years old: 6.1 per 100,000

Morbidity and Mortality
- TTP
  - Fatal in 10% to 20% cases
  - Chronic renal insufficiency in a subset of patients
- Shiga toxin–associated HUS
  - Fatal in approximately 1% to 4% of children (higher in adults)
  - Long-term sequelae (e.g., renal, neurologic) in up to one third of patients

Gender, Race, or Age Distribution
- TTP
  - Usually acquired in adults, but children rarely present with inherited ADAMTS13 deficiency
  - Male-to-female ratio is 2:3
- Shiga toxin–associated HUS
  - Usually children
  - No gender predilection

Clinical Features
- TTP
  - Hemolytic anemia
  - Thrombocytopenia
  - Neurologic symptoms
- Shiga toxin–associated HUS
  - Bloody diarrheal syndrome several days before onset of hemolytic anemia
  - Variable thrombocytopenia
  - Acute renal failure

Therapy
- TTP: plasmapheresis
- Shiga toxin–associated HUS: supportive care
- Atypical HUS: eculizumab (monoclonal anti-C5)

Pathologic Features

The blood smear findings in MAHA are similar, no matter what the underlying disorder. Schistocytes are the dominant finding. Schistocytes include RBC fragments and the deformed erythrocytes from which fragments have arisen. They take several forms, including triangulocytes and helmet cells. Triangulocytes are small fragments with a distinctly triangular shape (Fig. 1.40). Other, small RBC fragments lacking central pallor may just be referred to as *schistocytes*. Helmet cells, the largest form of schistocyte, are characterized by a large, smooth, variably concave defect interrupting an otherwise normal RBC contour (Fig. 1.41). The keratocyte is another type of schistocyte that displays two projections, or horns, protruding from one side of the cell, and it retains central pallor. Strictly speaking, the other types of schistocytes described should lack central pallor. Thus small irregular cells with retained central pallor are best considered nonspecific poikilocytes. Microspherocytes are also a common feature of MAHA. As with any hemolytic anemia of at least several days' duration, MAHA will be accompanied by polychromasia.

Ancillary Studies

TTP and HUS lack abnormalities on routine coagulation tests (prothrombin time and partial thromboplastin time), although

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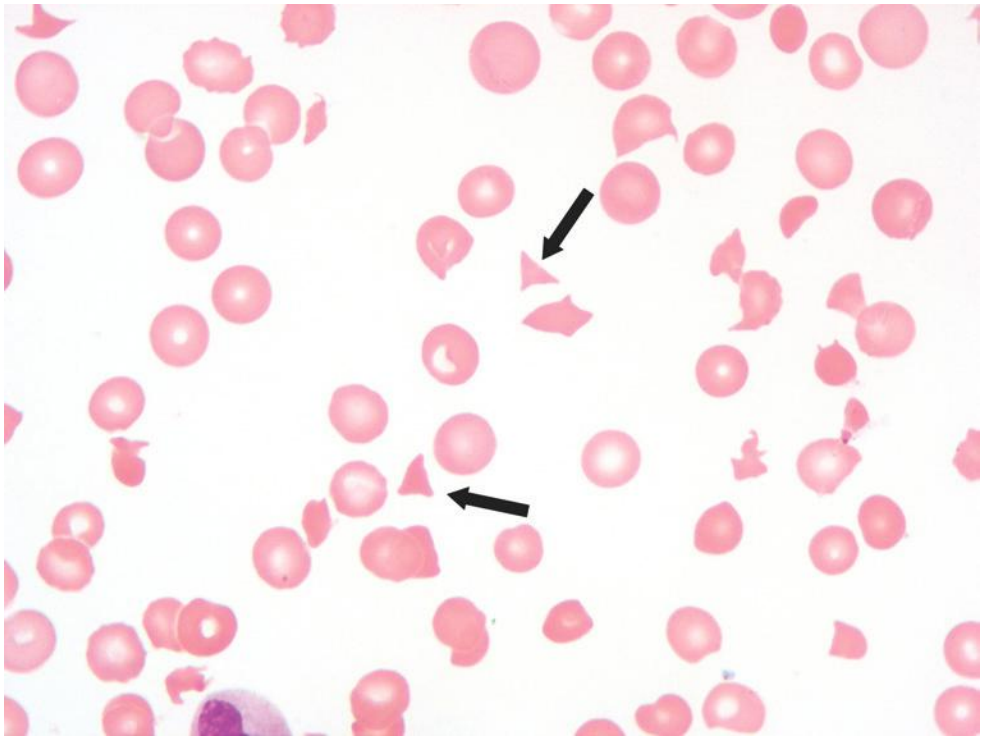


FIG. 1.40

Thrombotic thrombocytopenic purpura (TTP), peripheral blood findings. This image from a patient with TTP contains two triangulocytes *(arrows)* and other various schistocytes.

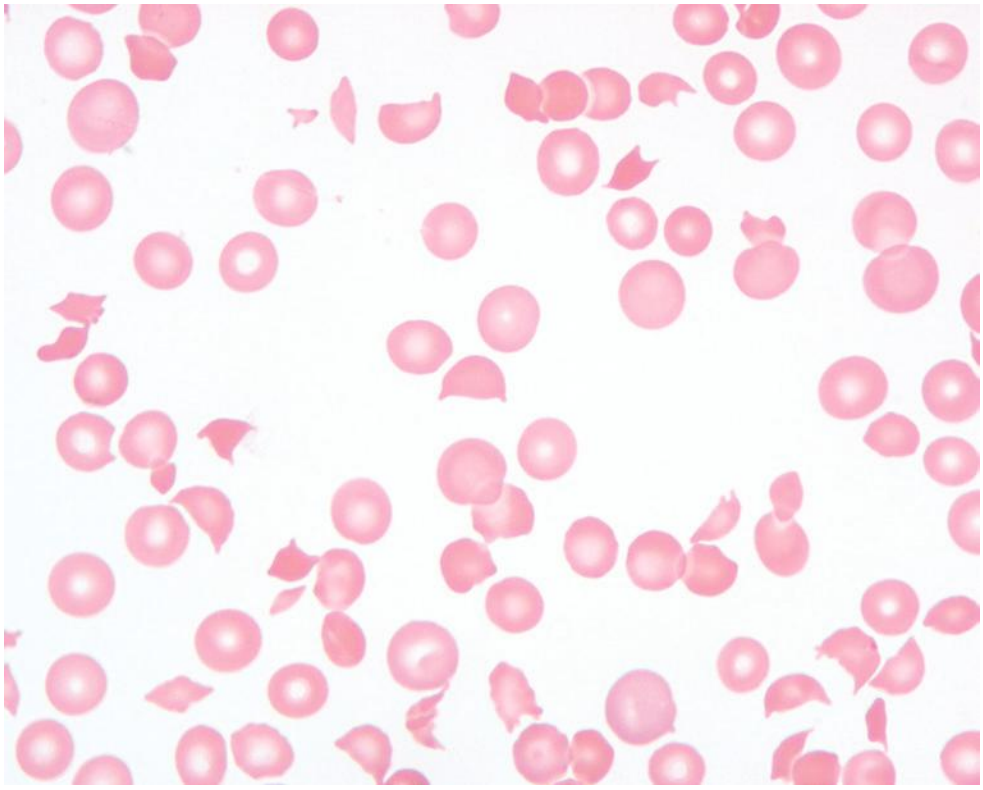


FIG. 1.41

Thrombotic thrombocytopenic purpura (TTP), peripheral blood findings. This smear from a patient with florid TTP demonstrates a classic helmet cell *(center)* and a variety of other schistocytes of varying size and shape.

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D-dimers may be elevated in Shiga toxin–associated HUS. Disturbed coagulation parameters and evidence of thrombolysis (increased D-dimers or fibrin-fibrinogen split products) are hallmarks of DIC. Assays for ADAMTS13 activity will show a dramatic reduction in the activity of this enzyme in TTP (<10%), with normal or mildly decreased levels in MAHA due to other causes. More details about the assays for ADAMTS13, including quantitation, autoantibodies, and testing for mutations of the *ADAMTS13* gene, is provided in Chapter 2. Positive serologic studies for *E. coli* O157:H7 or O104:H4 would support a diagnosis of HUS. Testing for complement-related mutations may help to establish a diagnosis of atypical HUS.

MICROANGIOPATHIC HEMOLYTIC ANEMIA—PATHOLOGIC FEATURES

Peripheral Blood Film Microscopic Findings

- Red blood cell fragmentation (helmet cells, triangulocytes, and other schistocytes)
- Spherocytes
- Polychromasia
- Thrombocytopenia

Differential Diagnosis

- Oxidant hemolysis
- Macroangiopathic hemolytic anemia

Differential Diagnosis

RBC fragmentation can occur as a result of so-called macroangiopathic hemolytic anemia (Fig. 1.42); this results from jet lesions or turbulent flow due to abnormalities of large vessels or cardiac valves. In current practice, this is most often encountered in patients with malfunctioning replacement valves. In addition, the differential diagnosis of MAHA includes oxidant hemolysis. As noted in the discussion of G6PD deficiency, bite cells and helmet cells may be indistinguishable on a cell-by-cell basis (see Fig. 1.37). However, the full spectrum of RBC fragments seen in MAHA, in particular the presence of triangulocytes, should serve to distinguish it from oxidant hemolysis. Furthermore, thrombocytopenia is not a feature of oxidant hemolysis. Occasional schistocytes can also be seen in many other conditions (e.g., burns, myelofibrosis, megaloblastic anemia, iron deficiency, and myelodysplasia), but they are not the predominant poikilocyte and instead are seen with a spectrum of other poikilocytes not expected in MAHA.

Prognosis and Therapy

Without therapy, TTP is fatal. However, since the advent of plasma exchange therapy, most patients with TTP (approximately 80%) achieve complete remission, and long-term survival is 80% to 90%. However, approximately one third

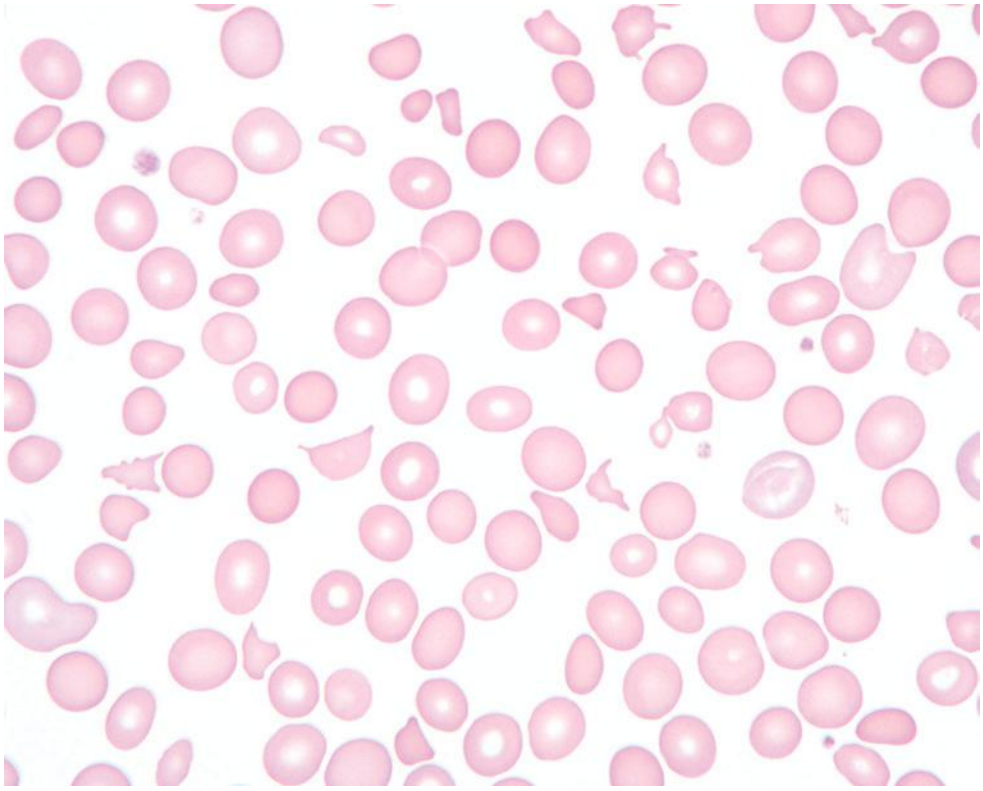


FIG. 1.42

Valve hemolysis, peripheral blood findings. Red cell fragmentation in a patient with a defective cardiac valve. The morphologic features are similar to those seen in microangiopathic hemolytic anemia.

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of the patients will ultimately relapse. Rituximab is useful for patients with refractory or recurrent TTP. Shiga toxin–associated HUS is typically a self-limited disorder requiring only supportive care, with a current mortality rate of 1% to 4%, but a subset of patients develop chronic renal insufficiency. Eculizumab (monoclonal anti-C5) is approved for atypical HUS, and long-term therapy may help prevent relapse and end-stage renal failure. The management of DIC and other conditions that cause secondary MAHA is directed toward treatment of the underlying disorder. The prognosis depends largely on the nature of the underlying condition and whether it can be treated rapidly and effectively. Replacement of clotting factors and platelets with component therapy may be needed for DIC.

PARASITIC HEMOLYSIS

A variety of microorganisms may produce hemolytic anemia through a variety of mechanisms; however, this section focuses on hemolysis caused by intracellular parasites, which are disorders that can be diagnosed based on morphologic findings on blood smear. Belonging to this group are *Plasmodium* species, which produce malaria, and *Babesia* species. The maturation and proliferation of these organisms play a role in RBC destruction.

Clinical Features

Malaria is an important worldwide public health problem, with 200 to 300 million people afflicted annually. An estimated 438,000 people died of malaria in 2015, a decline of 48% since 2000. In the United States, there are approximately 1700 cases annually. Malaria caused by *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae,* or *Plasmodium ovale* is characterized by periodic episodes of fever, sometimes preceded by a flulike prodrome. Splenomegaly is common. Anemia is also common and correlates with species, organism load, and patient-specific characteristics. Approximately 20% of patients with the most severe type of malaria, due to *P. falciparum,* demonstrate moderately severe anemia associated with the acute infection. Rarely, patients with fulminant *P. falciparum* infection develop acute intravascular hemolysis, a syndrome known as blackwater fever. Patients with malaria typically have a history of travel to Africa, Asia, or Central or South America.

The U.S. form of babesiosis, most commonly caused by *Babesia microti,* is a mild, self-limited disorder usually seen in the northeast part of the country. There were 1762 cases reported to the Centers for Disease Control in the United States for 2013, mostly from Connecticut, Massachusetts, Minnesota, New Jersey, New York, Rhode Island, and Wisconsin. Babesiosis is associated with a malaria-like syndrome of fever, chills, sweating, and headache, but the fever in babesiosis lacks the regular periodicity of that seen in malaria.

PARASITIC HEMOLYSIS—FACT SHEET

Definition
- Hemolytic anemia resulting, in part, from proliferation of parasitic organisms within red blood cells

Incidence and Location
- Malaria: approximately 1700 cases per year in the United States
- Babesiosis: 1762 cases reported in the United States in 2013

Morbidity and Mortality
- Malaria
  - *Plasmodium falciparum* may pursue fulminant course if untreated or if drug resistant
  - Highest morbidity and mortality among children in hyperendemic regions
- Babesiosis
  - Usually self-limited (asymptomatic, mild or moderate)
  - Up to 10% mortality in patients hospitalized for this infection in the United States

Clinical Features
- Malaria
  - Cyclic fevers, sometimes preceded by flulike syndrome
  - Moderately severe hemolytic anemia in 20% of *P. falciparum* infection
  - Rare fulminant intravascular hemolysis (blackwater fever)
- Babesiosis
  - Similar to malaria, but fevers not cyclic

Prognosis and Therapy
- Malaria
  - Various antimalarial agents
  - Excellent prognosis if treated promptly
- Babesiosis
  - No therapy necessary in most cases
  - Antibiotics in severe cases

Anemia, if present, is usually mild; however, occasional cases of babesiosis produce severe hemolytic anemia, usually in patients with prior splenectomy. A more severe European form of babesiosis is due to *Babesia divergens.*

Pathologic Features

Malarial parasites take a variety of morphologic forms that can be visualized on routine blood smears. Early ring-trophozoites are delicate, pale blue rings, 2 mm or less in diameter, with one red chromatin dot (Fig. 1.43). Occasionally, ring forms of malaria can contain a double chromatin dot, or two-ring trophozoites may be seen in the same RBC. Both of these findings are most common with *P. falciparum.* More mature trophozoites are larger with more abundant cytoplasm and a single chromatin mass (Fig. 1.44). The shape of the parasite at this stage varies depending on the species and the stage of maturation. Mature trophozoites are commonly

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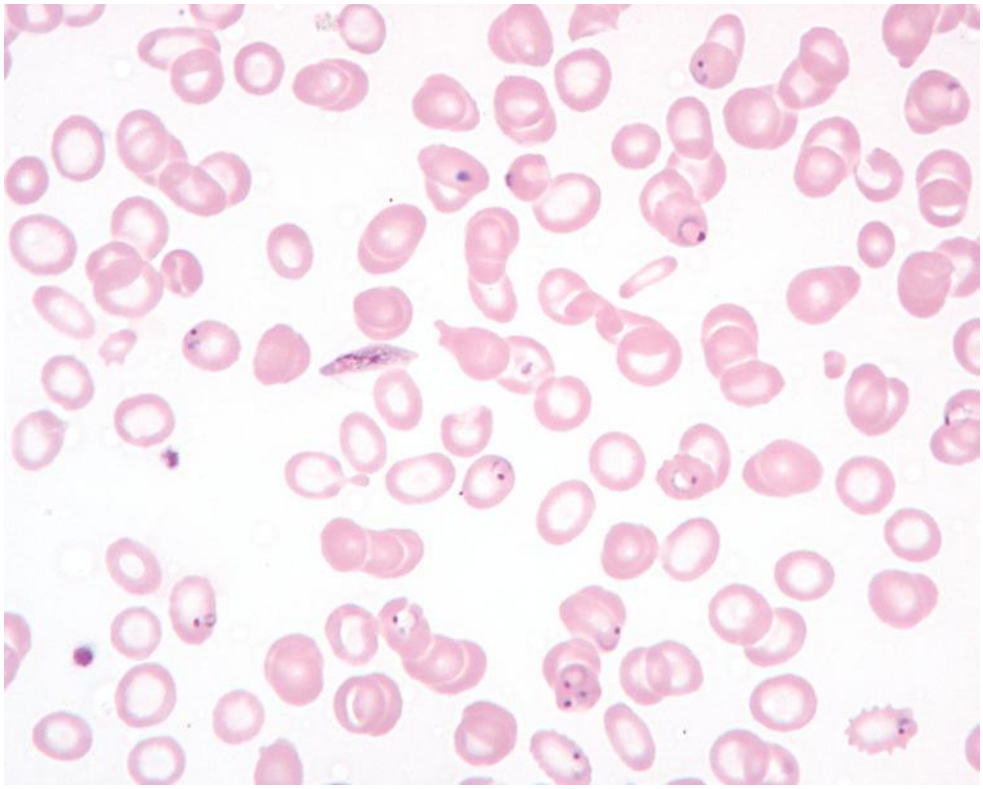


FIG. 1.43

Malaria, peripheral blood findings. This example of *Plasmodium falciparum* malaria with a high level of parasitemia demonstrates multiple red blood cells containing early trophozoites (rings). These have a pale blue, annular cytoplasmic body and a red chromatin dot. Also present is an elongate (banana-shaped) gametocyte, diagnostic of *P. falciparum.*

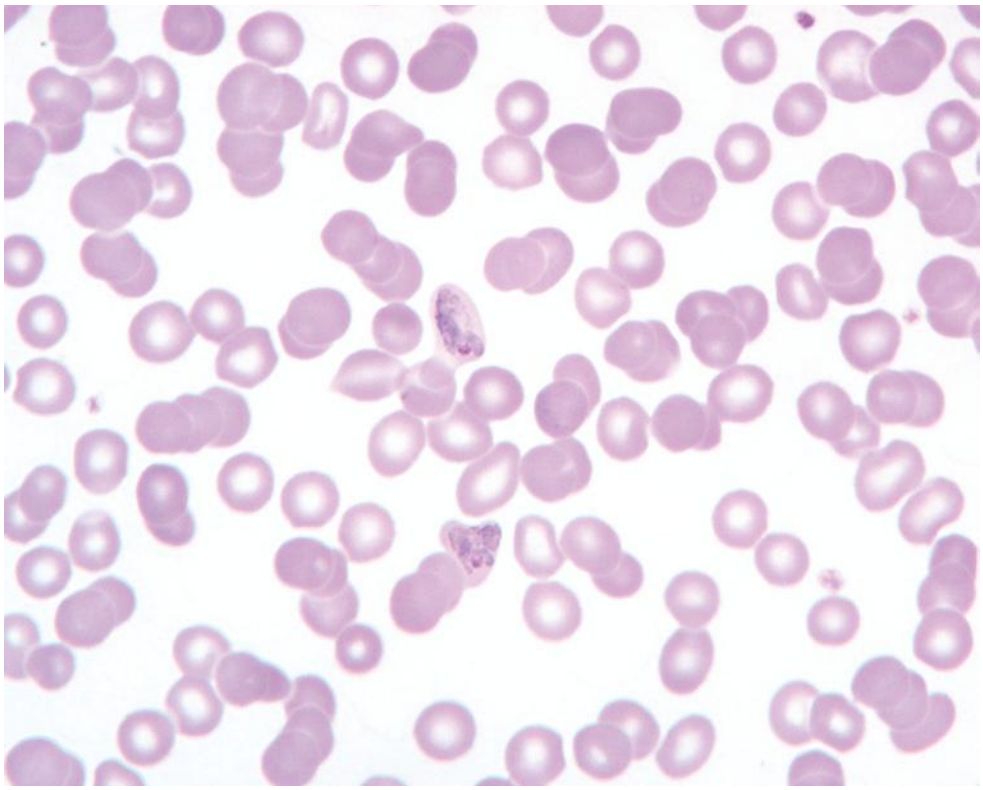


FIG. 1.44

Malaria, peripheral blood findings. Two mature, "ameboid" trophozoites of *Plasmodium vivax.* These have a large, irregular cytoplasmic mass and a distinct red chromatin mass.

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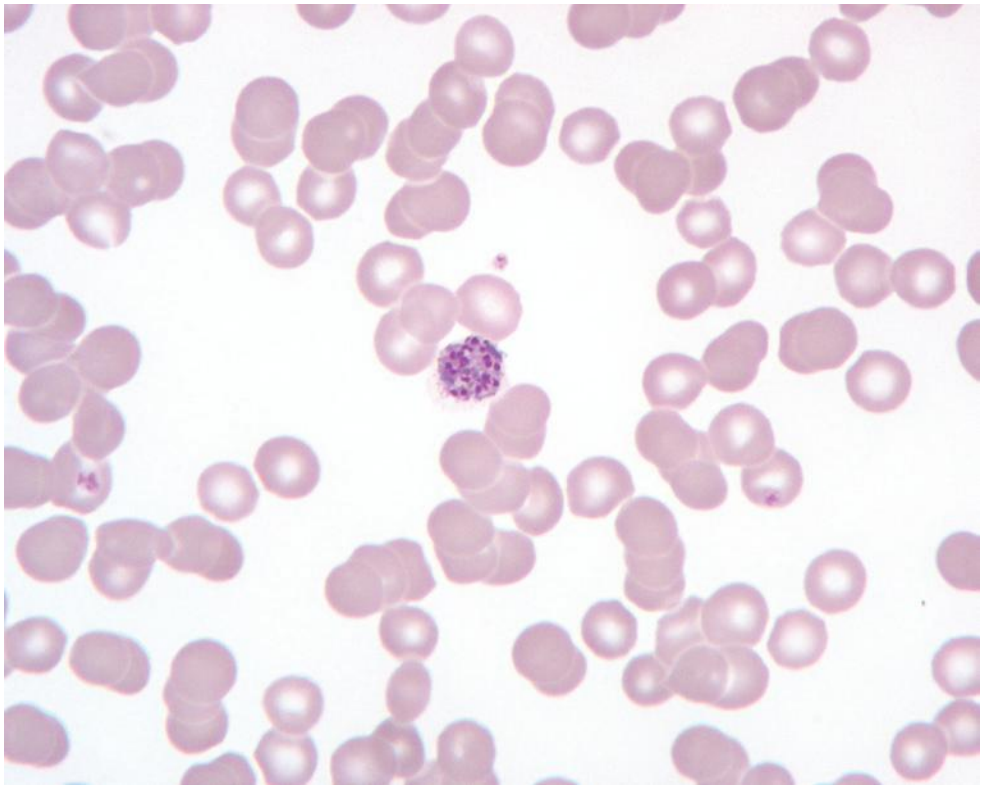


FIG. 1.45
Malaria, peripheral blood findings. This early schizont (dividing form of the organism) of *Plasmodium vivax* has a single cytoplasmic body with multiple chromatin masses.

PARASITIC HEMOLYSIS—PATHOLOGIC FEATURES

Microscopic Findings

- Malaria
  - Range of maturational forms seen in erythrocytes: immature trophozoites (rings), mature trophozoites, schizonts, gametocytes
  - Only ring forms and banana-shaped gametocytes in *Plasmodium falciparum*
- Babesiosis
  - Mainly delicate rings, resembling those in *P. falciparum* (1 to 12 per cell)
  - No maturational forms are seen as in malaria

associated with hemozoin pigment because of the breakdown of Hb. Schizonts, the dividing form of the organism, are characterized by two or more chromatin masses and undivided (early; Fig. 1.45) or divided cytoplasmic masses (Fig. 1.46). Finally, the gametocyte forms of the organism are round or elongate (i.e., banana shaped in the case of *P. falciparum*) structures with a compact or dispersed chromatin mass (Figs. 1.43 and 1.47). Infections with *P. vivax, P. ovale,* and *P. malariae* are associated with parasites at various stages of maturation, whereas *P. falciparum* manifests only ring trophozoites and gametocytes in the blood. A more detailed description of the maturational forms in various types of malaria can be found in many textbooks.

The predominant form of *Babesia* spp. seen in the blood is a small ring form (Fig. 1.48), closely resembling *P. falciparum*. However, *Babesia* spp. trophozoites may show wide morphologic variability, ranging from tiny forms composed of a minute cytoplasmic body with a barely visible nuclear dot to larger ameboid, pear-shaped, or elongate forms. As many as 12 *Babesia* spp. trophozoites may be seen in a single RBC (Fig. 1.49). Tetrads of *Babesia* spp. rings are rare but diagnostic.

Ancillary Studies

In addition to light microscopy of thin peripheral blood films, examination of thick films are used to help identify malaria in cases with low levels of parasitization. However, *Babesia* may be missed on thick smears because the parasites can be too small. Rapid test strips that use a drop of blood from a finger stick are available for malaria but do not permit quantification of parasitemia and should be followed by microscopic examination. Molecular tests for malaria and babesiosis can be performed at reference laboratories for definitive speciation in difficult cases. Serologic tests for malaria and *Babesia* are also available and may be helpful in certain circumstances, including as an aid to distinguish between *Plasmodium falciparum* and *Babesia* infection when microscopy and travel history are inconclusive. However, serologic testing for malaria is not used to distinguish between past and present infection.

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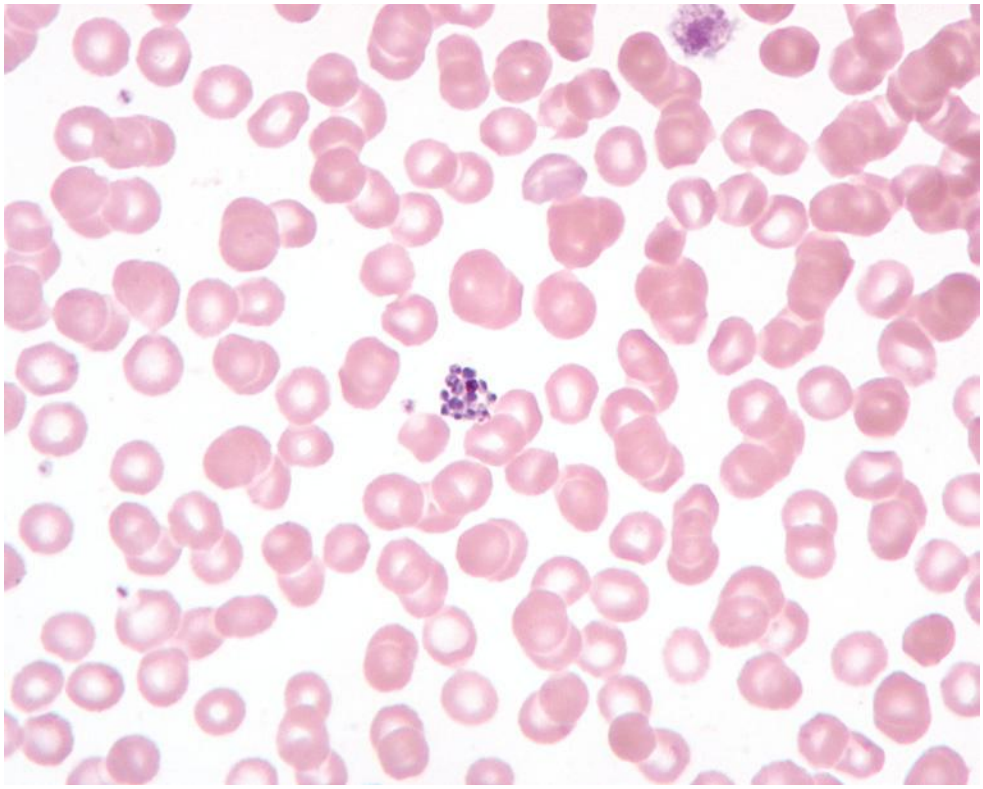


FIG. 1.46

Malaria, peripheral blood findings. This late (divided) schizont of *Plasmodium vivax* is a cluster of small round cytoplasmic masses, each containing a chromatin dot.

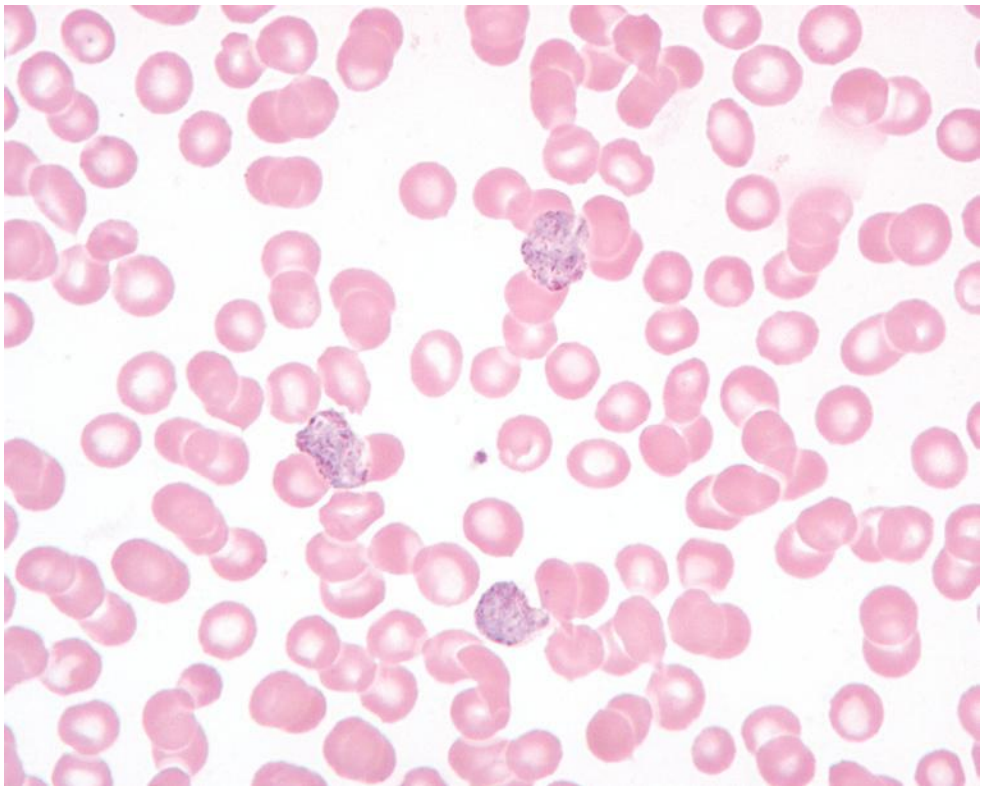


FIG. 1.47

Malaria, peripheral blood findings. This image contains three macrogametocytes of *Plasmodium vivax*. The chromatin masses are visible as compact, wedge-shaped pink masses compressed to one edge of the organism.

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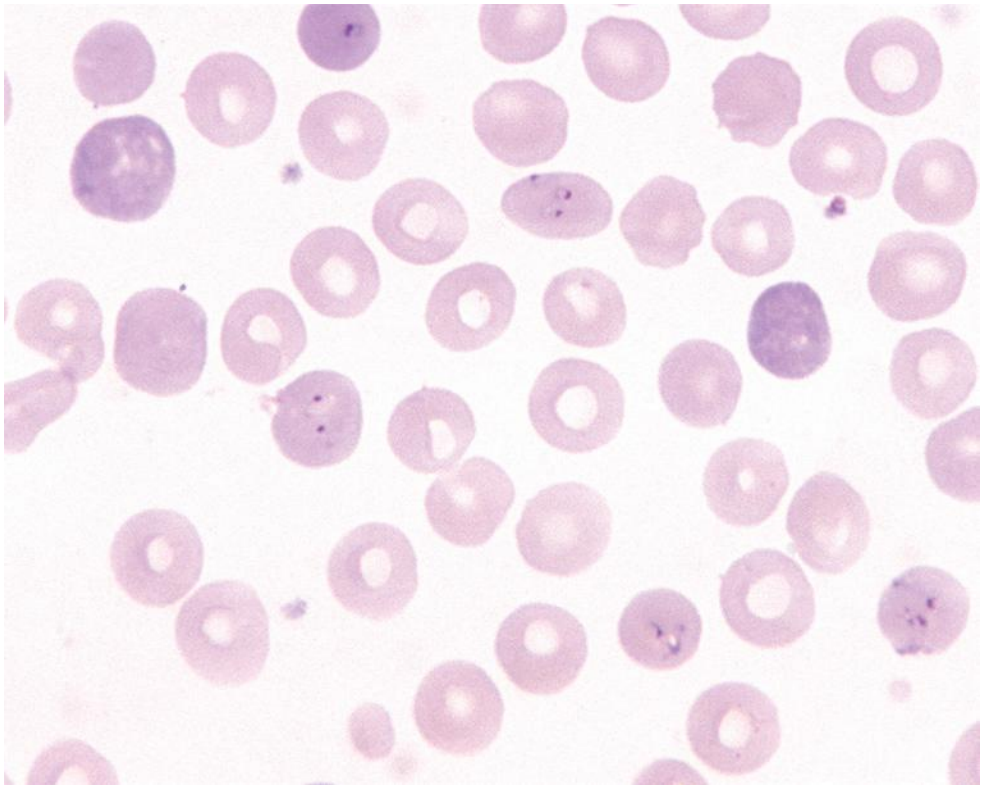


FIG. 1.48

Babesiosis, peripheral blood findings. Five erythrocytes in this image contain one to several delicate ring forms with a barely visible, pale blue cytoplasmic body and a red chromatin dot. These are similar to the ring forms of *Plasmodium falciparum*.

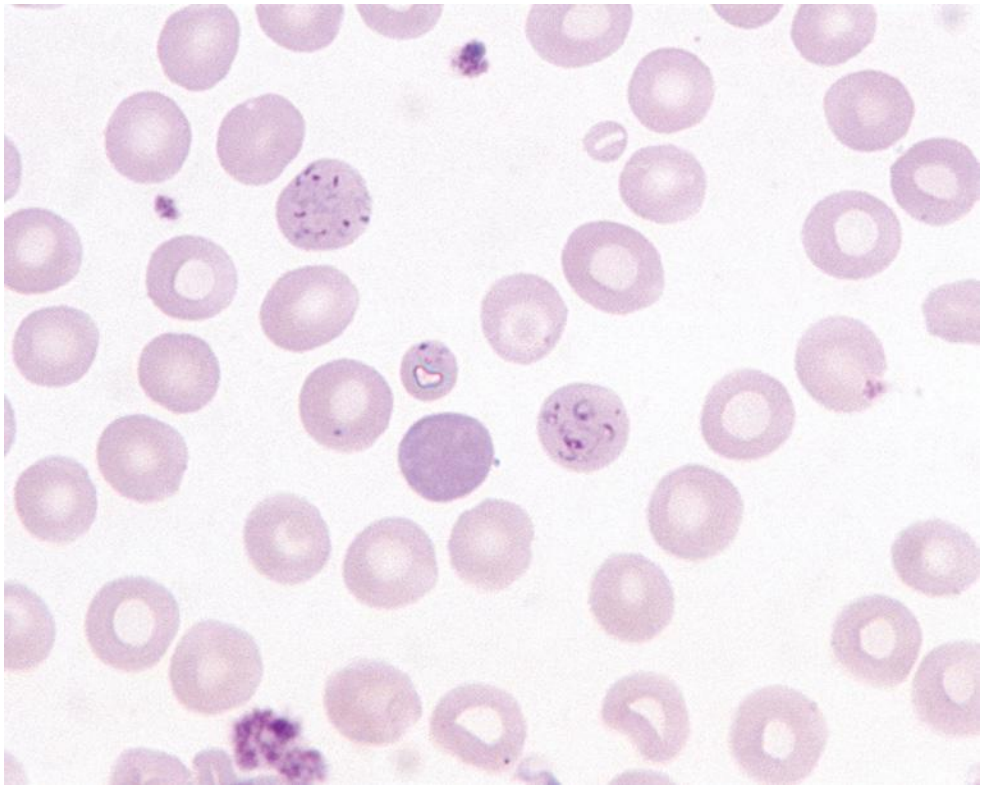


FIG. 1.49

Babesiosis, peripheral blood findings. Two infected erythrocytes in this field contain numerous ring forms. As many as 12 rings per erythrocyte can be seen in babesial infections.

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Differential Diagnosis

The differential diagnosis of malaria and babesiosis is largely with each other. Travel history and clinical manifestations (e.g., periodicity of fever) may be helpful. Morphologically, the presence of schizonts or gametocytes rules out babesiosis. Other distinguishing features in *Babesia* spp. infections include the generally smaller size of rings, absence of hemozoin, pleomorphism of the ring forms, many rings per RBC, and the occasional presence of extraerythrocytic ring forms.

Prognosis and Therapy

Treatment of malaria involves a variety of agents, including chloroquine, other quinoline derivatives, artemisinins, sulfonamides, pyrimethamine, and sulfones. For *P. falciparum* and *P. vivax*, the approach will depend on whether the infection is chloroquine sensitive or if it has been acquired in an area with chloroquine resistance. The prognosis is excellent if therapy is initiated promptly. *P. falciparum* may pursue a fulminant course if treatment is delayed or if the organisms are drug resistant. In areas of the world where malaria is hyperendemic, there is considerable morbidity and mortality, particularly in children.

Babesiosis generally is self-limited, but treatment with atovaquone plus azithromycin or clindamycin and quinine is used in more severe cases. Babesiosis carries a mortality rate of up to 10% among patients who are hospitalized for this infection in the United States. Elderly, immunosuppressed, or asplenic patients are at greater risk. European babesiosis has a mortality rate of approximately 40%.

ERYTHROCYTOSIS

Erythrocytosis, which is an increase in the concentration of RBCs and Hb in the blood, can result from either an absolute increase in red cell mass (a.k.a. absolute erythrocytosis or "polycythemia") or from a decrease in plasma volume (relative erythrocytosis). Erythrocytosis in which the concentration of RBCs is increased with little or no anemia can also occur in microcytic RBC states, most notably thalassemia minor or trait. However, this type of erythrocytosis is a consequence of the microcytosis and is not associated with an increased Hb concentration and is not discussed further in this text.

Relative erythrocytosis generally results from hemoconcentration due to decreased fluid intake and/or marked loss of body fluids. A contraction of plasma volume produces the appearance of erythrocytosis without any actual increase in the body red cell mass. A chronic form of relative polycythemia (Gaisböck's disease) is an obscure syndrome of uncertain cause.

Absolute erythrocytosis or polycythemia can be either primary or secondary. Primary erythrocytosis can be either congenital or acquired. The congenital form, primary familial polycythemia, is a consequence of mutations in the erythropoietin receptor (*EPOR*) that confer hypersensitivity to erythropoietin. Acquired absolute erythrocytosis, termed *polycythemia vera,* is a clonal stem cell disorder that belongs to the group of chronic myeloproliferative neoplasms. This disease is associated with an increase in all myeloid cell lines, but its primary clinical manifestations relate to erythrocytosis. This disorder is discussed in detail in Chapter 17. Secondary erythrocytosis is a consequence of increased erythropoietin resulting from one of several causes. Chronic hypoxia, caused by cardiac or pulmonary disease, sleep apnea, or residence at high altitude, results in a compensatory erythrocytosis. Certain abnormal Hbs, such as inherited high oxygen affinity variants or increased carboxyhemoglobin in smokers, fail to deliver oxygen to the tissues effectively and also result in a compensatory increase in erythropoietin production by the kidneys. Some patients may have aberrant erythropoietin production by neoplasms. Finally, there are rare congenital forms of secondary polycythemia other than those due to abnormal Hbs. For example, Chuvash polycythemia is due to a germline von Hippel–Lindau gene (*VHL*) mutation (R200W). The gene product is involved in regulation of cellular levels of hypoxia inducible factor alpha (HIF-α) subunits involved in the oxygene sensing pathway. The mutation results in elevated HIF1-α leading to elevated levels of erythropoietin and an augmented response to erythropoietin. Thus investigation for erythrocytosis includes a complete review of a patient's clinical history and CBC values. A normal or elevated erythropoietin (EPO) level is suggestive of a secondary cause of erythrocytosis. Analysis for *JAK2* mutation is important for the evaluation of polycythemia vera since only very rare cases (<1%) lack a *JAK2* mutation. For patients with clinical suspicion of congenital erythrocytosis, mutational analyses are becoming available as confirmatory tests.

The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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2

Hemostasis and Thrombosis

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■ BASIC PHYSIOLOGY OF HEMOSTASIS AND FIBRINOLYSIS

Physiologic hemostasis is initiated with injury to a blood vessel wall. This triggers a sequence of reactions involving plasma proteins, platelets, extracellular membrane components, and the vascular smooth muscle to ultimately form a stable fibrin plug blocking the defect. Simultaneously, inhibitors of hemostasis and the fibrinolytic system are initiated to limit the size of the clot and ultimately clear it after the blood vessel structure has been repaired.

The initial response to vascular injury includes vasoconstriction and the recruitment of platelets to adhere to the injury site. Platelet activation leads to platelet aggregation, in a process to be described in more detail later in this section. Together with platelet activation, the coagulation system, a group of plasma proteins, is sequentially activated to form a cross-linked fibrin clot that stabilizes the platelet aggregates (Table 2.1). These proteins are usually present in plasma as zymogens or are otherwise sequestered so as not to spontaneously initiate clotting. Most are serine proteases, although some serve as cofactors to other enzymes. By convention, activated factors are indicated by a lowercase "a" after the Roman numeral. The coagulation proteins are produced predominantly by hepatocytes, with the exception of factor VIII, which is produced in the reticuloendothelial system, and tissue factor, which is expressed in most tissues but not usually in contact with the blood flow. The production of factors II, VII, IX, and X; and protein S and protein C requires vitamin K–mediated γ-carboxylation. Deficiencies of vitamin K, either nutritional or pharmacologic, lead to decreased levels of these factors and a bleeding tendency; this is the mechanism behind the anticoagulant properties of warfarin, a vitamin K antagonist. Calcium is required for most enzymatic reactions.

COAGULATION CASCADE

Traditionally the multiple reactions leading to the formation of a durable fibrin clot were conceptualized as a sequential activation of coagulation proteins. This coagulation "cascade" consists of extrinsic and intrinsic pathways, which converge on a final common pathway (Fig. 2.1). We now know that several features of cascade are inaccurate in describing in vivo hemostasis; however, the traditional coagulation cascade is still conceptually helpful, especially when interpreting the two most common coagulation tests, the prothrombin time (PT) and activated partial thromboplastin time (APTT).

The intrinsic pathway begins with the contact factor pathway involving activation of factor XII by high-molecular-weight kininogen and prekallikrein. It is now known that this pathway is not a necessary part of physiologic hemostasis, as factor XII deficiencies do not lead to an increased risk of bleeding. However, auto-activation of factor XII when in contact with a negatively charged surface (e.g., kaolin) is the basis of the APTT. Factor XIIa then activates factor XI, which activates factor IX. In the presence of its cofactor, factor VIIIa, factor IXa catalyzes activation of factor X. The factor VIIIa/factor IXa complex is sometimes referred to as the intrinsic "tenase."

Factors I, II, V, and X operate within the common pathway. Factor Xa complexes with its cofactor factor Va to form a "prothrombinase," which mediates conversion of prothrombin (factor II) to thrombin (factor IIa). Thrombin then cleaves fibrin (factor I) into soluble monomers that are able to polymerize into a fibrin clot. Thrombin also catalyzes activation of several upstream factors, creating positive feedback. Factor IIa also activates factor XIII, which allows the enzyme to form covalent crosslinks within the fibrin clot to stabilize it.

Factor VII and tissue factor (TF) are unique to the extrinsic pathway, which is the reaction monitored by the PT. TF combines with activated factor VII to function as the extrinsic "tenase." As TF/factor VIIa will be described below, this function does not actually contribute to fibrin-clot formation in vivo owing to inhibition by tissue factor pathway inhibitor (TFPI); rather it is the ability of TF/factor VIIa to activate factor IX, which leads to thrombogenic production of fibrin.

Fibrin is a dimeric molecule wherein each subunit is composed of three polypeptide chains. These form a central E domain with two identical D domains at each end. Thrombin (factor IIa) cleaves fibrinopeptide A from the Aα chain and

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TABLE 2.1
Summary of Coagulation Factors

Factor	Name	Vitamin K–Dependent	Consequence of Deficiency	Function
I	Fibrinogen	No	Bleeding or clotting	Substrate
II	Prothrombin	Yes	Bleeding	Serine protease
III	Thromboplastin (tissue factor + phospholipid)	No	Bleeding	Receptor/cofactor
V	Labile factor	No	Mild bleeding	Cofactor
VII	Proconvertin	Yes	± Bleeding	Serine protease
VIII	Anti-hemophiliac factor	No	Bleeding	Cofactor
IX	Christmas factor	Yes	Bleeding	Serine protease
X	Stuart-Prower factor	Yes	Bleeding	Serine protease
XI	Plasma thromboplastin antecedent	No	Bleeding	Serine protease
XII	Hageman factor	No	Clotting	Serine protease
XIII	Fibrin stabilizing factor	No	Bleeding (delayed)	Transglutaminase

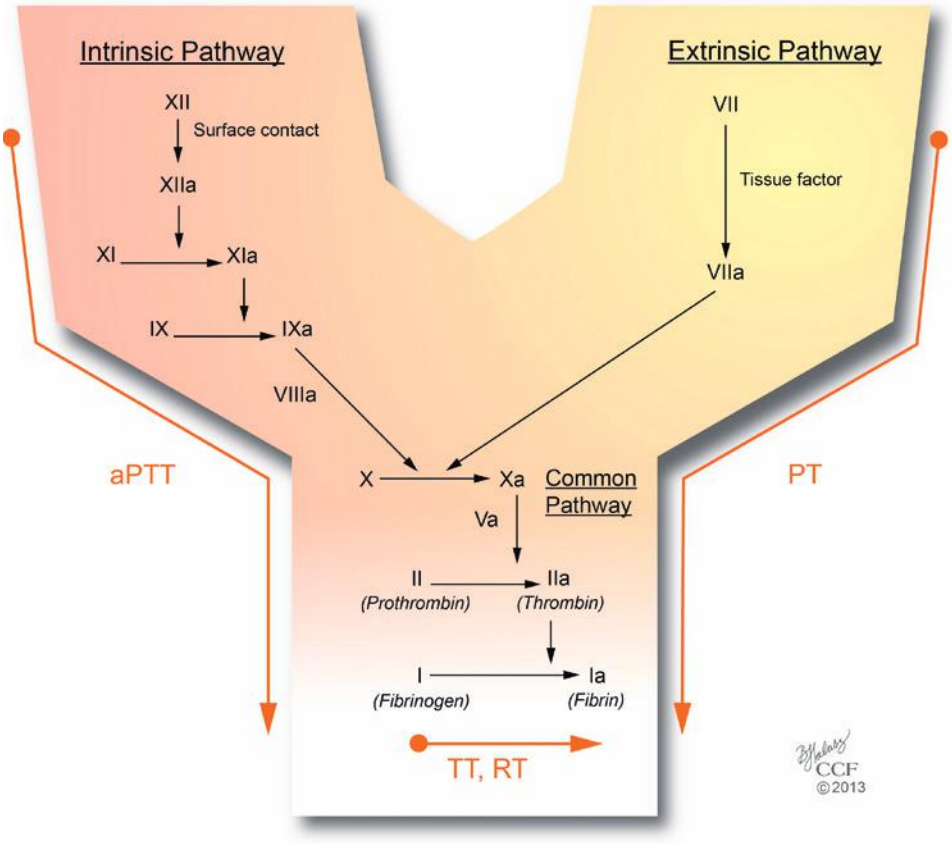


FIG. 2.1
Cascade model of coagulation and associated laboratory tests.

fibrinopeptide B from the Bβ chain, creating a soluble fibrin monomer. These then associate both in an end-to-end (D to D) as well as side-to-side (D to E) fashion to form polymers. Factor XIIIa creates stable cross-links between D domains to form a stable fibrin clot.

CELL-BASED MODEL OF COAGULATION

In vivo clot formation is much more complicated; it is dependent on both plasma proteins and associated cells, including endothelial cells and platelets. These cells contribute both required factors as well as provide scaffolding for the

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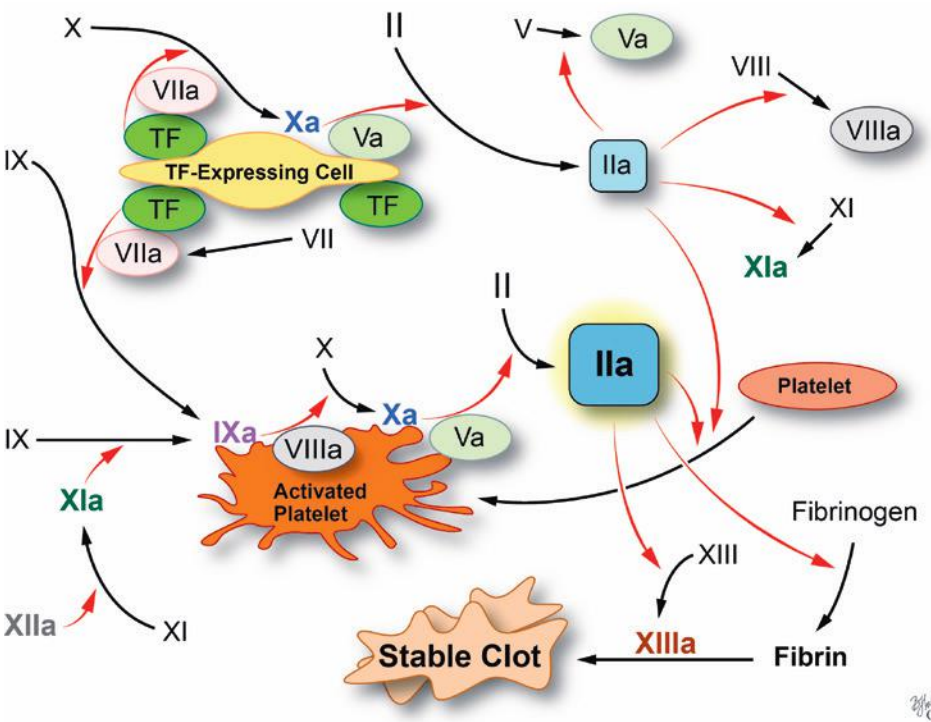


FIG. 2.2
Cell-based model of coagulation.

formation of coagulation protein complexes. Spatiotemporal proximity of enzymes to substrates and cofactors as well as inhibitors can tip the balance between prothrombotic and anti-thrombotic forces. The cell-based model of coagulation (Fig. 2.2) is initiated when TF-bearing cells, which are normally present outside the vascular space, are exposed to the blood after vascular injury. TF complexes with both factor VII and the small proportion of factor VII that circulates in the activated form. Inactive molecules of factor VII are then quickly activated after complexing with TF. TF/factor VIIa then catalyzes activation of factors X and IX. Factor Xa on the TF-bearing cells complexes with factor Va to catalyze the production of a small amount of thrombin (represented by the smaller box labeled "IIa" in Fig. 2.2). The production of thrombin is initially limited by inhibition of TF/factor VIIa by TFPI, which is normally present on endothelial cells and other cells that express TF. The TF-bearing cell-generated-IIa predominantly acts to activate other zymogens and platelets. However, once enough thrombin is generated on TF-bearing cells, this activates nearby platelets and coagulation factors. Activated platelets increase the negatively charged phospholipids on their membrane surfaces to facilitate the assembly of tenase and prothrombinase complexes. The amplification in this step is necessary to generate enough fibrin to form a clot.

The factor IXa generated by TF/factor VIIa diffuses and binds to the surface of nearby activated platelets, then complexes with factor VIIIa to catalyze activation of factor X on the platelet surface. Platelet-bound factor Xa then complexes with factor Va to generate thrombin (represented by the larger "IIa" box in Fig. 2.2) and ultimately quantities of fibrin sufficient to form a clot. The platelet-formed IIa also activates factor XIII, which then forms stable crosslinks within the clot.

PHYSIOLOGIC INHIBITION OF COAGULATION

Several inhibitory pathways are activated by the coagulation cascade as well, including the protein C and protein S pathway, serine protease inhibitors (aka serpins, most important anti-thrombin), and TFPI. Protein C and its cofactor, protein S, are both produced primarily in the liver in a vitamin K–dependent fashion. Thrombin has a weak ability to activate protein C; however, this is greatly enhanced when complexed with thrombomodulin. Thrombin initiates the protein C and protein S pathway when it binds to thrombomodulin and activates protein C. Activated protein C (APC) bound to endothelial cell protein C receptor interacts with its cofactor, protein S, which helps localize the complex to cell surfaces, putting it into closer proximity to factors VIIIa and Va as well as catalyze conversion of factors VIIIa and Va into inactive forms. Protein S is present in plasma, both a free form and bound to C4b-binding protein. Only the free form is capable of complexing with APC.

Antithrombin (AT) is a serpin that inactivates factors IIa, VIIa, IXa, Xa, XIa, XIIa, and kallikrein; however, its major impact is inhibition of factors IIa and Xa. AT acts as a so-called suicide inhibitor by forming a 1:1 covalent complexes between AT and serine proteases. When AT is bound to heparin, its inhibitory function is amplified by three orders of magnitude.

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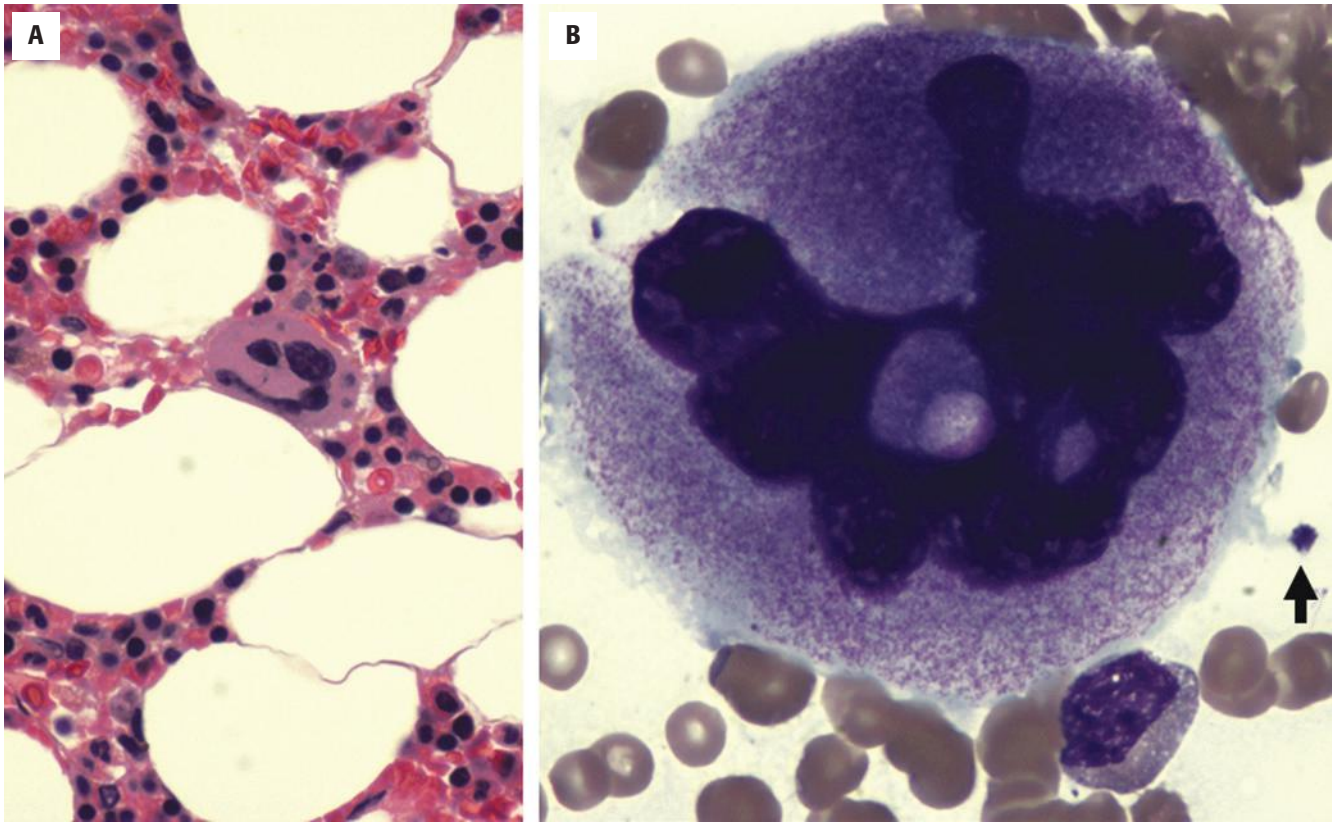


FIG. 2.3

Photomicrographs of normal megakaryocytes. A, Bone marrow biopsy specimen (hematoxylin and eosin; original magnification ×40). B, Bone marrow aspirate smear (Wright stain; original magnification ×100). Notice the granular appearance of the megakaryocyte cytoplasm; the *arrow* points to protoplatelet formation. The convoluted nucleus is aneuploid.

TFPI is a Kunitz-type serpin, a potent inhibitor of the TF/factor VIIa complex. The majority of TFPI is found on endothelial cells. Here TFPI first binds and inactivates uncomplexed factor Xa. TFPI-Xa can then bind and inhibit TF/factor VIIa. A smaller proportion of TFPI is also present in the plasma. Both TFPI and AT quickly inhibit factor Xa in the plasma, which is how the factor Xa/Va complex is limited to the TF-bearing cells.

FIBRINOLYTIC SYSTEM

The fibrinolytic system functions to remove the clot after the vasculature is repaired, as well as to degrade clots that form in the bloodstream. The final step in this pathway is the plasmin-mediated cleavage of fibrin, creating fibrin degradation products. Plasmin is produced from plasminogen by plasminogen activators (PAs): urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA). The latter is produced primarily in endothelial cells and secreted in response to a variety of stimuli, including molecules such as thrombin and histamine, as well as shear stress and venous occlusion. tPA is considered the major intravascular activator of plasminogen. Plasmin cleaves both PAs from single-chain to two-chain polypeptides, which increases their activity. Fibrin has a self-limiting property of localizing plasminogen and tPA on its surface, which markedly increases the ability of tPA to convert plasminogen to plasmin. Cleavage of fibrin exposes lysine residues to which plasminogen and tPA can also bind. Fibrinolysis can be inhibited by blocking this binding using lysine analogs (ε aminocaproic acid and tranexamic acid) or the naturally occurring thrombin-activatable fibrinolysis inhibitor. The fibrinolytic system is also regulated by inhibition of PAs by plasminogen activator inhibitors (most commonly PAI-1), produced by a variety of tissues, and direct inhibition of plasmin by serpins, such as α_2-plasmin inhibitor, which is present in platelet α-granules.

PLATELETS

Platelets are small (2-μm diameter), non-nucleated blood cells that play a vital role in hemostasis and are produced in the bone marrow from megakaryocytes (Fig. 2.3). Megakaryocytes are descended from pluripotent hematopoietic progenitors through a bipotential erythroid/megakaryocytic cell. Megakaryocyte maturation is accompanied by endomitosis, whereby the megakaryocyte progenitor DNA increases up to 256 times the normal chromosome complement through repetitive abortive mitoses, yielding a highly lobated, polypoid nucleus. During a terminal phase of megakaryocyte differentiation, platelets are produced from cytoplasmic projections known

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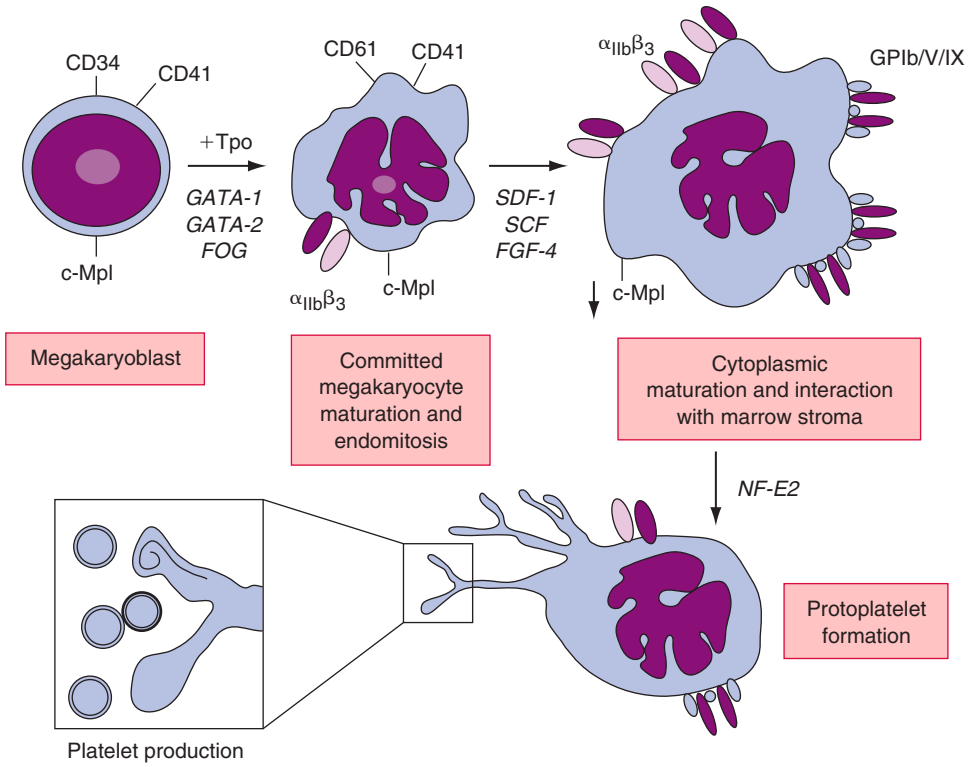


FIG. 2.4

Platelet maturation process. Megakaryoblasts express CD34, CD41, and the thrombopoietin receptor (c-Mpl). After this stage, the megakaryoblasts undergo committed maturation to megakaryocytes with expression of the α3 integrin CD61 and increased levels of αIIbβ3 (GPIIb/IIIa), dependent on transcription factors GATA-1 and GATA-2 together with the cofactor, FOG-1. This maturation is accompanied by endomitosis, resulting in an increase in DNA content accompanied by expression of the von Willebrand factor receptor, GPIb/V/IX. The cytoplasm enlarges (up to 100 to 150 μm) and becomes filled with platelet-specific organelles, proteins, and membrane systems. During a terminal phase of megakaryocyte differentiation, platelets are produced from cytoplasmic projections known as protoplatelets that form along one pole of the megakaryocyte cytoplasm under the guidance of transcription factor NF-E2. Boluses of platelet granules and membrane systems travel from the megakaryocyte cytoplasm down the tubules, yielding bulbous projections at tubule ends and along their length, giving a beaded appearance to the developing tubules. Longitudinal microtubule bundles composed of tubulin αβ-dimers form along the tubules that can move down the tubule. The microtubule bundles reorganize in the protoplatelet tip to form a microtubule coil that envelopes and defines a newly matured protoplatelet.

as protoplatelets (Fig. 2.4). Transverse bands of microtubules form along longitudinal bundles of microtubules in the protoplatelets, facilitating trafficking of granules and proteins to the distal end, with liberation of newly formed platelets from the tip of the protoplatelet into the bone marrow sinusoids.

After release, platelets circulate in a quiescent state but are activated rapidly upon blood vessel injury and play a crucial role in the primary hemostatic response. In their unactivated state, platelets are roughly discoid in shape and are coated by numerous surface glycoprotein (GP) receptors for adhesive ligands, including fibrinogen (GP IIb/IIIa or $\alpha_{IIb}\beta_3$), collagen (GP Ia/IIa and GP VI), and von Willebrand factor (VWF; GP Ib/IX/V). Platelets additionally have membrane receptors involved in signal transduction and biochemical activation, such as adenosine diphosphate (ADP) receptors ($P2Y_1$ and $P2Y_{12}$), thrombin receptors (PAR-1 and PAR-4), and adrenergic receptors. A cytoskeletal system is responsible for maintaining the discoid shape of resting platelets and is involved in shape change during platelet adhesion and activation. The platelet cytosol contains typical cellular organelles such as mitochondria, lysosomes, and glycogen but also contains platelet-specific granules, called α- and dense (δ) granules that play a major role in amplification of platelet activation and interaction with coagulation (Fig. 2.5). The cytosol also houses an open-canalicular membrane system that facilitates platelet granule release and a dense tubular system that stores metabolic enzymes and calcium.

Platelets promote hemostasis by four interconnected mechanisms: (1) adhesion to sites of vascular injury, (2) cellular activation and release of granule contents, (3) aggregation, recruitment, and amplification to form a hemostatic platelet plug, and (4) providing a procoagulant phospholipid surface (Fig. 2.6). Vascular injury and endothelial denudation is rapidly followed by platelet adhesion to the subendothelial extracellular matrix proteins, such as collagen, fibronectin, and VWF; their exposure leads to rapid binding to platelet surface receptors. At high shear rates VWF, a large, multimeric protein secreted abluminally from endothelial cells, facilitates platelet rolling and adhesion by binding to platelet surface GP Ib/IX/V. This is followed by binding of GP Ia/IIa and GP VI to collagen, platelet activation via GP VI, leading to inside-out signaling and firm adhesion via vascular wall–associated fibrin or fibrinogen through interaction with GP IIb/IIIa.

After adhering to the subendothelium, platelets initially are activated by thrombin and collagen via binding to distinct receptors. Binding of ligands to the various receptors leads

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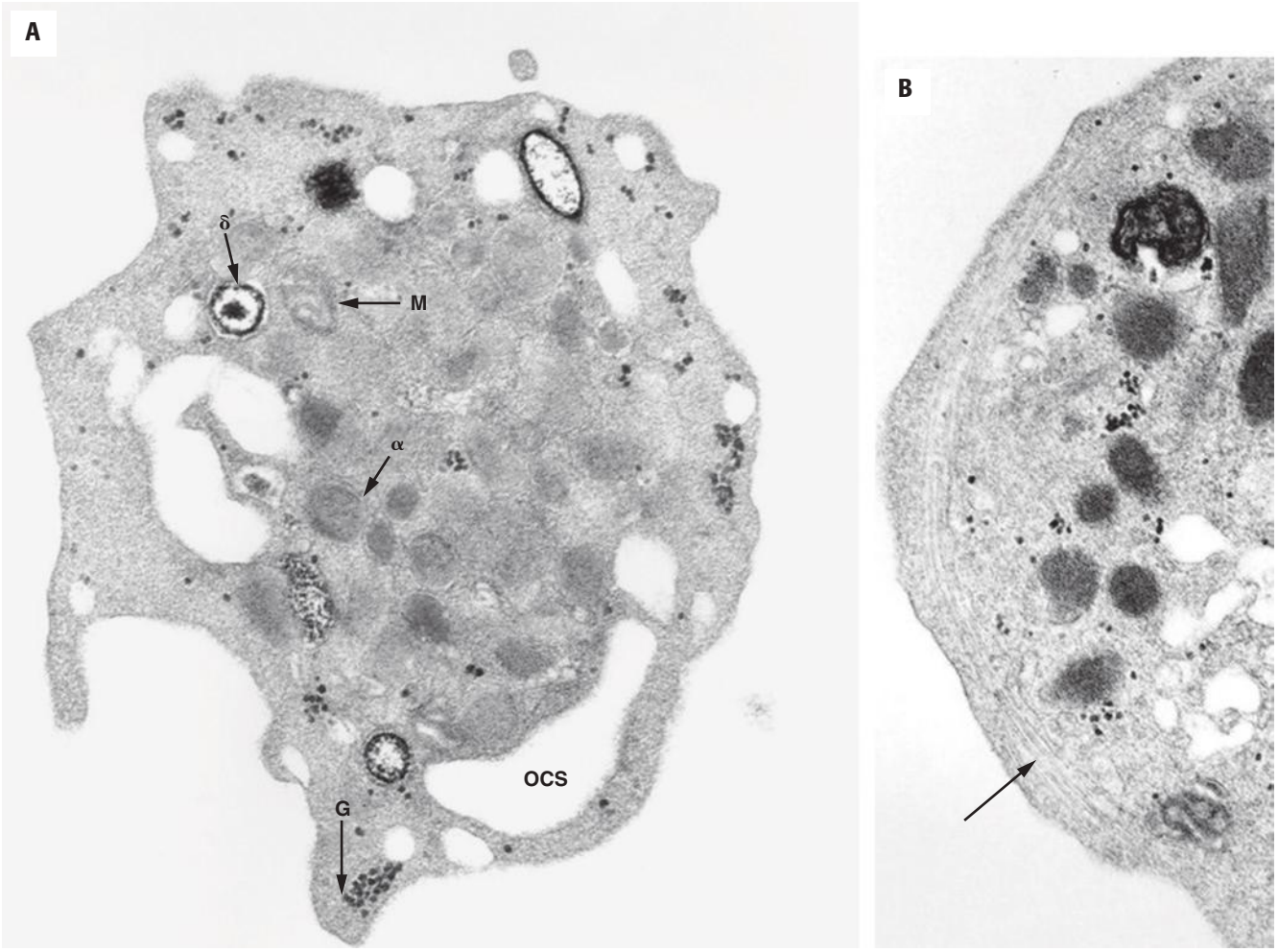


FIG. 2.5

Electron micrographs of normal platelets. A, Note the abundant dark α-granules (α), the surface-connected open canalicular system (OCS), the dense granules with electron-dense central core (δ), the mitochondria (M), and the glycogen (G) (original magnification ×22,000). B, Note the circumferential band of microtubules and microfilaments just under the plasma membrane (*arrow*; original magnification ×40,500).

to activation of many signaling pathways. Convergence of these pathways leads to activation of protein kinase C and increased cytosolic calcium. The increased cytoplasmic calcium activates phospholipase A_2 to liberate arachidonic acid from the platelet membrane, leading to thromboxane A_2 production and degranulation of α- and dense granules. Degranulation releases the contents of α-granules (including platelet factor 4, β-thromboglobulin, fibrinogen, VWF, among others), and dense granules (ADP, adenosine triphosphate [ATP], serotonin, calcium) into the surrounding milieu.

The release of ADP from the dense granules, together with calcium mobilization, leads to molecular reorganization of GP IIb/IIIa (Fig. 2.7), with activation of the receptor and subsequent platelet aggregation, whereby a GP IIb/IIIa receptor on one platelet is bound in a homotypic fashion to the same receptor on adjacent platelets via a central fibrinogen molecular bridge. In addition to ADP, other agonists, such as epinephrine, thrombin, collagen, and platelet-activating factor, can initiate platelet aggregation by interaction with membrane receptors. The release of ADP, thromboxane A2, and serotonin leads to the recruitment of many other platelets to the vessel wall with activation, amplification, and formation of a hemostatic platelet plug.

Activated platelets also play a procoagulant role that serves as a link between platelet function and coagulation activation. Platelet membrane phospholipids undergo a rearrangement during activation with a flip of phosphatidyl serine from the inner table to the outer table of the platelet membrane, providing a binding site for phospholipid-dependent coagulation complexes that activate both factor X and prothrombin.

LABORATORY EVALUATION IN HEMOSTATIC AND THROMBOTIC DISORDERS

COMMONLY USED LABORATORY ASSAYS RELATED TO HEMOSTATIC DISORDERS

Prothrombin time assesses the extrinsic and common pathways (factors VII, X, V, II, and fibrinogen). The PT is measured

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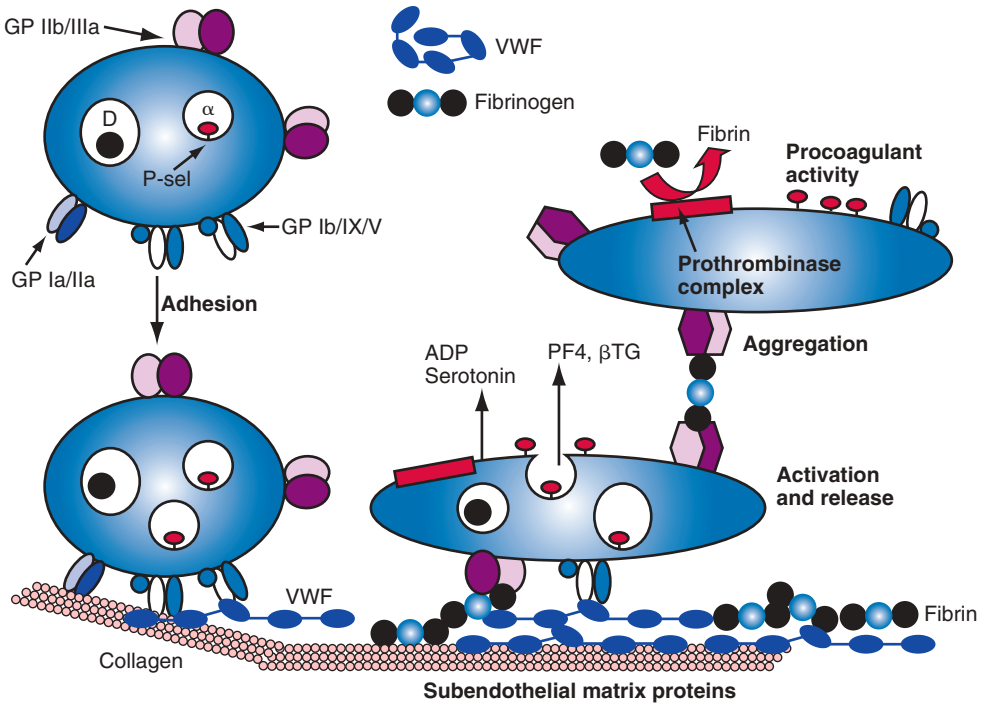


FIG. 2.6

Schematic diagram of the role of platelets in hemostasis. Platelet activation is stimulated by vascular injury through exposure of platelets to extracellular matrix proteins and adsorbed plasma proteins, including von Willebrand factor (VWF) and fibrinogen (Fib). Platelets adhere to VWF through the surface glycoprotein (GP) Ib/IX/V and to fibrinogen through GP IIb/IIIa. Platelet adhesion stimulates intracellular signaling, leading to degranulation of α-granules (platelet factor 4 [PF4] and β-thromboglobulin [βTG]), and phospholipid reorganization with formation of coagulation complexes and fibrin formation. Adenosine diphosphate (ADP) release from dense granules and activation of the GP IIb/IIIa receptor also occur, leading to aggregation of platelets to the adherent layer.

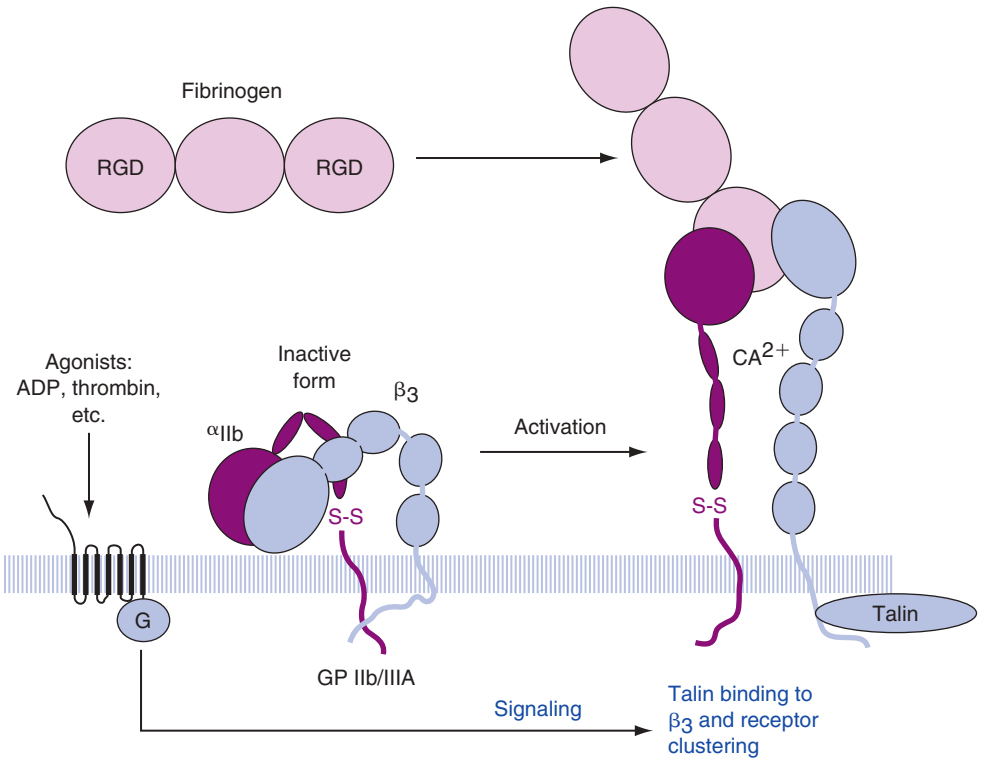


FIG. 2.7

Activation of glycoprotein (GP) IIb/IIIa (αIIb/β3 integrin), the receptor for fibrinogen. During platelet activation, an agonist binds to a surface receptor and initiates inside-out signaling. In the resting state, the GP IIb/IIIa receptor is in an inactive conformation and cannot bind to fibrinogen. After signaling, a molecular conformational change allows binding of the ligand (RGD peptide from the fibrinogen α-chain). This is followed further by cytoskeletal rearrangement and receptor clustering. *ADP*, Adenosine diphosphate.

in time (in seconds) by optical or mechanical methods when a fibrin clot is formed in the patient's 3.2% sodium citrate anticoagulated platelet-poor plasma after addition of calcium chloride and thromboplastin, a source of phospholipid and tissue factor. PT is useful to evaluate bleeding, factor deficiency or the presence of possible factor inhibitors and liver disease, or to monitor warfarin therapy. PT is most sensitive to detect factor VII deficiency. PT will be significantly prolonged when factor levels are less than approximately 40%. The international normalized ratio (INR) is calculated to standardize therapeutic

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monitoring of warfarin. An international sensitivity index (ISI) is determined by the manufacturer for each reagent/test system combination relative to the international standard for thromboplastin. The INR is then calculated as follows:

$$\text{INR} = (\text{Patient PT} / \text{Geometric mean normal PT for the laboratory})^{\text{ISI}}$$

Maintenance of a modest intensity INR of 2.0 to 3.0 is effective for management and prevention of most of cases with thromboembolism.

The activated partial thromboplastin time assesses the intrinsic and common pathways (factors XII, XI, IX, VIII, X, V, and II and fibrinogen). The APTT is measured in time (in seconds), by either an optical or mechanical method, when a fibrin clot is formed after the patient's plasma is incubated with a partial thromboplastin, a TF-free source of phospholipid, and a negatively charged surface, such as kaolin or silica, which can activate the contact factors, and then calcium chloride is added. The APTT is commonly used to evaluate factor deficiencies or the presence of factor inhibitors; monitor heparin, anti-Xa, and direct thrombin inhibitor (DTI) therapy; and detect lupus anticoagulant. Prolongation of the APTT can be observed when factor levels are decreased (<~30%). The APTT can be used to assess the therapeutic range of heparin (0.3–0.7 U/mL) by using an APTT/factor Xa inhibition assay dose-response curve. Shortened PT and APTT can be associated with activation of the clotting factors owing to preanalytic handling or associated with increased coagulation factor levels, such as pregnancy and acute phase reactions. Persistent short APTT results may be associated with a thrombotic risk. Falsely prolonged APTT and PT can be related to a polycythemic specimen with high hematocrit, presence of heparin, or under filled tubes. Severely hemolyzed, turbid, or lipemic samples may impact coagulation test results, particularly in optical methods.

The thrombin time (TT) measures the time (in seconds) that it takes to convert fibrinogen to fibrin after adding exogenous thrombin to the patient plasma. The reptilase time (RT) also measures the time it takes to convert fibrinogen to fibrin but differs from the TT in that the activation is thrombin independent and relies instead on an enzyme derived from a snake's venom. Both the TT and RT are prolonged by fibrinogen abnormalities, such as hypofibrinogenemia and dysfibrinogenemia, whereas the TT is prolonged and the RT is normal in the presence of unfractionated heparin or a DTI. Low-molecular-weight (LMW) heparin does not typically prolong the TT or the RT. Additionally, the TT may be prolonged by high concentrations of fibrin degradation products (FDPs), primary amyloidosis, and presence of inhibitors to thrombin, such as DTI drugs, and shortened in the presence of plasma volume expanders, such as dextran and hydroxyethyl starch.

Coagulation factors are measured to detect factor deficiency and degree of factor deficiency and to monitor factor replacement therapy. Factor levels can be assessed by antigenic/immunologic or functional tests. It is usually most appropriate to test a factor's functional activity first, and subsequent antigenic/immunologic testing with a clinical suspicion of qualitative factor abnormalities, which are far less common than quantitative factor deficiencies. The clot-based assays are the most commonly used functional assays and are typically determined by their ability to correct the clotting time of standard plasma deficient in only the factor of interest. Occasionally a factor assay shows increasing factor activity with increasing dilution, so-called dilutional effect or non-parallelism owing to dilution of inhibitors in the specimen. This pattern suggests the presence of coagulation inhibitors or lupus anticoagulants in the plasma. The reference range of factors is typically 50% to 150% but should be determined by individual laboratories using their instrument and reagent system.

The Bethesda assay is commonly used to evaluate the potency of factor VIII inhibitors. It can be used to assess the magnitude of the effect of any specific factor inhibitor by measuring the ability of the inhibitors in the patient's plasma to neutralize the factor of interest in normal plasma. By definition, one Bethesda unit (1 BU) is the quantity of inhibitor that neutralizes 50% of the factor of interest in normal plasma in 2 hours at 37°C. Although it is a standardized assay, significant variation is noted in results using different reagents/instruments. Lupus anticoagulants, heparin, anti-Xa drugs, and DTIs in the specimen can interfere with the clotting-based Bethesda assay.

Mixing studies are performed to determine the presence of factor deficiency or factor inhibitors, or nonspecific inhibitors such as lupus anticoagulants, if APTT or PT is prolonged in the specimen. To perform a mixing study, the APTT or PT is measured after mixing the patient's plasma and normal plasma at a ratio of 1 : 1. Mixing studies are often performed both immediately after the addition of normal plasma and after incubation at 37°C for 1 to 2 hours. If the prolonged result "corrects" into the normal reference range, the result suggests a deficiency of one or more coagulation factors in the patient's plasma. If the prolonged result does not correct, this suggests the presence of an inhibitor. Weak inhibitors, however, can be diluted by the mixing study, resulting in an apparent "correction" of the test. An immediate-acting inhibitor typically suggests the presence of heparin, an anti-Xa drug, a DTI, or a non-specific inhibitor such as a lupus anticoagulant. However, specific factor inhibitors (e.g., a factor VIII inhibitor) may be associated with complete or partial correction at the immediate phase and a marked inhibitor effect at the incubated or time-dependent phase.

LABORATORY ASSAYS RELATED TO THROMBOTIC DISORDERS

Dysregulation of the hemostatic system can lead to thrombophilia, the abnormal occurrence of a venous or arterial thrombosis. The reported prevalence and estimated relative risk of thrombophilic disorders are summarized in Table 2.2. In addition, individuals with a history of recurrent venous thromboembolism (VTE), a first VTE at younger than 50

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TABLE 2.2

Prevalence and Relative Risk Estimate of Thrombophilic Disorders

	PREVALENCE (%)		RELATIVE RISK	
	General Population	Patients With VTE	First VTE	Recurrent VTE
Antithrombin deficiency	0.05–0.2	0.5–4.9	5–50	1.9–2.6
Protein C deficiency	0.14–1.5	2–5	4–15	1.4–2.5
Protein S deficiency	0.03–0.5	1–3	1–10	1–2.5
Factor V Leiden mutation				
Heterozygous	3–8	10–25	3–8	1.4–1.6
Homozygous	0.02	1.5	10–80	2–3
Prothrombin G20210A mutation				
Heterozygous	2–4	5–8	2–4	1.4–1.5
Homozygous	0.02	<1.0	30	–
Lupus anticoagulant	1–8	–	3–10	2–6
Anticardiolipin/β2 glycoprotein 1 antibodies	3.4–5	8	0.7–2.4	1–6

VTE, Venous thromboembolism.

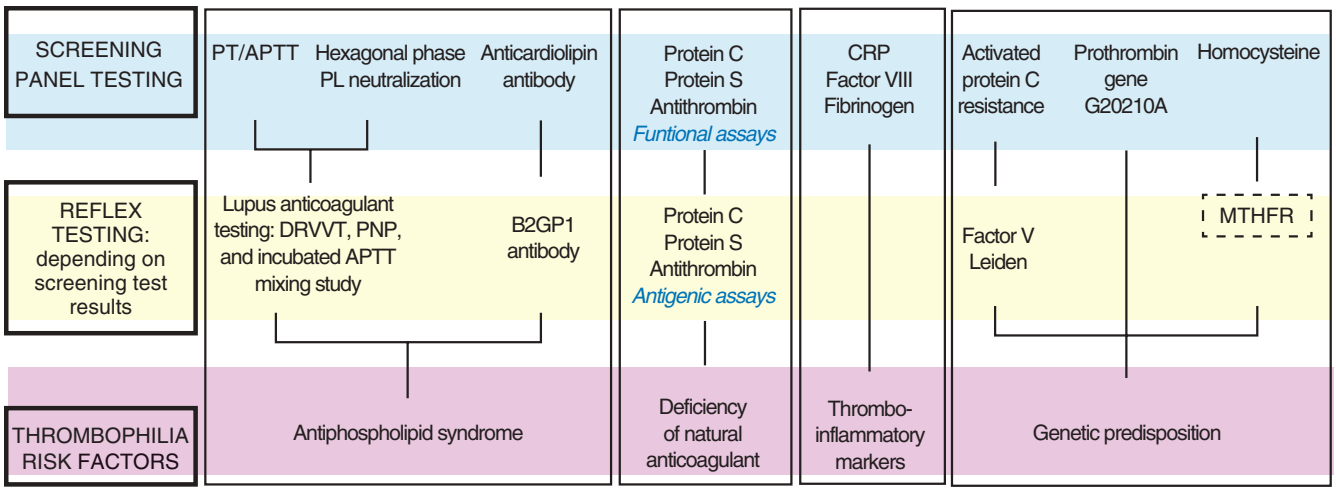


FIG. 2.8

Comprehensive diagnostic algorithm for evaluation of thrombophilia. *APTT,* Activated partial thromboplastin time; *B2GP1,* β 2 glycooprotein 1; *CRP,* c-reactive protein; *DRVVT,* diluted Russell's viper venom test; *MTHFR,* methylene tetrahydrofolate reductase; *PL,* phospholipid; *PNP,* platelet neutralization procedure; *PT,* prothrombin time.

years, an unprovoked VTE, or a VTE in an unusual site, such as cerebral, mesenteric, portal, or renal vein, or VTE in an individual with first-degree family members with VTE at a younger age are recommended for thrombophilia testing. No single laboratory assay is available to identify all thrombophilic disorders. Testing should be performed in a step-wise manner beginning with high-yield screening tests followed by appropriate specific confirmatory tests in order. Fig. 2.8 outlines a comprehensive diagnostic algorithm to maximize diagnostic potential in patients with thrombophilia. Laboratory assays to evaluate selected individuals with thrombophilia risk factors will be briefly discussed in the section of each diagnosis later. Comprehensive interpretation by coagulation specialists may be necessary to synthesize test results, correctly interpret them according to the patient's clinical condition, and provide appropriate guidance to clinicians.

EFFECT OF ANTICOAGULANTS ON COAGULATION ASSAYS

A variety of anticoagulants, including warfarin, unfractionated or LMW heparin, fondaparinux, and direct oral anticoagulants (DOACs) such as DTI (anti-IIa), rivaroxaban, apixaban, or

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edoxaban (anti-Xa), are used for treatment and to reduce the risk of recurrent VTE or thromboprophylaxis after major surgery. Warfarin has a narrow therapeutic window and requires routine monitoring and individualized dosing based on INR, whereas DOACs have an advantage of wide therapeutic window, fixed dosing, and no requirement of laboratory monitoring or dose adjustments. However, certain clinical scenarios do require measurement of drug levels such as episodes of bleeding, prior to or perioperative surgery or invasive procedures, concomitant use of drugs with known interactions, renal impairment, suspected overdose or non-compliance, and DOAC treatment failure (thrombosis during therapy). Anticoagulants can significantly affect the results of many coagulation assays. Some of these effects are consistent and may aid in therapeutic drug monitoring, whereas others merely complicate the laboratory evaluation of the patient's underlying hemostatic function. Table 2.3 summarizes the effect of various anticoagulants on coagulation assays.

EVALUATION OF PLATELET FUNCTION

In a patient with bleeding diathesis, a detailed personal and family bleeding history, including an assessment of the duration, pattern, severity of bleeding problems, and spontaneous or trauma-related bleeding, should be obtained before beginning a laboratory evaluation of platelet function. Platelet-mediated bleeding disorders typically manifest with a mucocutaneous bleeding pattern involving small vessels. Ecchymosis, petechiae, purpura, epistaxis, and gingival bleeding are commonly observed. This pattern is in contrast to that observed with coagulation protein disorders in which deep-tissue bleeding and hemarthroses are more common.

Many drugs and foods, such as aspirin, caffeine, and garlic, can affect platelet function. The clinical history should include investigation of systemic disorders, such as renal disease, hepatic failure, connective tissue disorders, myeloproliferative neoplasms (MPNs), myelodysplastic disorders, malignancy, and cardiovascular disease, as platelet dysfunction is associated

TABLE 2.3
Summarized Effect of Anticoagulants on Coagulation Assays

	Unfractionated Heparin	LMW Heparin	VKA	Dabigatran	Factor Xa Inhibitors (Rivaroxaban or Apixaban)	Fondaparinux
PT	–	–	↑↑	↑	↑↑	↑
APTT	↑↑	↑/–	↑	↑↑	↑-↑↑	↑
Thrombin time	↑↑↑	↑	–	↑↑↑	–	–
Fibrinogen (Clauss method)	–/↓	–	–	↓	–	–
Anti–factor Xa assay	↑	↑	–	–	–/↑	↑
Ecarin clotting time	–	–	–	↑↑	–	–
Intrinsic factors[a] VIII, IX, XI, XII	↓	↓	↓ (FIX)	↓↓	↓/–	↓
Extrinsic factors[b] II, V, VII, X	–	–	↓ (FII, FIX, FX)	↓	↓↓	–
Protein S (clot based) (immunologic)	 ↑ –	 – –	 ↓ ↓	 ↑↑ –	 ↑↑ –	 ↑ –
Protein C (clot based) (immunologic)	 ↑ –	 – –	 ↓ ↓	 ↑↑ –	 ↑ –	 ↑ –
Antithrombin Factor Xa based Factor IIa based	 –/↓ –/↓	 – –	 – –	 – ↑	 ↑ –	 – –
APC ratio[a]	↑	–	↑/–	↑	↑	↑
DRVVT	↑	↑	↑	↑	↑↑	–
Mixing study, PT/APTT	Incomplete correct (APTT)	Incomplete correct (APTT)	Incomplete correct (PT)	Incomplete correct	Incomplete correct	Incomplete correct
D-dimer	–	–	–	–	–	–

[a]APTT-based assay, [b]PT-based assay.
APC, Activated protein C; *APTT,* activated partial thromboplastin time; *DRVVT,* diluted Russell's viper venom test; *F,* factor; *LMW,* low molecular weight; *PT,* prothrombin time; *VKA,* vitamin K antagonist.

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with many of these diseases. In addition, some specific clinical features, such as albinism, deafness, nephritis, and susceptibility to infections, may help in the differential diagnosis of the inherited platelet disorders.

Investigation of platelet disorders should start with measurement of the platelet count and review of peripheral smear morphology. On a properly prepared Wright-stained blood smear, the platelets are of roughly uniform size (≈2-μm diameter) with abundant purple-staining granules. The accepted normal range of the platelet count is generally 150 to 400 × 10^3/μL of blood, although values much lower than this can be adequate for hemostasis. The mean platelet volume (MPV; normal 7–11 fL) is an indication of platelet size. The MPV can be an indication of platelet turnover. In patients with rapid turnover, the platelets will be larger in general because of the larger size of newly produced platelets. True congenital macrothrombocytopenias usually have uniformly large platelets with a high MPV. This can result in falsely low platelet counts, because the large platelets may be counted as leukocytes or erythrocytes by automated cell counters. True thrombocytopenia must be distinguished from pseudothrombocytopenia or spurious in vitro platelet clumping (Fig. 2.9). A more accurate platelet count can be established by collecting the blood sample in either citrate or heparin anticoagulants or by collecting blood directly from a finger stick into a diluent. In the initial evaluation of platelets, it is desirable to perform a screening test to evaluate platelet function, especially in laboratories that cannot perform more definitive platelet testing. The PFA-100 (Platelet Function Analyzer; Siemens USA, Washington, DC) measures the closure time required for platelets to adhere to a membrane coated with aggregation agonists (collagen/epinephrine or collagen/ADP), activate, aggregate, and occlude the aperture. Inhibition of platelet cyclooxygenase by aspirin-like drugs prolongs closure times with the collagen/epinephrine cartridge, whereas von Willebrand disease (VWD), intrinsic platelet dysfunction and non-aspirin drugs may give an abnormal closure time with both cartridges. Severe platelet disorders, such as Glanzmann thrombasthenia and Bernard-Soulier syndrome, give markedly prolonged closure times (typically >300 seconds), but the device is not as sensitive to milder disorders, such as storage pool disorders or signaling defects. The PFA-100 results can be affected by low platelet counts and low hematocrits, but they are not affected by heparin.

The thromboelastograph (TEG) (Haemonetics, Braintree, MA) measures the viscoelastic properties of whole blood and is sensitive to coagulation proteins, platelets, and fibrinolysis. TEG provides a comprehensive real-time analysis of hemostasis from initial thrombosis to fibrinolysis. The maximal amplitude (MA) is a parameter dependent on platelet number, function, and fibrinogen concentration, but it has limited sensitivity to antiplatelet drugs.

Platelet aggregation measures the ability of agonists to cause in vitro platelet activation and platelet–platelet binding. As such, platelet aggregation is often useful to distinguish intrinsic platelet disorders involving surface glycoproteins, signal transduction, and platelet granules. Platelet aggregation studies can be performed in whole blood by an impedance technique or in platelet-rich plasma by a turbidimetric technique, often called "light transmission aggregometry." Platelet aggregation can be combined with studies of dense granule ATP release with a lumi-aggregometer using a firefly luciferin-luciferase reagent. Many factors can affect the platelet aggregation results, such as thrombocytopenia, thrombocytosis, processing temperature, stirring rate, and processing time (testing should be completed within 4 hours of phlebotomy). In addition, clinicians ordering the tests should advise patients

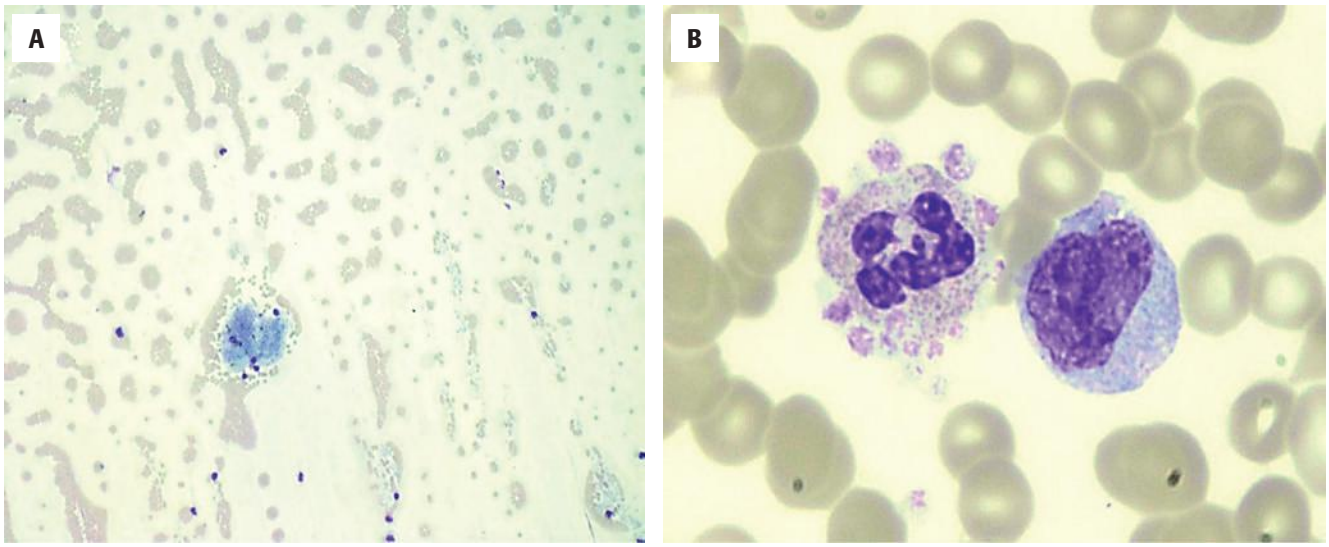


FIG. 2.9

A, Pseudothrombocytopenia showing large clumps of platelets along the feathered edge or sides of the peripheral blood smear (Wright stain, ×10). B, Platelet satellitism showing platelets binding to cytoplasmic membranes of neutrophils.

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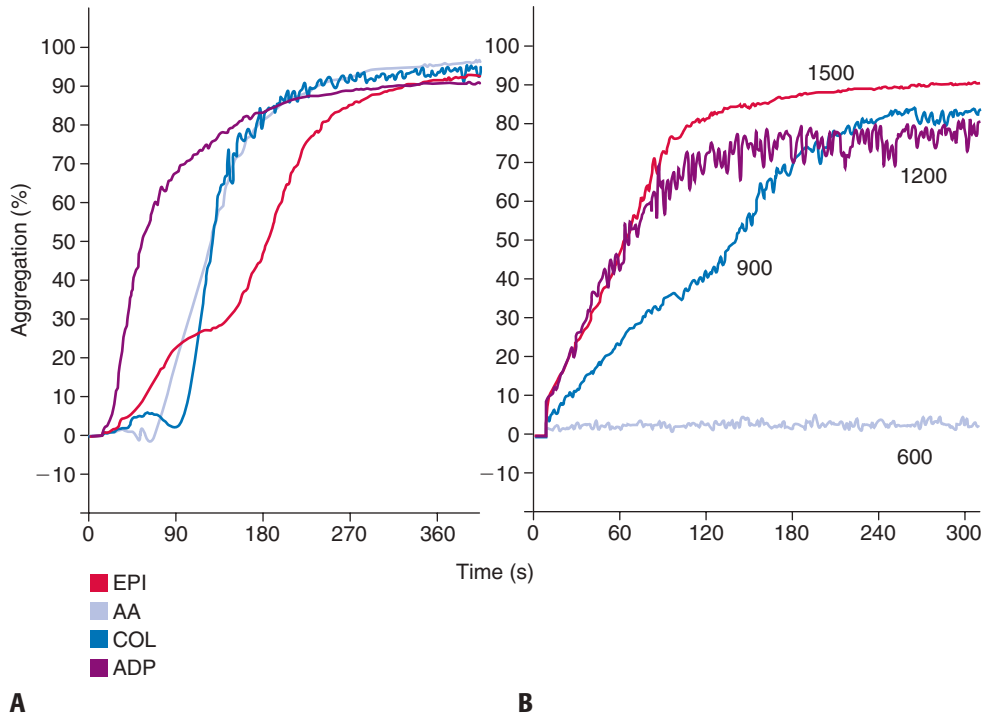


FIG. 2.10

A representative platelet aggregation. A, Platelet aggregation with adenosine diphosphate (ADT; magenta, 5 μmol/L), epinephrine (EPI; red, 100 μmol/L), collagen (COL; blue, 2 mg/mL), and arachidonic acid (AA; lavender, 0.5 mg/mL). ADP shows two waves of aggregation. After the initial wave of aggregation, platelet ADP release from dense granules stimulated the final and irreversible wave of aggregation. Collagen aggregation characteristically shows an initial shape change. Normal aggregation for all agonists is more than 70% aggregation. B, Platelet aggregation with several concentrations of ristocetin. Ristocetin stimulates a conformational change in von Willebrand factor, leading to aggregation through the glycoprotein Ib/IX/V complex. Note that aggregation is virtually absent at low ristocetin concentration (600 μg/mL), becoming progressively stronger until complete aggregation is reached somewhere between 1200 and 1500 μg/mL. (Optimal platelet aggregation using a Helena PACKS-4 aggregometer).

to discontinue, if possible, any medication, such as aspirin or nonsteroidal antiinflammatory agents that might interfere with assessment of the test results.

The initial agonists typically used include ADP, collagen, arachidonic acid, epinephrine, and ristocetin. An expanded agonist panel to investigate specific disorders could utilize agonists such as thrombin receptor antagonist peptide (TRAP6), alpha thrombin, the thromboxane analog U46619, convulxin for GPVI deficiency, platelet-activating factor for protein kinase C defects, and A231878 for calcium mobilization defects. Optimal platelet aggregation shows a biphasic pattern for the agonists ADP and epinephrine (Fig. 2.10A); the initial increase in aggregation is caused by primary aggregation in response to activation of the GP IIb/IIIa platelet membrane receptor, whereas the second wave of aggregation is the result of platelet degranulation with recruitment of additional platelet aggregates. At higher agonist concentrations, the two waves merge into one wave of aggregation. Other agonists, such as arachidonic acid, thrombin receptor agonists, and collagen, usually show only a single wave of aggregation. Collagen characteristically shows an initial shape change before the wave of aggregation; this is seen as a transient increase in turbidity. Ristocetin-induced platelet aggregation (RIPA) utilizes the antibiotic ristocetin that facilitates the binding of von Willebrand factor (VWF) to the GP Ib/IX/V complex (Fig. 2.10B). Addition of escalating concentrations of ristocetin to platelet-rich plasma allows detection of both increased and decreased sensitivity to ristocetin. A normal result requires the presence of both functional VWF and normal GP Ib/IX/V, so that RIPA can detect both von Willebrand disease and some platelet dysfunctions, such as Bernard-Soulier syndrome.

Other techniques to study platelet function include flow cytometry, electron microscopy, and next-generation sequencing. Flow cytometry has been used to study platelet structure and function based on the detection of cell surface proteins with fluorescently labeled antibodies as well as detection of platelet activation. Electron microscopy can be used for the ultrastructural evaluation of platelets, including assessment of platelet granules and cellular organelles. Genomic approaches can also be used to identify specific inherited platelet defects, and next-generation sequencing is becoming more available for diagnosis of platelet disorders (Table 2.4).

New diagnostic guidelines for evaluation of inherited platelet disorders from the International Society of Thrombosis and Hemostasis (ISTH) published in 2015 indicate that first-step tests should include a blood smear, light transmission aggregometry with a limited agonist panel (ADP, collagen, arachidonic acid, ristocetin), assessment of platelet granule release, and surface glycoprotein analysis by flow cytometry. Second-step tests should include platelet aggregation with an expanded agonist panel, flow cytometry with additional

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TABLE 2.4
Identified Genomic Syndromes Associated With Platelet Dysfunction

Syndrome	Affected Gene and Chromosome Location	Platelet Count/Size	Clinical Features
Defects of Platelet Production			
GATA1-related thrombocytopenia and erythroid defects	*GATA1,* Xp11.23	Decreased/large size	Abnormal aggregation
Paris-Trousseau and Jacobsen syndromes	*FLI-1,* 11q23	Decreased/large size	Giant alpha granules
Familial platelet disorder with acute myelogenous leukemia	*RUNX1,* 21q22	Decreased/normal size	Abnormal aggregation Other: development of AML
Amegakaryocytic thrombocytopenia with radioulnar synostosis	*HOXA11,* 7p15-14	Decreased/normal size	Reduced megakaryocytes Other: radioulnar synostosis, hearing loss
Congenital amegakaryocytic thrombocytopenia	*c-MPL,* 1p34	Decreased/normal size	Reduced megakaryocytes Other: evolution to aplasia
Thrombocytopenia and absent radii	*RBM8A*; large deletion of 1q21.1	Decreased/normal size	Reduced megakaryocytes Other: bilateral radial aplasia
Defects of Platelet Surface Receptors			
Bernard-Soulier syndrome	*GPIBA* (17p13), *GPIBB* (22q11), *GP9* (3q21)	Decreased/large size	Decreased aggregation to ristocetin
Platelet-type von Willebrand disease	*GPIBA,* 17p13	Variable thrombocytopenia	Decreased binding of GPIba to VWF
Glanzmann thrombasthenia	*ITGA2B, ITGB3*	Normal	Absent aggregation to ADP, epinephrine, collagen
Scott syndrome	*ABCA1, ANO6*	Normal	Decreased platelet procoagulant activity
Collagen receptor disorders	*ITGA2, GP6*	Normal	Decreased aggregation to collagen
ADP receptor disorders	*P2Y12*	Normal	Decreased aggregation to ADP
Thromboxane receptor defect	*TBXA2R,* 19p13.3	Normal	Decreased aggregation to arachidonic acid
Platelet Organelle Disorders			
Gray platelet syndrome (α-granule defect)	*NBEAL2* and *GFI1B*	Decreased/large size	Pale gray platelets, decreased α-granules Other: marrow fibrosis
Hermansky-Pudlak syndrome	*HPS1* to *HPS9,* 10q23.1-123.3	Normal	Abnormal aggregation; δ-SPD Other: albinism, pulmonary fibrosis
Chediak-Higashi syndrome	*CHS (LYST),* 1q42.1-q42.2	Normal	Abnormal aggregation; δ-SPD Other: albinism, infections
Quebec platelet syndrome	*PLAU,* 10q22.2	Decreased	Decreased aggregation to epinephrine; degraded α-granules owing to duplication of urokinase in α-granules
Cytoskeletal and Signaling Pathways			
Myosin heavy chain (MYH9) disorders	*MYH9,* 22q12-13	Decreased/large size	Mild aggregation dysfunction Other: neutrophil inclusions, deafness, nephritis
Wiskott-Aldrich syndrome (and X-linked thrombocytopenia)	*WAS,* Xp11.23-p11.22	Decreased/small size	Storage pool and cytoskeletal disorder; abnormal aggregation Other: neutropenia, eczema, immunodeficiency

ADP, Adenosine diphosphate; *AML,* acute myeloid leukemia; *VWF,* von Willebrand factor.

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antibodies such as anti-GPIa/IIa, GPIV, and GPVI, measurement of serum thromboxane B2, and electron microscopy. Third-step tests would include testing available in specialized centers, such as biochemical studies, receptor binding assays, and molecular genetics studies.

BLEEDING DISORDERS

HEMOPHILIA A AND B

Clinical Features

Hemophilia A and B are inherited bleeding disorders caused by deficiencies in factor VIII (hemophilia A) or factor IX (hemophilia B). Both diseases are inherited in an X-linked recessive manner. However, up to one third of both hemophilia A and hemophilia B cases may be the result of a spontaneous mutation, and there will be no family history of the disease. Hemophilia A is the most common inherited bleeding disorder, occurring in 1 in 5000 male births. Hemophilia B is less common, occurring in approximately 1 in 30,000 male births. Neither disease shows a predilection for specific ethnic groups.

In both diseases, the type and frequency of bleeding are determined by the levels of the deficient factor. Severe hemophilia is defined by a factor level less than 1 IU/dL, whereas patients with moderate hemophilia have levels of 1 to 5 IU/dL, and those with mild hemophilia have levels of 6 to 40 IU/dL. Severe disease is the most common and accounts for approximately half of cases. The most serious bleeding risk with severe disease occurs early, with 1% to 4% of neonates experiencing intracranial hemorrhage, potentially causing permanent neurologic defects. Extracranial hemorrhage can also occur in severely affected neonates; both of these consequences are associated with traumatic delivery.

Severe hemophiliacs also suffer from spontaneous (not associated with trauma) hemorrhage into muscles and joints. The former usually occur in the muscles of the legs, buttocks, and forearms and during the period when infants increase their activity at 6 to 8 months of age. Hemarthroses continue to be a problem throughout the life of a severe hemophiliac, increasing with age, and are a serious cause of morbidity in these patients. The most commonly affected joint is the ankle followed by the elbow and knee. Repeated episodes of bleeding into a joint cause inflammation, which eventually damages the tissues in the joint causing hemophilic arthropathy and eventually joint deformity, permanent impairment, and pain.

Moderate hemophilia is diagnosed in older patients and is clinically heterogeneous. Although most patients experience bleeding only after surgery or trauma, some may have spontaneous bleeding similar to that seen in severely affected patients. In mild hemophilia, bleeding only occurs after surgery or trauma. These patients tend to be diagnosed later in life, sometimes on routine coagulation screening. Female carriers of hemophilia tend to have factor levels around 50% and may experience menorrhagia, traumatic hemarthroses, bruising, hematomas, and postsurgical/postpartum bleeding.

HEMOPHILIA A AND B—FACT SHEET

Definition
- Inherited bleeding disorder caused by deficiency of factor VIII (hemophilia A) or factor IX (hemophilia B)

Incidence
- Hemophilia A: 1 in 5000 male births
- Hemophilia B: 1 in 30,000 male births

Morbidity
- Life-threatening intracranial hemorrhage in neonates with severe disease
- Joint deformity and pain associated with recurrent hemarthroses
- Development of anti–factor VIII or IX inhibitors in a subset of patients (see next section)

Gender, Race, and Age Distribution
- Males (rarely females with loss of one X chromosome or imbalanced X chromosome inactivation
- No ethnic preference
- Congenital

Clinical Features
- Intracranial, extracranial, and post circumcision bleeding in severely affected neonates
- Hemarthroses or intramuscular hematomas, either associated with trauma (mild disease) or spontaneous (severe and some moderate disease)
- Postsurgical and trauma-associated bleeding

Prognosis and Therapy
- 1% to 4% of patients with severe disease will have neonatal intracranial hemorrhage
- Severely affected patients have life-long risk of hemarthroses, with accumulating permanent joint damage
- Bleeding episodes treated with factor replacement with either plasma concentrates or recombinant protein
- Prophylactic factor administration for severely affected children reduces frequency of hemarthroses

Pathologic Features and Test Results

Deficiencies in factors VIII or IX cause a prolongation of the APTT with a normal PT and TT. APTT mixing studies will be negative in both immediate and incubated phases unless the patient has developed specific factor inhibitors (see next section). It is recommended to use clot-based assays rather than chromogenic assays for factor measurement because of better accuracy at low factor levels; special

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HEMOPHILIA A AND B—PATHOLOGIC FEATURES

Coagulation Assays

- Prolonged APTT with normal PT and TT
- Decreased factor VIII or IX levels
- Negative immediate and incubated mixing studies
- Normal platelet count and morphology
- Normal von Willebrand factor antigen levels and activity

Differential Diagnosis

- Type 2N or 3 von Willebrand disease (for hemophilia A)
- Acquired factor inhibitors

low-level curves can be made with clot-based assays to detect very low levels of FVIII (<1%). Neonates have reduced levels of vitamin K, so a mild decrease in factor IX seen at birth should be confirmed after 6 months of age. Platelet counts and VWF activity and antigen are normal.

Genetic analysis is recommended in patients because different types of gene defects are associated with different levels of risk for developing inhibitors. Large deletions and nonsense mutations are associated with higher risk; patients with missense and splicing mutations have lower risk. The most common mutations in severe hemophilia A are inversions of intron 22 (40% to 50% of severely affected patients) and intron 1. There are no specific common defects for mild and moderate hemophilia A, so these patients, as well as those patients with severe hemophilia A without intron 1 or 22 abnormalities, undergo complete sequencing of the *F8* gene. Over 2000 unique mutations have been detected; however, in 2% to 18% of patients a gene defect may not be determined. Over 1000 mutations causing hemophilia B have been described, and these patients usually have the eight exons, intron-exon boundaries, and promoter region of the *F9* gene sequenced. The Centers for Disease Control and Prevention (CDC) Hemophilia Mutation Project maintains lists of described *F8* and *F9* mutations (www.cdc.gov/ncbddd/hemophilia/champs.html).

Differential Diagnosis

Because factor VIII circulates in association with VWF, deficiencies or defects in VWF can lead to decreased levels of factor VIII. Types 1 and 3 VWD are caused by low or essentially absent VWF levels, respectively. Type 2N VWD is caused by a mutation in the VWF that prevents its binding to factor VIII. These patients may have normal VWF antigen and activity levels with decreased factor VIII. Investigating the family history may be helpful in distinguishing the two because type 2N VWD is inherited in an autosomal rather than X-linked fashion. Demonstrating decreased VWF:factor VIII binding capacity is diagnostic for type 2N VWD, whereas *F8* sequencing will be positive for a mutation in most patients with hemophilia A.

Patients with acquired factor VIII inhibitors (described in the next section) may show bleeding episodes similar to those seen in hemophiliacs; however, these present later in life will have laboratory evidence of an inhibitor (positive mixing study and Bethesda assay).

Prognosis and Therapy

Life-threatening bleeding in hemophilia includes events of intracranial and paratracheal hemorrhage usually associated with trauma. Risk of hemarthroses increases with age, and hemophilic arthropathy occurs in approximately half of patients with severe disease. Rarely, patients may have an unexpectedly mild course. These include cases of hemophilia B Leyden, in which a mutation in the F9 promoter causes severe hemophilia B in childhood, but factor IX levels increase to mild range after puberty, likely related to hormonal changes. In addition, some hemophiliacs who also have inherited thrombophilia, such as factor V Leiden, may have fewer bleeds.

The mainstay of treatment is intravenous factor replacement, usually with recombinant proteins, although in some countries purified plasma-derived products are still used. This can be given "in demand" as treatment for bleeding episodes or prophylactically in patients with a high risk for spontaneous bleeding. Prophylactic factor replacement has been shown to reduce joint damage in patients who exhibit hemarthroses. Maintaining a factor level greater than 1 IU/dL reduces frequency of hemarthroses in severe disease. The earlier the patient starts prophylaxis, both in terms of age and length of time after first bleed, the greater the benefit, and prophylaxis is often started at 2 years of age. Development of specific factor inhibitors, a risk for all patients with hemophilia, is discussed further in the next section.

For milder disease there are a few other options. Desmopressin acetate (DDAVP) induces release for factor VIII from endothelial storage sites and may be used to raise levels of factor VIII adequate for hemostasis in some cases of mild hemophilia A. Antifibrinolytics, such as tranexamic acid and ε aminocaproic acid, may also help with oral bleeding and epistaxis.

Other treatment strategies are currently being investigated, both for treatment of hemophilia A and as inhibitors of factor VIII. Emicizumab is an antibody that mimics factor VIIIa function by maintaining interaction between factors IXa and X, and it has shown efficacy for treatment of bleeding as well as low immunogenicity. Other strategies include inhibition of antithrombin production using small interfering RNA molecules and an antibody that inhibits TFPI activity. Gene therapy is also an attractive strategy, especially since even a small increase in factor levels can have a major impact on bleeding risk. Studies for gene therapy are actively recruiting.

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ACQUIRED HEMOPHILIA/SPECIFIC FACTOR INHIBITORS

Clinical Features

Antibodies against specific coagulation factors can develop both in hemophiliacs receiving factor replacement as well as spontaneously in non-hemophiliacs (referred to as "acquired hemophilia"). In both instances factor VIII is the most common target, although patients with hemophilia B less frequently develop anti-IX antibodies. These antibodies are usually IgG and neutralize the coagulant properties of the target factor and increase the risk of severe bleeding episodes.

Up to 30% of severe hemophiliacs will develop an inhibitor, usually within 20 to 30 days of first exposure to factor replacement. In patients with non-severe hemophilia B receiving on-demand factor therapy, the incidence increases with the number of exposures. Risk of inhibitor development is related to the type of *F8* mutation, with large deletions showing higher risk, as well as other polymorphisms in *HLA* and other immune system genes. People of African descent and non-white Hispanics show somewhat higher risk, as do patients receiving intensive or on-demand therapy compared to prophylactic therapy. It had been suggested that recombinant factor VIII therapy was related to higher risk of inhibitor development than plasma-derived forms; however, data are conflicting.

Cumulative incidence of developing a factor IX inhibitor in patients with hemophilia B is 4% to 5%. These usually occur more quickly, after only 9 to 11 exposure days. However, patients with non-severe hemophilia B rarely develop inhibitors. Anaphylactic reactions are a unique feature in those patients who develop inhibitors.

Acquired hemophilia has an incidence of 1 in 1.3 to 1.5 million and shows a bimodal distribution, with over half of cases occurring after age 60, with incidence increasing with age. In older patients, approximately half of cases have an associated underlying condition, most commonly with autoimmune disease (17% to 19% of cases), and malignancy (7% to 15%), as well as some drugs (e.g., phenytoin and interferon [IFN]) and graft-versus-host disease. The second smaller peak occurs in postpartum women, and it can occur up to several months after delivery. There appears to be a slight overrepresentation in Hispanic and African patients compared to the population. No correlation exists between severity of bleeding and factor VIII level or inhibitor titer. Subcutaneous and mucosal bleeding is most common, although retroperitoneal, muscular, and intraarticular bleeding can also occur. Life-threatening bleeding is a risk unless the antibody is eradicated.

Rare cases of inhibitors against factor V have also been reported. In the past, many of these cases were associated with use of bovine thrombin as an incisional sealant. Sporadic factor V inhibitors are also seen, with a suggestion of an association with autoimmune diseases, malignancy, and some classes of antibiotics. Both show variable risk of bleeding, although severe bleeding is usually confined to sporadic cases. Bovine-thrombin–associated cases may also have inhibitors against other coagulation factors.

Pathologic Features and Test Results

Factor VIII and IX inhibitors will cause a prolongation of the APTT and markedly decreased levels of the affected factor by clottable assays. APTT mixing studies may be corrected in the immediate phase but will show prolongation after 1 hour of incubation. PT, TT, and platelet count should be normal. Patients with hemophilia A are often monitored for inhibitor development. Occasional cases may be detected if there is inadequate factor recovery after factor replacement therapy. Bethesda assays will be positive.

Factor V inhibitors cause prolongation of both the PT and APTT and positive mixing studies for both. Factor V levels are decreased, and a factor V-specific Bethesda assay will be positive. TT will be normal unless anti-thrombin antibodies are also present.

Differential Diagnosis

Deficiencies of any of the intrinsic pathway factors will cause a prolonged APTT with normal PT, as will heparin. Lupus anticoagulants will also cause a prolonged APTT with normal PT and have positive APTT mixing studies. However, these will usually be prolonged in both the immediate and incubated phases, and phospholipid dependence tests will be positive.

Prognosis and Therapy

Treatment of factor inhibitors includes both episodic treatment for bleeding episodes as well as therapy to eradicate the inhibitor. Treatment of bleeding in patients with congenital hemophilia A with inhibitors is based on the nature of the antibody. Low-titer inhibitors can be overwhelmed by using excess factor replacement therapy, whereas high-titer inhibitors require use of bypassing agents such as recombinant factor VIIa or prothrombin complex concentrates (PCCs). Immune tolerance can be achieved using frequent injections of factor VIII concentrates and can be effective in up to 65% to 70% of patients.

In acquired hemophilia A, 30% of affected individuals will experience mild bleeding, and fatal hemorrhage can occur in 9% to 22% of patients. Bypassing agents are first-line therapy for bleeding events. Rarely, low-titer cases may be treated with high doses of factor VIII. Immunosuppressive therapy is used to eradicate the inhibitor. Traditionally, this has been using steroids with or without cyclophosphamide, with rituximab increasingly being added to the regimen.

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TABLE 2.5
Summary of Major Features of Specific Factor Deficiencies

Factor	Gene and Location	Incidence	Abnormal Tests	Bleeding Severity (Common Types)	Treatment
Prothrombin	*F2* (11p11)	1 in 2 million	PT, APTT	Mild to moderate (Mu, H, HA, Me)	PCC
V	*F5* (1q24.2)	1 in 1 million	PT, APTT	Moderate (U, Me, Mu, S)	FFP (or platelets)
V and VIII	*LMAN1* (18q21.3) *MCFD2* (2p21)	1 in 2 million	PT, APTT	Mild to moderate (B, E, D, Me)	FVIII and FFP
VII	*F7* (13q34)	1 in 500,000	PT	Mild to severe (B, E, Me)	FFP, rVIIa, PCC
X	*F10* (13q34)	1 in 1 million	PT, APTT	Mild to severe (U, E, Me, HA, H, S, T)	FFP, rVIIa, factor X
XI	*F11* (4q35.2)	1 in 500,000 (Ashkenazi Jews)	APTT	Mild to moderate (S, T, Me, D)	FFP
XIII	*F13A1* (6p25) *F13B* (1q31)	1 in 2 million	Factor XIII activity, clot lysis	Moderate to severe, often delayed (U, E, H, D, Me, S, T)	Cryo, FFP, factor XIII concentrates

APTT, Activated partial thromboplastin time; *B,* bruising; *cryo,* cryoprecipitate; *D,* post dental procedures; *E,* epistaxis; *FFP,* fresh frozen plasma; *H,* hematomas; *HA,* hemarthrosis; *Me,* menorrhagia; *Mu,* mucosal; *PCC,* prothrombin complex concentrate; *PT,* prothrombin time; *U,* umbilical stump; *rFVIIa,* recombinant factor VIIa; *S,* postsurgical; *T,* post trauma; *U,* umbilical stump.

Inhibitor eradication can be achieved in 50% to 80% of patients; however, 20% will relapse.

Recombinant porcine factor VIIIa can be used to treat bleeding episodes in both congenital hemophiliacs and acquired hemophilia. However, in both populations many patients will show baseline cross-reactivity to the protein, and more develop antibodies after therapy.

The investigational therapies previously described for use in hemophilia A (emicizumab, siRNA for antithrombin, and anti-TFPI antibodies) are also being studied for use in patients with factor VIIIa inhibitors.

OTHER FACTOR DEFICIENCIES

Inherited deficiencies of other clotting factors are generally rare, usually inherited in an autosomal-recessive manner, and include deficiencies of fibrin, factors II, V, V and VIII combined, VII, X, and XIII. The major features of specific factor deficiencies are listed in Table 2.5. Bleeding severity can vary among affected individuals and may not correlate with the amount of factor present. Deficiencies of factor XII, prekallikrein, and high-molecular-weight kininogen can also occur and present with a markedly prolonged APTT but do not have increased risk of bleeding. Factor XIII deficiencies are also often associated with miscarriage.

Fibrinogen abnormalities include type I defects, afibrinogenemia or hypofibrinogenemia, which are quantitative decreases in fibrinogen, and type II defects, dysfibrinogenemia or hypodysfibinogenemia, which are qualitative defects. These disorders are caused by mutations in *FGA*, *FGB,* and *FGG* genes on chromosome 4q31-q32. Afibrinogenemia will cause prolongation of TT, PT, and APTT, whereas hypofibrinogemia may cause prolongation of these tests. Afibrinogenemia can have variably severe bleeding, most commonly at the umbilical stump or mucosal sites. Patients may also have musculoskeletal or central nervous system bleeding and often exhibit poor wound healing. Patients with hypofibrinogenemia have milder bleeding. Dysfibrinogenemia may be asymptomatic or have mild bleeding but can also have thrombotic events. There will be a discordant decrease in clottable fibrinogen activity compared to antigenic fibrinogen levels. Reptilase time will be prolonged with defects in proteolysis of fibrinopeptide A, which are associated with bleeding risk. Defects in proteolysis of fibrinopeptide B do not confer bleeding risk. Replacement therapy for fibrinogen includes fibrinogen concentrates and cryoprecipitate.

Acquired deficiencies in factors can be seen in a variety of conditions. Liver disease affecting hepatic synthetic capability will cause decreased levels of all coagulation factors except factor VIII. Levels of multiple factors are decreased in disseminated intravascular coagulation (DIC; discussed later) and after massive transfusions. Vitamin K–dependent factors (factors II, VII, IX, and X and proteins C and S) are decreased with vitamin K deficiency, warfarin therapy, or rare inherited defects in the γ-glutamyl carboxylase pathway. Decreases in various factors, but most commonly factor X, can be seen in patients with amyloidosis.

VON WILLEBRAND DISEASE

Clinical Features

Von Willebrand disease is the most common autosomally inherited bleeding disorder, with an estimated incidence of symptomatic disease of 1 in 10,000. The disease is caused by deficiency (partial deficiency in type 1, near absence in

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type 3) or dysfunction (type 2) of von Willebrand factor. Bleeding is usually mucocutaneous, most commonly epistaxis, gum bleeding, and bruising, and can occur at any age. Menorrhagia and postpartum hemorrhage can also be seen in affected women. Severe bleeding can occur with surgery and dental procedures, and some patients, especially those with concurrent decreased factor VIII, may exhibit musculoskeletal or joint bleeds.

VWF is a protein involved in both primary and secondary hemostasis. In the former, VWF in the extracellular matrix binds to platelets via the GPIbα subunit of the GPIb/IX/V receptor. VWF monomers bind to each other via disulfide bonds to form multimers of up to 40 subunits. It is the high-molecular-weight (HMW) multimers that have the most function for binding platelets. In secondary hemostasis, VWF in the plasma serves as a carrier for factor VIII, which is quickly cleared from the plasma if not bound to VWF. VWF is produced in endothelial cells that secrete it into plasma as well as maintain additional stores within Weibel-Palade bodies that can be released upon stimulation. Megakaryocytes also synthesize VWF; it is present in platelet α-granules and secreted upon activation.

The *VWF* gene is present on chromosome 12, with partial non-functional duplication on chromosome 22. The presence of this pseudogene requires careful primer selection when performing amplification and sequencing assays. The gene is large (178 kB) and contains 52 exons. There are many single-nucleotide polymorphisms within the gene, some of which are more prevalent in certain ethnic groups, but which do not confer risk of bleeding. The D1472H (c.4414G>C) sequence variant, an A1 domain polymorphism of exon 28, is reported in 63% of African-American and 17% of healthy white individuals and associated with reduced ristocetin-dependent binding of VWF to GP1bα, leading to significant decrease in the ratio of VWF ristocetin cofactor (VWF:RCo) to VWF antigen (VWF:Ag) and misdiagnosis as type 2M VWD. However, this polymorphism is not associated with increased bleeding symptoms. Despite these confounding factors, sequencing can be used to confirm a diagnosis of VWD and distinguish type 2N VWD from hemophilia A.

The expression of VWF in the plasma is also affected by other genes besides *VWF*, most notably the ABO blood group genes. It is known that patients with blood type O have approximately 25% lower VWF levels than non-O. VWF levels can also vary if increased VWF is released in states of stress or exercise.

The basic assays used to interrogate VWF are immunologic VWF:Ag assays (by enzyme-linked immunosorbent assay [ELISA]) and assays for VWF activity, as well as quantitation of clottable factor VIII. The traditional method to measure VWF activity is the VWF:RCo assay. In this assay, formalin-fixed reagent platelets are mixed with platelet-poor patient plasma and ristocetin, an antibiotic that facilitates VWF binding to GPIbα. Aggregation of the reagent platelets is measured. Because HMW multimers are most efficient at binding platelets, deficiencies of HMW multimers will lead to decreased VWF:RCo. The VWF:RCo assay is widely available and the most commonly used assay to measure VWF function. However, it suffers from relatively poor precision. To improve measurement of VWF activity, functional ELISA tests for VWF binding to GPIbα and collagen have been developed. These involve coating microplate wells with either GPIbα or collagen (types 1 and 3). Patient plasma is added, and patient VWF binds to the immobilized target. After washing, bound VWF is measured using an anti-VWF antibody. Although the functional GPIbα-binding assay has shown discordant results and is not widely used, the collagen binding assay (VWF:CBA) shows greater precision and a better lower limit of detection than the VWF:RCo, and it is recommended to be added to the basic VWD panel. Addition of VWF:CBA to VWD panels has been shown to reduce the likelihood of missed diagnosis or inaccurate typing of VWD disease. Automated VWF activity assays based on ristocetin independent VWF binding to GPIbα are recently available and show a good correlation with traditional VWF:RCo assay. The size distribution of VWF multimers can be demonstrated by electrophoresis. Multimers are separated on sodium-dodecyl-sulfate agarose cells and visualized. The patient multimers can be compared to standards to detect loss of HWM and intermediate-molecular-weight (MW) multimers (Fig. 2.11A).

Type I VWD is the most common subtype, accounting for approximately 75% of cases; it is caused by decreased amounts of functionally normal VWF. Inheritance usually shows an autosomal-dominant pattern. In 65% of cases, mutations within the *VWF* gene can be found in the coding regions, promoter, and splice sites. The remaining patients without identifiable VWF mutations generally have milder disease. These cases may result from variants in introns or other genes. Type 1C VWD, considered a variant of type 1, is associated with increased clearance of VWF from the plasma and short plasma VWF half-life (<3 hours). Increased testing for this variant suggests that some cases originally considered type 1 but lacking *VWF* mutations may actually represent type 1C.

In type 3 VWD there is a severe decrease or absence of VWF. These cases are usually inherited in a recessive or codominant manner and are the least common type of VWD. These patients also suffer from a severe decrease in factor VIII (<10%). Over 80 mutations have been described, resulting in insertions, nonsense and missense mutations, and total or partial gene deletions.

Type 2A VWD is the second most common type after type 1 (10%) and the most common type 2 disorder. The ultimate manifestation is loss of the intermediate MW and HMW VWF multimers leading to disproportionate VWF activity compared to the amount of antigen detected. Mutations leading to type 2A VWD can alter dimer/multimer assembly, confer increased susceptibility to ADAMTS13 cleavage, and cause intracellular VWF sequestration.

Type 2B VWD results from gain-of-function mutations in GPIbα that lead to increased affinity of VWF for the platelet receptor. The resulting increase in VWF binding to platelets causes a decrease of HWM multimers in the plasma. Clearance of platelets bound to VWF HMW multimers may also lead to mild thrombocytopenia. Similar to type 2A, there is a decrease in VWF activity compared to VWF antigens present.

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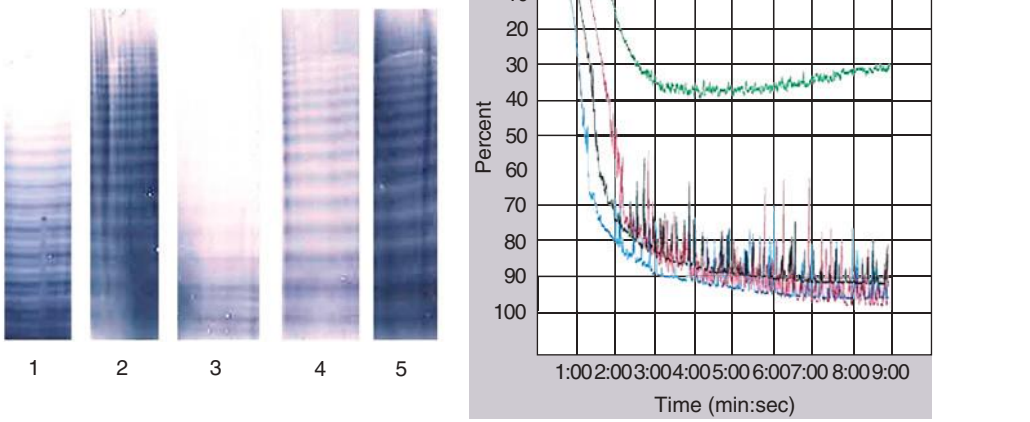


FIG. 2.11

A, Von Willebrand factor multimer analysis. 1: Type 2B showing loss of high-molecular-weight multimers; 2 and 5: normal controls; 3: type2A showing loss of high- and intermediate-molecular-weight multimer; 4: type 1 or 2M showing normal pattern of multimers with decreased amount compared to control. B, Ristocetin-induced platelet aggregation showing increased aggregation at the lowest concentration of ristocetin (0.6 mg/mL) in type 2B patient (multimer; A-1).

Type 2M VWD has a decrease-of-function mutation involving either the GPIbα binding site or collagen binding sites in A3 and A1 domains of the VWF molecule. This causes a relative decrease in VWF activity compared to VWF antigen levels.

Type 2N VWD results from point mutations that inhibit VWF from binding to factor VIII. As a result, there may be normal levels of VWF:Ag but decreased factor VIII, which can be difficult to distinguish from hemophilia A. Family studies may be helpful in distinguishing between these possibilities because type 2N VWD is inherited in an autosomal-recessive manner, whereas hemophilia is X-linked.

An acquired form of VWD can develop in association with a variety of medical conditions and via several different mechanisms. The condition usually occurs later in life (median age 62 years). Approximately 50% of cases have been reported as associated with lymphoproliferative or plasma cell disorders. In these patients, as well as in some patients with autoimmune disease, antibodies develop that inhibit and/or increase clearance of VWF. Other mechanisms for the development of acquired VWD include decreased VWF production (hypothyroidism, valproate therapy), mechanical destruction of HMW multimers (aortic stenosis, artificial values, left ventricular assist devices), and adsorption of HMW multimers onto platelets (essential thrombocythemia, other thrombophilia) or other malignant tumors.

Pathologic Features and Test Results

Normally the ratio of VWF:RCo or VWF:CBA to VWF:Ag should be 0.6 or greater, although this may vary among assays, and this cutoff should be determined individually for each laboratory. Types 1 and 3 VWD should show proportional

VON WILLEBRAND DISEASE—FACT SHEET

Definition
- Bleeding disorder caused by decreased (type 1), absent (type 3), or dysfunctional (types 2) von Willebrand factor

Incidence
- 1 : 10,000 symptomatic patients

Morbidity
- Predominantly mild mucocutaneous bleeding
- Potential for severe surgical or gastrointestinal bleeding
- Postpartum hemorrhage requiring transfusion
- Severe case may develop hematomas or hemarthroses with resulting joint damage

Gender, Race, and Age Distribution
- There is no specific gender or racial predilection for the disease. Patients present at variable ages, usually depending on the severity of the disease

Clinical Features
- Easy bruising, epistaxis, and gum bleeding are the most common manifestations
- Menorrhagia is common and may be the presenting symptom in some women
- Increased risk for bleeding during surgical or dental procedures
- Rarely, severe gastrointestinal bleeding
- Severe cases, especially with decreased factor VIII may show spontaneous bleeding into muscles and joints, similar to what is seen in hemophiliacs

Prognosis and Treatment
- Patients rarely experience life-threatening bleeding
- DDAVP can be used in some patients to temporarily increase plasma VWF concentrations
- Factor VIII/VWF concentrates may be required for some bleeding episodes and are increasingly being used prophylactically

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decreases in both VWF:Ag and activity (VWF:RCo and/or VWF:CBA). Given the variability of VWF expression owing to variants in other loci, most notable the ABO blood groups, the National Institutes of Health issued guidelines recommending that both VWF:Ag and activity level of less than 30 IU/dL should be used to define type 1 VWD levels. Patients with VWF:Ag and activity level of below the normal range but greater than 30 IU/dL should be noted, as these patients may have increased risk of bleeding, and repeat testing may be indicated if there is a high suspicion for VWD.

Type 2 VWD, with the exception of type 2N, will usually show a decrease in the VWF:RCo (or VWF:CBA)/VWF:Ag ratio, reflecting the dysfunction of the VWF molecules present in the plasma. Type 2A and 2B show a decrease of HMW multimers on electrophoresis, whereas the distribution pattern of multimers in 2M and 2N is normal. Type 2A should also show proportional loss of intermediate MW multimers, but this may be subtle. The ristocetin-induced platelet aggregation assay is useful for distinguishing 2B from 2A (see Fig. 2.11B). This assay uses several concentrations of ristocetin, including much lower levels of ristocetin than VWF:RCo, concentrations that would not normally induce significant platelet aggregation. In type 2B VWD, because of the increased affinity of the defective VWF, there will be abnormally increased platelet aggregation in lower levels of ristocetin, whereas type 2A shows minimal levels of aggregation similar to those in healthy patients. Type 2B is also unique among VWD in that it is often accompanied by thrombocytopenia.

Type 2M VWD will show an abnormally low antigen/activity ratio and a normal response to the RIPA. The distinguishing feature from type 2A VWD is the maintenance of a normal multimer distribution. In type 2N VWD, a specific diagnosis can be made using an ELISA, which measures VWF binding to factor VIII, which will be decreased in type 2N but not other VWD or hemophilia A.

A variant type, 1C, shows an abnormal ratio of VWF propeptide (VWFpp) to VWF:Ag. VWFpp is released during processing of a mature VWF monomer, so increased VWFpp/VWF:Ag indicates increased clearance of VWF multimers from the circulation.

Acquired VWD can show different patterns according to associated mechanisms and will usually show laboratory results suggestive of a type 2 VWD with reduction in VWF activity/antigen ratios and loss of HMW multimers. However, acquired VWD related to decreased VWF production, such as hypothyroidism patients, can mimic type 1 VWD.

VON WILLEBRAND DISEASE—PATHOLOGIC FEATURES

- Normal platelet count, except in some type 2B
- VWF test results for the major subtypes are summarized in Table 2.6

Differential Diagnosis

The tendency toward mucocutaneous bleeding is clinically similar to what is seen in platelet defects. Platelet count should be normal in VWD (with the exception of some type 2B) as would platelet aggregometry and flow cytometry. Both platelet disorders and VWD can show abnormal closure times on the PFA-100, and this assay is sometimes used as a screen for

TABLE 2.6
Summary of Laboratory Abnormalities in von Willebrand Disease

	Type 1	Type 2A	Type 2B	Type 2M	Type 2N	Type 3
VWF:Ag	↓	↓	↓	↓	N or ↓	↓↓↓
VWF:RCo	↓	↓↓	↓↓	↓↓	N or ↓	↓↓↓
VWF:RCo/VWF:Ag	≥0.6[a]	<0.6	<0.6	<0.6	≥0.6	≥0.6
Factor VIII (clottable)	N or ↓	N or ↓	N or ↓	N or ↓	↓↓	↓↓↓
Platelet count	N	N	N or ↓	↓↓	N	N
Multimer distribution pattern	N	Loss of high and intermediate MW multimers	Loss of high MW multimers	N	N	Absent
RIPA	↓ or N	↓	↑ in lowest level of ristocetin	↓ or N	↓ or N	↓↓↓
Other					Decreased VWF:factor VIII-binding capacity	

[a]The 0.6 cutoff for antigen-to-activity ratio is commonly used; however, the appropriate ratio should be determined in each laboratory.
↑, Increased; *↓,* decreased; *MW*, molecular weight; *N,* normal; *RIPA,* ristocetin-induced platelet aggregation; *VWF:Ag,* antigen assay; *VWF:RCo,* ristocetin cofactor assay.

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these disorders, although it cannot reliably differentiate VWD from platelet dysfunction. The basic panel of VWF:Ag, VWF:RCo, VWF:CBA, and clottable factor VIII should show some abnormality in nearly all cases of VWD.

As mentioned before, type 3 and 2N VWD may mimic hemophilia, but the basic VWD panel will be markedly abnormal in type 3, and the pattern of inheritance in type 2N would prompt additional studies such as VWF:FVIII binding tests.

Platelet-type VWD is similar to type 2B in that it causes increased affinity between VWF and the GPIb/IX/V receptor; however, in platelet-type VWD the defect is caused by a mutation in the GPIbα subunit. The pattern of test results will be identical to type 2B using standard assays. Sequencing or ristocetin independent VWF-GP1b binding assay may be required to distinguish between these possibilities.

Acquired VWD is usually distinguished from inherited VWD by clinical factors including the spontaneous development of a bleeding disorder later in life or the presence of medical conditions known to be associated with acquired VWD.

Prognosis and Therapy

In some patients, usually type 1, DDAVP administration can be used to induce release of VWF stored in endothelial cells and temporarily increase circulating VWF, either as treatment for bleeding or in preparation for a procedure. DDAVP is contraindicated in patients with severe renal impairment and also in type 2B VWD because it can induce platelet aggregation. Patients may undergo a DDAVP "challenge" in which VWF levels are measured before and after administration to see if they respond.

Patients with severe bleeding or requiring surgery with high risk of hemorrhage usually require therapy with factor VIII–containing VWF concentrates (factor VIII/VWF). Increasingly, these concentrates are also being used prophylactically in patients with a history of severe bleeding who are unresponsive to DDAVP or in those in whom DDAVP is contraindicated. There is far less experience with prophylactic factor replacement in VWD compared to hemophilia, and VWF levels are not as predictive of bleeding risk as they are in hemophilia. However, increasing evidence indicates that prophylactic regimens of factor VIII/VWF reduce bleeding rates, decrease numbers of transfusions required, and prevent arthropathy when started in young patients. Also, despite the fact that VWF and factor VIII increase throughout a pregnancy, women with VWD are susceptible to postpartum hemorrhage that may require transfusion within 24 to 28 hours of delivery. These patients may be treated with prophylactic factor VIII/VWF for 3 to 5 days

Treatment of acquired VWD focuses on eliminating the underlying cause, although patients may require temporary factor replacement or DDAVP for bleeding episodes or surgical prophylaxis. For patients with lymphoproliferative disorders or plasma cell dyscrasias, treatment of these conditions leads to remission in 70% of cases. Intravenous immunoglobulin can be used to treat cases associated with autoimmune disease or lymphoproliferative disorder. Disease will resolve in most patients with acquired VWD owing to high shear stress, hypothyroidism, drug reaction, or thrombocytosis will resolve when the underlying cause is removed.

DISSEMINATED INTRAVASCULAR COAGULATION

Clinical Features

Disseminated intravascular coagulation (DIC) refers to a syndrome in which there is widespread activation of the coagulation system. As a result, there is both fibrin deposition in multiple organs, leading to their dysfunction, and consumption of coagulation proteins and platelets that can lead to hemorrhage. Fibrin deposition is exacerbated by concurrent dysfunction in the fibrinolytic system. DIC can occur either as an acute (decompensated), life-threatening disorder caused by rapid activation of the coagulation system with a resultant catastrophic decrease in coagulation proteins or have a more chronic course in which the body is able to compensate for ongoing coagulation and fibrinolysis. The latter may be asymptomatic and present only with persistent laboratory abnormalities.

The major causes of DIC include sepsis or severe infection, burns and trauma (including fat embolism), organ destruction (e.g., acute pancreatitis), malignancy, both solid tumors and hematolymphoid, obstetric complications (amniotic fluid embolism, abruptio placentae, preeclampsia/eclampsia), vascular abnormalities (Kasabach-Merritt syndrome, large aneurysms), severe liver failure, severe toxicity (e.g., snake bite), and immunologic reactions (transfusion reaction, transplant rejection). Purpura fulminans is a life-threatening manifestation of DIC that occurs in patients with inherited or acquired (as in meningococcal sepsis) protein C deficiency. There is extensive thrombosis with resulting hemorrhagic necrosis of the skin.

Pathologic Features and Test Results

In most settings, DIC is diagnosed using a combination of results from commonly available assays including the platelet count, PT, fibrinogen, and D-dimer. Since none of these assays is specific for DIC, the results must be interpreted together and within the clinical context. Additionally, fibrinogen is an acute phase reactant and may not be decreased in some cases. The ISTH developed a scoring system for "overt" (acute) DIC. The first decision point is whether or not the patient has an underlying disorder associated with DIC. If not, the algorithm should not be used. If the patient does have a DIC-associated condition, then the assay results are scored

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TABLE 2.7
ISTH Scoring System for Overt DIC

	0	1	2	3
Platelet count ($\times 10^9$/L)	>100	<100	<50	—
FDP or D-dimer	No increase	—	Moderate increase	Strong increase
Prolonged PT (sec)	<3	>3 but <6	>6	—
Fibrinogen (mg/dL)	>100	<100	—	—

FDP, Fibrinogen degradation product; *DIC,* disseminated intravascular coagulation; *ISTH,* International Society for Thrombosis and Hemostasis; *PT,* prothrombin time.

ACUTE DISSEMINATED INTRAVASCULAR COAGULATION—FACT SHEET

Definition
- Condition of simultaneous activation of the coagulation and fibrinolytic system leading to thrombin formation and coagulation protein consumption

Incidence
- Described as occurring in 1% of admissions to Japanese tertiary care centers
- Up to 7% of solid tumors
- 0.03% to 0.35% of pregnancies
- Over 80% of bacterial sepsis with systemic inflammatory response syndrome
- 30% to 40% of trauma

Morbidity
- Multiorgan system failure with potential for long-term organ dysfunction
- Uncontrollable bleeding
- Purpura fulminans in inherited or acquired protein C deficiency
- Venous thromboembolism (more common in chronic DIC)

Gender, Race, and Age Distribution
- No predilection

Clinical Features
- Petechiae and ecchymoses
- Bleeding including oozing around catheter and drain sites
- Concomitant microvascular thrombosis; less often large vessel thrombosis
- Evidence of organ failure, most commonly the kidneys, then liver and lungs
- Shock

Prognosis and Treatment
- Prognosis is related to the underlying disorder
- 40% to 80% mortality in cases associated with severe sepsis or trauma
- Treatment is centered on the underlying disorder
- Supportive therapy
- Transfusion to control bleeding

DISSEMINATED INTRAVASCULAR COAGULATION—PATHOLOGIC FEATURES

Complete Blood Count and Peripheral Smear Findings
- Thrombocytopenia (usually <100 × 10^9/L or rapidly developing)
- Fragmented red blood cells (schistocytes) in a subset of cases

Coagulation Assays
- Prolonged PT and APTT
- Decreased factor levels except acute phase reactants (factor VIII and potentially fibrinogen)

Markers of Fibrin Degradation
- Increased fibrin degradation products
- Increased D-dimer (specific for degradation of cross-linked fibrin rather than fibrinogen degradation)

Decreased Levels of Natural Anticoagulants
- Decreased protein C and antithrombin

Differential Diagnosis
- Thrombotic microangiopathy (TTP/HUS)
- Heparin-induced thrombocytopenia
- Liver dysfunction or vitamin K deficiency
- Acute thrombosis
- Massive hemorrhage

according to the scale in Table 2.7. If the score is 5 or greater, the findings are consistent with overt DIC and should be repeated daily to monitor disease. If the score is less than 5, it is suggestive but not definite for chronic DIC, and the score should be repeated once or twice.

Other abnormalities can be seen, but the assays are not as widely available. These include decreases in natural anticoagulants, including antithrombin and protein C. AT is the major inhibitor of thrombin and is quickly reduced during sustained periods of thrombin generation. Production of AT is decreased during the acute phase response, and AT is

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proteolyzed by neutrophil-elastase. Low protein C, also an indicator of DIC, is also seen in most cases of severe sepsis. The protein C pathway forms a linkage between the coagulation and inflammation pathways. Inflammation decreases thrombomodulin (TM) expression on endothelial cells. This prevents the normal TM-mediated activation of protein C and interferes with other anticoagulant functions of TM. The relative decrease of fibrinogen versus plasminogen may indicate the relative activation of the coagulation versus fibrinolysis system. Thromboelastography has also been used in conjunction with other coagulation assays. This has the advantage of providing information on both the procoagulant and fibrinolytic state of the plasma.

Differential Diagnosis

Thrombotic microangiopathy as seen in thrombotic thrombocytopenic purpura (TTP)/hemolytic uremic syndrome (HUS) will have schistocytes on the peripheral smear, and renal dysfunction and neurologic symptoms can be seen in both TTP/HUS and DIC. However, in TTP/HUS, PT and APTT are normal. A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) may be decreased, and there may be fever. Heparin-induced thrombocytopenia (HIT) usually shows only isolated thrombocytopenia, with normal coagulation assays. The timing relative to heparin administration and positive antiplatelet factor 4 antibody by ELISA distinguish HIT from DIC. Hepatic insufficiency can lead to prolongation of both PT and APTT and decreased levels of multiple factors. However, factor VIII level is typically normal to elevated because it is synthesized in the endothelial cells, not the liver. In these cases, hepatic enzymes aspartate aminotransferase/adenosine triphosphate (AST/ALT) will often be abnormal, bilirubin will be increased, and there will be hypoalbuminemia. In acute thrombosis or massive hemorrhage, clinical features, imaging studies, low factor levels, thrombocytopenia, prolonged PT and APTT, and decreased fibrinogen but normal to elevated D-dimer will be helpful to diagnosis.

Prognosis and Therapy

Because DIC is an intermediary mechanism of many different diseases, the prognosis is mainly related to the underlying disease. Definitive treatment is aimed at the underlying cause of the disorder with adjunctive supportive care. In severe cases, other interventions are necessary. Patients with active hemorrhage or those who are about to undergo interventions with high risk of hemorrhage may benefit from transfusions of platelets, plasma, or cryoprecipitate. Heparin is used to treat thrombosis in patients with chronic DIC. However, use of anticoagulants outside of this setting is controversial.

FIBRINOLYTIC DISORDERS

Clinical Features

As with the coagulation system, perturbations of the fibrinolytic system can lead to either bleeding or thrombosis, depending on where in the system the defect lies. Increased activity of plasmin or decreased fibrinolytic inhibitors can lead to excess bleeding, whereas deficiencies of plasmin or its activators or increased activity of fibrinolytic inhibitors can lead to thrombosis. Although heritable deficiencies of the fibrinolytic system are relatively rare, acquired imbalances of the system can be seen in a variety of disorders.

Deficiency of plasminogen activator inhibitor-1 (PAI-1) is a very rare condition that can lead to mild to moderate bleeding. Both quantitative and qualitative defects have been described. In cases with identifiable mutations, increased bleeding is only seen in homozygotes. Bleeding is often delayed and usually occurs after surgery or trauma; spontaneous bleeds do not usually occur. Some patients may also exhibit delayed wound healing.

Inherited α_2-antiplasmin deficiency is a rare disorder. In homozygotes, bleeding may be severe and can present early in childhood, often first manifesting as umbilical stump bleeding. Bleeding occurs after surgery, trauma, or dental procedures and is often delayed. Rarely, children may develop intramedullary bleeding into the diaphysis of long bones, causing pain and lucent lesions. Heterozygotes may be asymptomatic or have mild bleeding.

Acquired α_2-antiplasmin deficiency has been reported in cases of acute promyelocytic leukemia with t(15;17)(q22;q12); PML-RARA (APL), systemic amyloidosis, severe liver dysfunction, and renal disease. Thrombin-activatable fibrinolysis inhibitor may also be decreased in liver disease; however, any tendency toward increased fibrinolysis may be offset by concurrent increased levels of PAI-1 and decreased plasminogen.

Pathologic Features and Test Results

Results of screening tests, such as PT, APTT, and TT, may or may not be abnormal in fibrinolytic disorders. Thromboelastography can be used as a global measurement of both procoagulant and fibrinolytic function. This test may be more widely available and/or have a shorter turn-around time than specific tests for PAI-1 and α_2-antiplasmin. However this is more of a global screen and cannot provide information about specific fibrinolytic disorders. Quantitative PAI-1 disorders are detectable using measurement of the antigen by ELISA. However, it is difficult to diagnose qualitative defects since available PAI-1 assays have low sensitivity for small quantities of PAI-1, and reference ranges often include zero.

α_2-antiplasmin deficiency can similarly be detected using assays for α_2-antiplasmin antigen and activity. As with other

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coagulation protein disorders, qualitative defects show normal antigen levels with decreased activity, whereas quantitative disorders show decreased levels of both antigen and activity. The euglobulin lysis time may also be shortened. This test involves cold precipitating the "euglobulin fraction" from acidified plasma. The precipitate contains plasminogen, tissue plasminogen activator, fibrinogen, and most of the α_2-antiplasmin. The precipitate is re-suspended in buffer and clotted with thrombin, and time to clot lysis is recorded.

Differential Diagnosis

Factor XIII deficiency has similar clinical findings to hyperfibrinolytic disorders in that it causes delayed bleeding. As with hyperfibrinolytic disorders, PT, APTT, and TT are usually normal. However, factor XIII deficiency is characterized by decreased factor XIII levels and abnormal urea clot stability in severe cases. Hyperfibrinolysis is a component of DIC. However, isolated hyperfibrinolysis does not show decreased antithrombin or thrombocytopenia.

Prognosis and Therapy

Fibrinolytic inhibitors, tranexamic acid, and ε-aminocaproic acid are the most commonly used treatments for bleeding in both inherited and acquired fibrinolytic disorders. These function by preventing binding of plasminogen to fibrin. The drugs can be given on demand or as prophylaxis for surgical or dental procedures. Fresh frozen plasma can also be given, but fibrinolytic inhibitors are preferred. The lucent bone lesions resulting from intramedullary bleeding in α_2-antiplasmin deficiency can be treated with surgical evacuation and topical tranexamic acid and fibrin.

Acquired hyperfibrinolysis may increase morbidity in patients with other disorders. Hyperfibrinolysis detected by thromboelastography has been shown to be an independent predictor of mortality of trauma patients and has also been associated with need for massive transfusion.

THROMBOPHILIC DISORDERS

ANTIPHOSPHOLIPID SYNDROME

Clinical Features

Antiphospholipid syndrome (APS), the most common cause of acquired thrombophilia, is a noninflammatory autoimmune disease defined by the persistent presence of antiphospholipid antibodies (APAs) in the plasma. The APAs are autoantibodies directed against phospholipid–protein complexes and associated with increased risk of both arterial and venous thrombosis as well as recurrent pregnancy loss. APAs can arise spontaneously (primary) or in association with other conditions such as autoimmune disorders (secondary). There are two main types of APAs: lupus anticoagulants (LAC) and antibodies against anticardiolipin (ACA)/β2 glycoprotein 1 (B2GP1). However, LACs are extremely heterogeneous and directed against a wide variety of target proteins with affinity for anionic phospholipids. Antibodies against B2GP1 are the primary antigenic target and are associated with higher risk of thrombosis than antibodies against ACA, prothrombin, protein C (PC), protein S (PS), or annexin V.

Although there is a strong association between APS and thrombotic complications, the pathogenetic mechanism between the two remains unclear. Recent studies have shown evidence that B2GP1 binds to anionic phospholipid membranes and inhibits VWF adhesion to platelets. Antibodies against B2GP1 may exert a prothrombotic effect by neutralizing B2GP1 inhibition of VWF adhesion to platelets and activating platelets, monocytes, endothelial cells, and/or complement. Other potential mechanisms of thrombophilia in APS include inhibition of anticoagulant activity of PC or PS, or disruption of annexin V on phospholipid surfaces, leading to binding to the phospholipid membrane and formation of procoagulant complexes.

APS is associated with a wide spectrum of clinical manifestations from asymptomatic thrombocytopenia to life-threatening catastrophic APS. Early recognition of APS is important because treatment can reduce mortality and morbidity. Diagnostic criteria have been developed to use as a tool for making a correct diagnosis and include both clinical and laboratory criteria; at least one of each is required for APS diagnosis. Two clinical hallmarks of APS include proven venous, arterial, or small vessel thrombosis and pregnancy morbidity. VTE, the most frequent form, is most often located in deep veins of the lower extremities in up to 50% of patients with APS and then followed by pulmonary embolism (PE). Arterial thrombosis is less common, but if the cerebral circulation is involved, this may lead to stroke or transient ischemic attack. The most common pregnancy complication is recurrent miscarriage before 10 weeks of gestation; however, unexplained late fetal deaths, intrauterine growth restriction, or premature births are also related to APS. Other clinical manifestations of APS that are not part of the diagnostic criteria include thrombocytopenia, arthritis, livedo reticularis, migraine, heart valve thickening, autoimmune hemolytic anemia, epilepsy, pseudovasculitic lesions, cardiomyopathy, renal involvement, pulmonary hypertension, and rarely chorea or cerebellar ataxia. Less than 1% of patients with APS can develop life-threatening catastrophic APS, which is characterized by rapid occurrence of microvascular thrombosis, multiorgan involvement, and systemic inflammatory response to ischemic tissue necrosis. Approximately 12% of patients with thrombosis and 10% to 15% of patients with recurrent fetal losses were found to have APS. APS is reported in up to 50% of cases of systemic lupus erythematosus (SLE) and 5% to 20% of other autoimmune disorders. Paradoxically,

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ANTIPHOSPHOLIPID SYNDROME—FACT SHEET

Definition
- Antiphospholipid syndrome is an acquired autoimmune disease defined by the persistent presence of antiphospholipid antibodies against phospholipid–protein complexes causing thrombophilia

Prevalence
- 1% to 5% in healthy general population
- Incidence: new approximately 5 patients/100,000 persons/year
- 12% of thrombosis and 10% to 15% of patients with recurrent miscarriage

Morbidity and Mortality
- May be associated with thrombosis and pregnancy mortality

Gender, Race, or Age Distribution
- Affects females more commonly than males
- Generally affects young and middle-aged adults

Clinical Features
- Venous, arterial and small vessel thrombosis, commonly in deep veins of the lower extremities, PE, and less often, but life-threatening involvement in the cerebral circulation
- Pregnancy mortality including recurrent early or a single late (beyond 10 weeks) pregnancy loss or premature birth
- Thrombocytopenia in 30% to 40% of patients with APS
- Autoimmune hemolytic anemia in approximately 10% of patients with APS

Prognosis and Treatment
- One third of antiphospholipid antibody–positive patients will develop thrombotic events
- Higher risk of thrombosis or PE in patients with two or three types of APAs (LAC, anticardiolipin, and/or B2GP1 antibodies)
- Long-term anticoagulation with a vitamin K antagonist (warfarin) is the mainstay of therapy for thrombotic APS

ANTIPHOSPHOLIPID SYNDROME—PATHOLOGIC FEATURES

Complete Blood Cell Count and Peripheral Smear Morphology
- Often thrombocytopenia
- Autoimmune hemolytic anemia

Bone Marrow Findings
- Not essential for the diagnosis; however, bone marrow findings may be helpful to differentiate from ITP

Platelet Function Tests
- Not helpful

Ancillary Studies
- Prolonged APTT
- APTT mixing study; usually prolonged at both immediate and delayed phase
- Phospholipid-dependent confirmatory test (DRVVT, hexagonal phase phospholipid test, platelet neutralization test)
- Factor assays to rule out deficiency or inhibitors; heparin or anti-Xa assay to exclude heparin or other Xa inhibitor effects

Differential Diagnosis
- Many clinical conditions causing transient APS
- ITP
- Autoimmune hemolytic anemia
- Drug effect, such as heparin, direct thrombin inhibitors (DTIs), and anti-Xa

LACs prolong clot-based assays in vitro while predisposing to high risk of thrombosis in vivo. Approximately one-third of patients who are APA positive will eventually develop thrombotic events. High titer of ACAs, older age, or patients with SLE have an increased risk of thrombosis. Although rare, bleeding can also occur in patients with severe thrombocytopenia. Some patients with infections, malignancy, autoimmune disorders, or receiving certain medications may be positive for APAs; however, these are usually transient and do not increase the risk of thrombosis.

Pathologic Features and Laboratory Studies

Thrombocytopenia is the most common finding occurring in 30% to 40% of APS cases. The platelet counts are usually greater than $50 \times 10^3/\mu L$ without clinical manifestation or therapy requirement. Interestingly, up to 20% of APS cases can be initially diagnosed as immune thrombocytopenic purpura (ITP). The mechanism of thrombocytopenia in APS cases is not completely understood; it may be binding of APA to platelet phospholipids or inducing platelet activation or aggregation, leading to platelet destruction. Another possible explanation is related to immune-mediated thrombocytopenia with the presence of autoantibodies directed against various platelet membrane glycoproteins.

Other hematologic manifestations associated with APS are autoimmune hemolytic anemia (AIHA), bone marrow (BM) necrosis, thrombotic microangiopathy including TTP, HUS, or HELLP (*h*emolysis, *e*levated *l*iver enzymes, and *l*ow *p*latelet) syndrome. However, the pathogenic role remains undetermined. AIHA has been reported in 6% to 10% of APS cases, and APAs may play a role in the destruction of red cells. Many studies demonstrated a strong association with AIHA, particularly in patients with SLE, high frequency of IgG ACA, and high risk of venous thromboembolic disease. BM necrosis has rarely been reported in association with APS, particularly in the context of catastrophic APS with systemic thrombosis. Patients presenting with thrombotic microangiopathy and demonstrable APA have been reported.

Laboratory diagnostic criteria include positive testing in plasma for one of the following on two or more occasions, at least 12 weeks apart: (1) LAC; (2) ACA (IgG or IgM) in medium or high titer; or (3) B2GP1 antibodies (IgG or IgM) in medium or high titer. A repeated positive test after at least

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a 12-week interval is required for diagnosis because transient low-level increases in APA occur in a variety of clinical conditions, including acute phase response, and may not confer increased risk of thrombosis.

Lupus Anticoagulant Testing

The consensus criteria from the ISTH require the following four criteria to confirm the presence of LACs. First, there should be prolongation of at least one phospholipid-dependent clotting test (e.g., APTT, diluted Russell viper venom time [DRVVT] screen, or hexagonal phase phospholipid neutralization screen). The assays are usually performed with low concentrations of phospholipid to improve sensitivity. Second, there is evidence of inhibitory activity in the patient plasma demonstrated by mixing patient plasma with normal pooled plasma (NPP; i.e., immediate and incubated APTT mixing study or DRVVT mixing study). Third, phospholipid dependence of the inhibitor should be demonstrated by the shortening of clotting time after addition of more phospholipid (e.g., DRVVT confirmatory ratio, hexagonal phase phospholipid neutralization ratio, or platelet neutralization). Fourth, the presence of specific factor inhibitors (especially factor VIII inhibitors) and anticoagulant drugs (heparin, direct factor Xa inhibitor, or DTI) should be excluded. Although many different guidelines aim to standardize methodologies and improve the quality of LAC testing, no single test provides high sensitivity in detecting LACs and identifying LACs remains a diagnostic challenge. Therefore, at least two different sensitive screening tests using different assay principles are recommended to maximize diagnostic potential. If these screening tests are positive, mixing and confirmatory tests are performed using the same assays.

The 2014 Clinical and Laboratory Standards Institute (CLSI) guideline for LAC recommends screening, and confirmatory assays have priority over mixing studies because of false correction of mixing study in some LAC cases. APTT and DRVVT-based assays are preferred. DRVVT is more specific for detection of high risk of thrombosis in patients with LAC and is considered the most robust test to detect LACs. Subsequent immediate and incubated APTT mixing study can differentiate factor(s) deficiency with corrected results and the presence of non-specific (i.e., LAC, heparin, or paraproteins) or specific inhibitor (i.e., factor inhibitors or DTI) with no or partially corrected results. LAC typically does not correct APTT in an immediate mixing study after a 1 : 1 mixing of the patient's plasma and NPP; however, up to 15% of LAC can be time dependent. Criteria for "correction" or "no correction" are validated in a laboratory using sensitive normalized ratio in each mixing test assay. If fewer than four diagnostic criteria are met but clinical suspicion for LAC exists, the results can be interpreted as "indeterminate" and should be repeated at a later date.

LACs, particularly with weak positive results, can be transient, and clinical conditions associated with transient LACs should be excluded before making a diagnosis of APS. Acute thrombotic events or acute phase responses with elevated factor VIII can cause false-negative results. The platelet-poor plasma utilized for LAC testing should have a platelet count less than $10 \times 10^3/\mu L$ because residual platelets or microparticles can absorb and neutralize LACs. The presence of anticoagulants or specific factor inhibitor can lead to false-positive results. It is important to know that the presence of strong LAC in the plasma can affect factor assays, and the presence of specific coagulation factor inhibitor, most commonly factor VIII and second most commonly factor XI, can affect LAC assays. Rare patients can have simultaneous LAC and specific factor inhibitor. Factor assays using APTT reagent that is insensitive to LAC can resolve this problem. Nonparallelism in multiple APTT-based factor assays may demonstrate laboratory artifacts induced by LAC in the plasma. Thrombin time and anti-Xa assays can help to identify anticoagulant effect such as heparin, warfarin, DTI, or factor Xa inhibitors or specific inhibitors such as factor VIII inhibitor. Commercially available heparin neutralizers can quench heparin concentrations up to 1.0 U/mL; however, similar reagents are not available for DTIs or specific factor Xa inhibitors. Therefore, LAC testing should not be performed on individuals taking these drugs. Individuals on long-term vitamin K antagonist therapy should be tested at least 1 to 2 weeks after discontinuation of therapy and after the international normalized ratio (INR) has normalized to less than 1.5.

Anticardiolipin Antibody and β2 Glycoprotein 1 Antibody Testing

Anticardiolipin antibody recognizes a complex of cardiolipin, a naturally occurring phospholipid bound to B2GP1 protein. Specific antibodies against cardiolipin and B2GP1 (IgG or IgM) are measured by commercially available solid-phase ELISA. Assays for ACA are generally considered sensitive; however, because the antigen target of ACAs is a B2GP1-cardiolipin complex, B2GP1 antibody assays are considered more specific than ACA assays. It is recommended that both ACA and B2GP1 antibody assays be performed to maximize sensitivity and specificity. Current guidelines include only medium and high levels of antibodies (99th percentile or >40 IgG or IgM phospholipid units, but not IgA) as diagnostic criteria to improve the test specificity. IgG B2GP1 (domain 1) antibody is more strongly associated with thrombosis, whereas lower titers of IgM antibody may not be associated with thrombosis. Positive tests should be repeated after an interval of at least 12 weeks because transient APAs can occur in relation to infection or drugs; these transient APAs are not associated with thrombotic risk. Additionally, false-positive results for ACAs can be associated with a high level of rheumatoid factor and cryoglobulins.

Differential Diagnosis

The diagnosis of APS requires clinicopathologic correlation because both clinical (either vascular thrombosis or pregnancy

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morbidity) and laboratory criteria must be met. Many clinical conditions are associated with transient LACs, such as malignancy, infection or inflammation with significant elevation in C-reactive protein, immune-mediated PS deficiency, end-stage renal disease, autoimmune disorders including SLE (25%), rheumatoid arthritis or Sjögren syndrome, and drugs (e.g., procainamide, phenytoin, or hydralazine). Although bleeding, not clotting, is associated, immune-mediated factor II or factor X deficiency can cause transient LACs. Excluding clinical conditions causing transient LACs and the confirmatory test for the persistent presence of LACs should be performed at an interval of at least 12 weeks. Thrombocytopenia is commonly observed in patients with APS. Some APS patients can be initially diagnosed as ITP. However, there are conflicting data whether ITP patients with persistent APA or LAC have an increased risk of developing APS and thrombotic events.

Prognosis and Therapy

Lupus anticoagulant is strongly associated with venous thrombosis and stroke, whereas ACAs are more frequently associated with pregnancy morbidity than with thrombosis. Individuals with high-titer IgG ACAs (>40 IgG phospholipid units) have been found to have a rate of thrombosis of 6.1% per year. Patients with two or more positive results in LAC, ACA, or B2GP1 antibodies were more strongly associated with thrombosis. Patients with LAC and B2GP1 antibody had a significantly increased risk for thrombosis (odds ratio 4.1, 95% confidence interval 1.3–13.5). Patients with triple positive results represent an even higher risk for future thrombotic events, with a cumulative incidence of 44.2% after 10 years, and pregnancy mortality. Triple positive results have been reported in approximately 30% of APS-affected women and are considered a predictor of PE. Patients with thrombotic APS have a higher risk for subsequent cardiovascular mortality and bleeding related to severe thrombocytopenia or anticoagulant therapy.

Treatment of APS patients is based on the patient's clinical status, presence of underlying disease such as autoimmune disorder and history of thrombotic events. For patients without prior thrombotic events, therapy will focus on prevention of thrombosis and reducing additional vascular risk factors. The mainstay of therapy in APS with venous thrombotic events is indefinite anticoagulation with a vitamin K antagonist, most commonly warfarin with a target INR of 2.5 (2.0–3.0). Up to 29% of patients not receiving long-term anticoagulants have been reported to experience recurrent thrombotic events. Recent DOACs can be used cautiously in some patients with complications or problems for frequent monitoring. Patients with pregnancy complications will develop thrombosis, approximately 3% to 7% per year, and can be managed with long-term low-dose unfractionated or LMW heparin and low-dose aspirin. Hydroxychloroquine, intravenous immunoglobulin (IVIG), statins, or newer promising therapy including B-cell monoclonal antibodies such as rituximab or complement cascade inhibitors can be cautiously used in certain patients with refractory forms of APS. Although most APS patients with thrombocytopenia do not require intervention, steroids, IVIG, dapsone, rituximab, or splenectomy can be considered in severe or symptomatic patients. Untreated catastrophic APS has a high mortality rate of over 50%. The first-line therapy for catastrophic APS is heparin and high-dose corticosteroids; plasma exchange and/or IVIG can reduce the mortality.

NATURAL ANTICOAGULANT DEFICIENCY

ANTITHROMBIN DEFICIENCY

Clinical Features

Antithrombin deficiency was the first recognized inherited risk factor for VTE and has the highest risk for VTE among the known hereditary thrombophilias. AT deficiency is inherited in an autosomal-dominant fashion. Most cases are heterozygous because homozygosity for AT deficiency is almost universally fatal in utero. AT deficiency is associated almost exclusively with venous thrombosis. It usually manifests as deep venous thrombosis (DVT) of the extremities and PE but can also occur in unusual sites, such as cerebral sinuses and mesenteric, portal, and renal veins. Thrombosis usually occurs at a young age (<50 years), but it is uncommon during the first two decades of life and may or may not have a provocative event.

Pathologic Features and Laboratory Studies

There are two major types of inherited AT deficiencies. Type 1 AT (quantitative) deficiency is most commonly caused by a lack of the AT gene product showing proportionately reduced functional and antigenic levels. It is frequently observed in the heterozygote state, resulting in approximately 40% to 60% activity and antigen levels. Type 2 AT deficiency is a qualitative deficiency, resulting in lower functional activity than antigen levels. Type 2 AT deficiencies are further classified into three subtypes: type 2a caused by mutations in AT reactive site, type 2b with mutations in the heparin-binding site, and type 2c with pleiotropic mutations. Among these defects, mutations in heparin-binding sites are associated with a low risk of VTE. However, subtyping is difficult owing to a lack of commercial assays. More than 250 distinct mutations related to AT deficiency are described in the AT gene (*SERPINC1*; chromosome 1q25.1). The most common mutation is a missense mutation that occurs in 50% to 60% of patients, leading to circulating dysfunctional proteins or disruption of the gene product. However, genetic analysis by sequencing is not typically performed.

The initial diagnostic step to investigate AT deficiency is a functional assay capable of detecting both type 1 and type 2 abnormalities. If it is decreased, an antigenic (immunologic)

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assay is performed to differentiate type 1 and type 2 deficiencies. Currently available functional assays use a chromogenic method based on AT inhibition by thrombin or factor Xa in the presence of heparin (heparin cofactor activity). Antigenic assays are based on ELISA or automated latex particle immunoturbidity assays; however, these antigen assays cannot detect patients with type 2 deficiency when used as an initial screening test.

Differential Diagnosis

Acquired causes of AT deficiency need to be differentiated from hereditary AT deficiency. Drugs such as heparin or L-asparaginase can cause reduce dAT activity. AT activity can be reversibly reduced by up to 30% during full-dose unfractionated heparin therapy but will not be reduced during LMW heparin therapy. Other causes of low AT levels include reduced synthesis in liver disease and increased AT loss in nephrotic syndrome, protein-losing enteropathy, DIC, sepsis, burn, trauma, hepatic venoocclusive disease, thrombotic microangiopathies, cardiopulmonary bypass surgery, hematomas, or metastatic tumors. AT levels can be low in premenopausal women, during oral contraceptive use and pregnancy. As with low PC and low PS, confirmatory testing should be repeated on a new specimen after any potential confounding conditions have resolved.

Prognosis and Therapy

Approximately 58% of VTE episodes in patients with AT deficiency occur spontaneously, whereas 42% show an association with a transient risk factor, which can be potentially preventable. AT deficiency in pregnant women has an estimated six-fold increased risk of thromboembolic events and a markedly increased risk of fetal loss. Patients with concurrent defects, such as factor V Leiden mutation, are associated with a higher risk of VTE and at younger ages, with a median age of 16 years. There is no recommended prophylaxis for AT-deficient individuals. Initial management of thrombosis in AT deficiency is similar to other causes of VTE: (1) consideration of thrombolytics, (2) initial therapy with heparin or fondaparinux, and (3) transitioning to vitamin K antagonist (warfarin) with maintenance of INR 2.0 to 3.0. Long-term anticoagulation can reduce a substantial recurrence rate of 2.7% per year.

PROTEIN C DEFICIENCY

Clinical Features

Protein C is a vitamin K–dependent glycoprotein primarily synthesized as an inactive form by the hepatocytes. Activation of functional PC requires interaction with the thrombin-thrombomodulin-endothelial PC receptor complex. Activated protein C, with its cofactor PS, regulates thrombin generation by degradation of activated coagulation factors Va and VIIIa.

PC deficiency is an important known risk factor for VTE including DVT and PE. The first thrombotic event usually presents between the ages of 10 and 50 years. PC and vitamin K–dependent clotting proteins are significantly lower than other coagulant proteins in healthy children, reaching adult range after puberty. Patients with moderate or severe PC deficiency can present with rapidly progressive neonatal purpura fulminans or DIC. In addition to severely decreased PC, patients with neonatal purpura fulminans can develop markedly elevated D-dimer, thrombocytopenia, hypofibrinogenemia, and prolonged PT. Histologically, fibrin clots in small venules of the subcutaneous fat or even in large vessels are present in purpura fulminans lesions. Adult heterozygous PC deficiency can present with recurrent DVT, PE, parenchymal thrombi, and DIC. Ischemic arterial stroke, mesenteric thrombi, and pregnancy-associated thrombosis also develop.

Pathologic Features and Laboratory Studies

There are two types of PC deficiencies. Type 1 PC (quantitative) deficiency, characterized by reduced functional activity and antigen levels, is more common (75% to 80%) than type 2 (qualitative) deficiency (10% to 15%). Type 2 deficiency results in reduced activity and normal antigen level. PC deficiency may be inherited in an autosomal-dominant or -recessive fashion with incomplete penetrance. Most patients are heterozygotes with functional PC levels of 30% to 65% of normal.

PC assays measure either PC activity (functional) or antigen quantity (immunologic). As an initial screening test, functional PC levels can be measured by clotting time–based or chromogenic assays. Clotting time–based assay can detect both type 1 and type 2 deficiency. However, they can give falsely increased results with anticoagulant therapy, LAC and FVL mutation, and falsely decreased results with elevated factor VIII levels or low PS (in acute phase response). The chromogenic assay is less affected by interfering substances than the clotting time–based assay and is more reproducible; however, it can detect only functional PC defects related to the peptide binding site and therefore can miss some type 2 deficiencies. If the result of a functional PC assay is low, an antigenic PC assay is required.

PC mutational analysis is available in few laboratories by sequencing and may be helpful to identify heterozygous carriers. Approximately 250 mutations within the *PROC* gene (chromosome 2q14.3) are reported in PC deficiency, with a high frequency of single nucleotide substitution within the coding region in type 1, and missense mutations in type 2 deficiencies. Studies based on mutational analysis reported about 50% risk for thrombosis in carriers from symptomatic families by the age of 45 years. However, 10% to 30% of

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PC-deficient families did not demonstrate any mutation in the *PROC* gene.

Differential Diagnosis

Acquired PC deficiency is more common than hereditary PC deficiency. The acquired causes should be excluded first before making a diagnosis of hereditary PC deficiency. Both liver dysfunction and vitamin K deficiency (including warfarin therapy) can decrease PC levels. Because of the short half-life (6–8 hours) of PC, PC levels are more rapidly reduced in liver disease, anticoagulation therapy, and vitamin K deficiency compared to other coagulation proteins. Conversely, PC levels rapidly recover to the normal range after discontinuation of anticoagulation therapy or correction of vitamin K deficiency. Even so, to ensure the most accurate results, PC levels should not be measured for at least 10 days after stopping anticoagulants such as warfarin. PC levels are lowered in recent or current thrombosis, DIC, L-asparaginase therapy, nephrotic syndrome, and during the intra- or immediately postoperative period; neonates also have relatively low PC levels (range 17% to 53%). Oral contraceptive use and pregnancy (>20% by 20 weeks' gestation) can increase the PC level. Initially, low PC assays should be repeated after any such conditions have resolved.

Prognosis and Therapy

Neonatal purpura fulminans, DIC, or acute thrombosis cases can be managed by PC replacement using fresh frozen plasma or human plasma-derived viral inactivated PC concentrate. Symptomatic PC deficiency can be treated with anticoagulant using unfractionated or LMW heparin, warfarin maintaining an INR 2.0 to 3.0, or DOACs. Warfarin therapy should be preceded by at least 5 days of heparin administration because of increased risk for warfarin-induced skin necrosis (WISN) or recurrent thrombotic complications (Fig. 2.12). WISN has been reported in 0.01% to 0.1% of patients on warfarin with a high morbidity and mortality. It usually occurs 3 to 10 days after initiating warfarin and begins with paresthesia, edema, ecchymosis, and hemorrhage in subcutaneous adipose tissue. Thromboprophylaxis should be considered in all high-risk individuals. For pregnant women, LMW heparin is the drug of choice because of the high rate of allergy, HIT, or osteoporosis in association with prolonged use of unfractionated heparin. Other complications related to long-term anticoagulant therapy should be considered in these patients.

PROTEIN S DEFICIENCY

Clinical Features

Protein S is a vitamin K–dependent glycoprotein containing γ-carboxyglutamic residues that act as a cofactor to activated PC and accelerating PC proteolysis of factor Va and VIIIa approximately two-fold. Approximately 60% of PS in the plasma is bound noncovalently to C4b binding protein (C4bBP) with high affinity; the remaining free (unbound) PS is the predominantly active form. Other functions of PS include anticoagulant activity by direct binding of

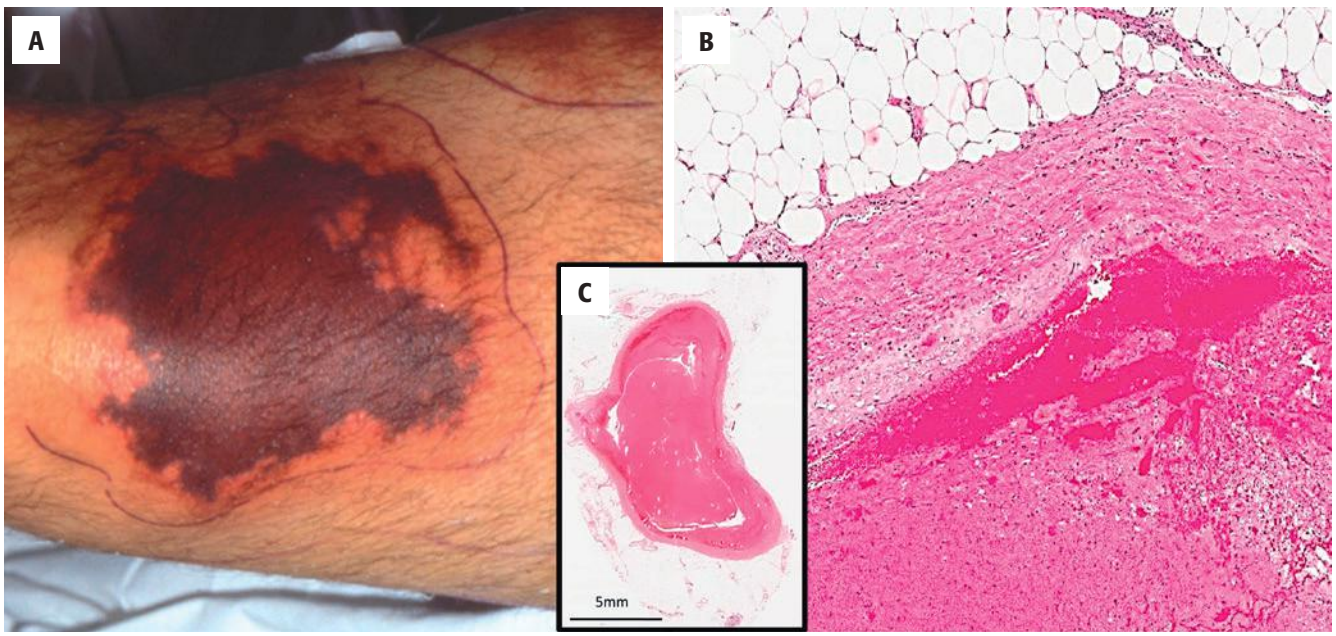


FIG. 2.12

A, A representative picture of a patient with warfarin-induced skin necrosis in the lower extremity. B, Histologic features in deep vein thrombosis (DVT) in a patient with recurrent history of DVT and pulmonary embolism with an inferior vena cava filter on warfarin therapy. C, Occlusion of common iliac vein by organizing thrombi composed of fibrin clots. *(A, Picture courtesy Dr. Bartholomew. B & C, H&E stain.)*

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factors V, VIII, and X acting as a cofactor for the TFPI, and enhancement of fibrinolysis by inhibiting plasminogen activator inhibitor or decreasing thrombin activatable fibrinolysis inhibitor.

PS deficiency typically presents with recurrent DVT, PE, or both, with about half of the thrombotic events unprovoked. Homozygous patients are very rare, but they typically present in newborns with purpura fulminans and DIC. Hereditary PS deficiency is transmitted in an autosomal-dominant fashion. Approximately half the patients with PS deficiency become symptomatic at 55 years of age, depending on the phenotype. Affected family members have 5- to 11-fold higher risk of VTE than their healthy relatives. Women with deficiency of PC, PS, or AT taking oral contraceptives are at a particularly increased risk for VTE.

Pathologic Features and Laboratory Studies

The diagnosis of PS deficiency is complicated by the complex interaction of PS with binding proteins in plasma and inconsistent genotype-phenotype association. There are three types of PS deficiencies. Type 1 and type 3 are quantitative deficiencies with both low free PS antigen and low PS activity, together accounting for 95% of cases. Type 1 deficiency can be further differentiated from type 3 deficiency as total PS antigen is low in type 1 deficiency whereas it is normal in type 3 deficiency. Type 3 deficiency may be related to excess binding of PS to C4bBP, with decreased free PS. Type 2 deficiency is a qualitative defect with low PS activity with normal antigenic free and total PS levels. As with PC, the functional activity assay, which can detect quantitative and qualitative PS deficiencies, is generally considered an initial test, and the antigenic (immunologic) assay is performed only if functional activity is low. Functional PS activity measured by clotting time–based assay is sensitive but not specific. Functional PS levels range between 35% and 60% in heterozygous patients. However, because of the influence of PS activity in a variety of conditions and interference by anticoagulants and high factor VIII in the clot-based assay, some experts consider free PS antigen as an initial screening (and surrogate marker of PS activity). If it is low, PS activity is measured to confirm deficiency. However, using free PS antigen may not detect the rare type 2 defect.

Almost 200 mutations in *PROS1* gene (chromosome 3q11.2) resulting in PS deficiency, more often type 1 than other types, have been identified in approximately 70% of patients with PS deficiency. The PS Heerlen polymorphism (Ser460Pro), associated with reduced levels of free PS resulting in type 3 deficiency, is frequently found with a prevalence of 0.52% in the general population. PS Tokushima (Lys-155Glu), resulting in type 2 deficiency and associated with DVT, is frequently observed in general Japanese populations with an estimate prevalence of 1.6%.

Differential Diagnosis

As with PC deficiency, acquired causes of PS deficiency are more common and should be excluded before making a diagnosis of PS deficiency. PS will also be decreased in many clinical conditions that cause decreased PC (see above). In addition, PS will be decreased in the acute phase response because its binding protein C4bBP is an acute phase reactant; when C4bBP is increased it lowers both PS activity and free antigen. PS is also decreased with elevated factor VIII (>250%) and infectious and autoimmune conditions such as HIV infection, Crohn's disease, or ulcerative colitis. PS levels are usually lower in women, especially during hormone replacement therapy, oral contraceptive use, and the second or third trimester of pregnancy. Common misdiagnosis of PS deficiency occurs when PS is measured immediately after an acute thrombotic event and before starting anticoagulation. In this state, PS will be reduced because of thrombotic consumptive process or underlying acquired conditions. PS level should be repeated 4 to 6 weeks later after any such conditions causing acquired PS deficiency are resolved.

Prognosis and Therapy

VTE has been associated with a high rate of morbidity such as post-thrombotic syndrome, pulmonary hypertension, or PE. The management of VTE in PS deficiency is similar to that in PC deficiency.

ACTIVATED PROTEIN C RESISTANCE AND THE FACTOR V LEIDEN MUTATION

Clinical Features

Activated protein C, in the presence of cofactor PS, cleaves and inactivates activated coagulation factors Va and VIIIa in the tenase complex, resulting in downregulation of further thrombin generation. APC resistance (APC-R) is observed in approximately 20% of patients with a first episode of DVT and 50% of familial thrombosis. Greater than 90% of APC-R cases have been associated with a point mutation in the factor V gene, known as factor V Leiden (FVL). FVL causes a DNA polymorphism (G1691A) substituting amino acid arginine to glutamine at position 506 (R506Q), one of three arginine sites (R306, R506, and R679) cleaved by APC (Fig. 2.13). Elimination of an APC cleavage site by FVL results in a protein that degrades factor Va at a 10-fold slower rate and impedes APC-mediated inhibition of thrombin generation.

FVL is the most common risk factor for thrombosis to date in general US and European populations. FVL is more commonly present in a heterozygous form among white

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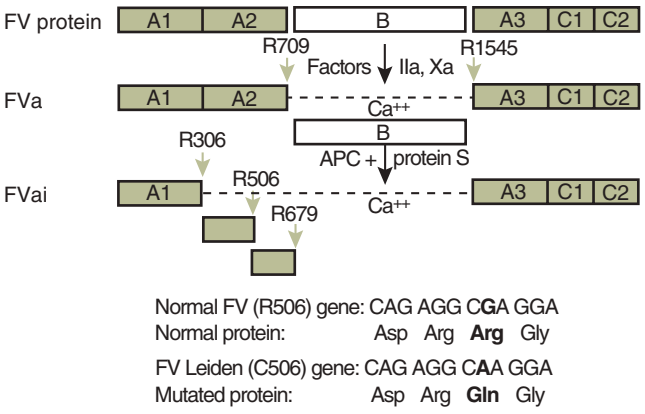


FIG. 2.13

Schematic representation of activation and degradation of normal factor V (FV) and factor V Leiden (FVL). FV circulating as a single chain high-molecular-weight protein has little procoagulant activity. It is activated by thrombin or factor Xa through proteolysis at Arg709, Arg1018, and Arg1545. Activated factor V (FVa) is a heterodimer that is non-covalently associated in a calcium-dependent manner. FVa is inhibited by the activated protein C (APC) and protein S (PS) complex and cleaved by APC in three peptide bonds at Arg306, Arg506, and Arg 679. FVL mutation eliminates one of three APC cleavage sites by a point mutation, resulting in impairment of degradation of FVa. (Abbreviation: *FV,* factor V; *FVL,* factor V Leiden; *FVa,* activated factor V; *FVai,* inactivation of activated factor V.)

populations and is extremely rare in African, Australian, or South Asian populations. FVL is the most commonly known hereditary risk factor for venous thrombosis; typical presentation is venous thrombosis in the extremities or, less commonly, in unusual sites such as cerebral, retinal, hepatic, ovarian, or renal veins. Approximately 30% of patients with VTE will develop recurrent thrombosis within the subsequent 10 years. FVL is found in 20% to 60% of women with pregnancy-associated VTE or VTE during oral contraceptive use. The thrombotic risk is further increased in the presence of a second risk factor. Women with FVL (heterozygous) using oral contraceptives appear to have a 30- to 60-fold increased risk of thrombosis. Some studies show that the risk of PE is not as great as the risk of DVT in FVL patients. FVL is not a risk factor of arterial thrombosis. In addition to FVL, various less common factor V (FV) mutations including the FVR2 haplotype (H1299R), which occurs in approximately 12% of white populations in the US; FV Liverpool (I359T); FV Cambridge (R306T); and FV Hong Kong (R306G) have been reported. However, these mutations have not been shown to increase thrombotic risk in epidemiologic studies. Rare pseudo-homozygous FVL, with a combination of heterozygous FVL, low factor V activity (~50% of normal), and low APC-R ratio in the range typical of homozygous FVL, will demonstrate a similar clinical phenotype to FVL homozygote with an increased thrombotic risk.

Pathologic Features and Laboratory Studies

Laboratory assays for APC-R and FVL include functional assays and genotyping for FVL by DNA analysis. The functional assay for APC-R is based on prolongation of the APTT by degradation of factor Va and VIIIa using exogenously supplied APC. The calculated ratio of APTT in patient plasma and normal plasma before and after adding APC is typically 2.0 or higher in healthy individuals; 1.5 to 2.0 in heterozygous individuals for FVL, and less than 1.5 in homozygous individuals. Each laboratory should determine its own cut-off for an abnormal result. Elevated factor VIII, low PS (<20%), and causes of prolonged baseline APTT, such as heparin, warfarin, DTI, LAC, liver dysfunction, or low factor levels, can cause a falsely low APC-R ratio. A second-generation assay for functional APC-R utilizes predilution of patient plasma with FV-deficient plasma (also containing heparin neutralizer) and provides higher sensitivity and specificity. This assay is less affected by active thrombosis, surgery, inflammatory disorders, factor deficiencies, heparin, or warfarin.

Identification of FVL as the cause of APC-R is confirmed by a DNA-based method such as polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP), allele-specific PCR (AS-PCR) genotyping, melting curve analysis, or sequencing. Non–PCR-dependent and simple microtiter plate-based invader technology using fluorescence resonance energy transfer (FRET) shows reliable detection for FVL mutation. However, owing to the use of specific primers, this test method will only detect specific mutations (i.e., FVL) and will not detect other rare FV mutations related to functional APC-R. In general, a cost-efficient functional assay for APC-R is recommended as an initial screening test and DNA analysis for FVL as a confirmatory test in individuals with abnormal APC-R results, strong LAC, or prolonged baseline APTT and in relatives of family history of known FVL. Fig. 2.14 shows an example of heterozygous FVL using melting curve analysis.

Differential Diagnosis

Venous thromboembolism by FVL needs to be differentiated by other inherited or acquired thrombophilic disorders. Inherited disorders, such as prothrombin gene G20210A mutation, abnormalities in natural anticoagulants, and dysfibrinogenemia, are described in other sections. Disorders causing acquired APC-R in the absence of FVL in a small percentage of patients (~5%) include APAs, high homocysteine, or high clotting factors. Acquired APC-R can also be caused by development of autoantibodies against factor V following exposure to bovine thrombin, or with untreated hematologic malignancies such as myeloma, LAC, pregnancy, oral contraceptives, active thrombosis, elevated factor VIII, and mutations in the factor VIII gene. Persistently elevated factor VIII (>150% of normal) is associated with four- to five-fold increase of risk in VTE, whereas high levels of both factor IX and factor XI, and high prothrombin (>115% of normal) are associated with an approximately two-fold increased risk in VTE.

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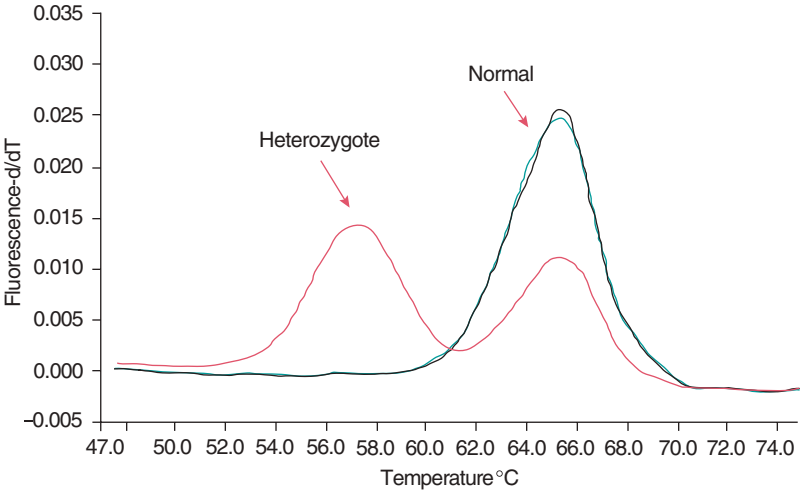


FIG. 2.14

Melting curve analysis for factor V Leiden mutation. Normal individual *(blue line)* demonstrates a peak at the normal melting temperature (temperatures: 65 ± 2.5°C), whereas a heterozygote individual shows double peaks, with the first peak representing the mutant allele (57 ± 2.5°C) and the second peak representing the wild-type allele (65 ± 2.5°C). The reactions were performed in LightCycler capillaries using the LightCycler instrument (Roche Diagnostics).

Prognosis and Therapy

The treatment of individuals with FVL depends on clinical manifestations. Long-term anticoagulation with unfractionated or LMW heparin, warfarin, or DOACs is recommended for individuals with first or recurrent unprovoked VTE. However, potential benefits and risks from long-term warfarin use should be considered because of a risk for major bleeding (1% to 3% per year) in warfarin users. The presence of FVL or prothrombin gene G20210A does not impact therapy duration. Because of the high prevalence of FVL in the general population, genetic counseling for affected individuals and family members is recommended.

PROTHROMBIN GENE G20210A MUTATION

Clinical Features

The prothrombin gene mutation is a gain-of-function mutation resulting in elevated functional prothrombin (factor II) levels owing to increased synthesis; it is associated with an increased risk of venous thrombosis. A point mutation that changes guanine to adenine at the 20210 position (G20210A) of the prothrombin gene occurs in an intron near the 3′ end of the gene. This alters mRNA formation by affecting 3′ end processing and/or enhancing translation efficiency, resulting in increased plasma prothrombin levels. However, the exact mechanism of how increased prothrombin expression causes hypercoagulability remains unclear. Prothrombin G20210A is the second most common hereditary risk factor for venous thrombosis. Similar to FVL, prevalence of prothrombin G20210A varies by ethnic group; 2% to 5% of Europeans carry the mutation, whereas it is less common in African-Americans and rare in Asians or Native Americans. Heterozygous individuals show a 3-fold increase in risk of venous thrombosis; however, the risk will be dramatically increased when the patients carry additional inherited or acquired risk factors. For example, in addition to prothrombin G20210A, VTE risk will be increased 20-fold in individuals with concurrent FVL and 15- to 16-fold with pregnancy or use of oral contraceptives.

Pathologic Features and Laboratory Studies

Prothrombin levels will be increased in individuals with prothrombin G20210A: 20% to 30% in heterozygotes and approximately 70% in homozygotes. However, measurement of prothrombin activity is not suggested because of overlap of prothrombin levels in individuals with and without mutations. The prothrombin gene testing to detect the G20210A single nucleotide polymorphism (SNP) can be performed by various PCR-based methods (Fig. 2.15A). A commonly utilized method is PCR followed by DNA sequencing by gel electrophoresis (Fig. 2.15B), restriction endonuclease digestion, and radioisotope probing. Technologic advances in molecular diagnostic testing have led to automated genotyping analyses based on various PCR methods coupled with fluorescence polarization methods or the Invader assay. DNA microarray technology can detect multiple genetic markers simultaneously using various DNA chip platforms with a relatively low cost as a single test compared to conventional DNA assays. DNA analysis is not recommended as a screening test in the general population, but it is indicated to identify high-risk individuals requiring anticoagulant therapy or prophylaxis. The advantage of DNA analysis is that it cannot be affected by other conditions such as active thrombosis, surgery, inflammatory conditions, or anticoagulant therapy. However, these tests require expensive equipment and skilled personnel, have risks of contamination, and may require reflex confirmatory assays

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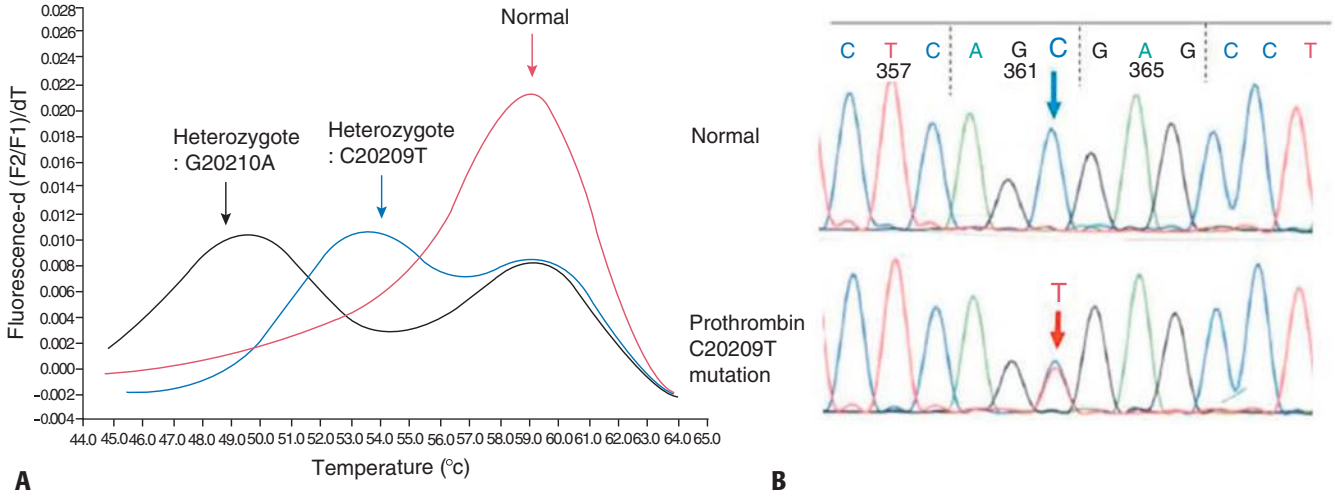


FIG. 2.15

A, Melting curve analysis for prothrombin gene mutation. A healthy individual *(red line)* demonstrates a peak at the normal melting temperature (59 ± 2.5°C), whereas a heterozygote individual with G20210A shows double peaks, with the first peak representing the mutant allele (49 ± 2.5°C) and the second peak representing the wild-type allele (59 ± 2.5°C). In comparison, a heterozygote individual with C20209T shows shifted temperature in the mutant allele (53.59°C). The reactions were performed in LightCycler capillaries using the LightCycler instrument (Roche Diagnostics). B, Sequencing for prothrombin gene mutation. *Red arrow* indicated nucleotide shows two peaks with a C20209T sequence variant in one base upstream from position 20210, one presenting tyrosine (T) base, and the other a cytosine (C) base.

(i.e., sequencing) if there is an ambiguous or atypical pattern by PCR.

Prognosis and Therapy

The management of individuals with prothrombin G20210A is similar to individuals with other thrombophilic disorders, such as FVL, depending on clinical manifestations. Long-term anticoagulation with unfractionated or LMW heparin, warfarin, or DOACs is recommended for individuals with increased risk of VTE.

DYSFIBRINOGENEMIA

Clinical Features

Dysfibrinogenemia is a very rare disorder in a heterogeneous group of disorders that results in structurally and functionally altered fibrinogen. It is associated with mutations throughout the three genes *FGB*, *FGA*, and *FGG* coding for the fibrinogen subunits (Bβ, Aα, and γ). The prevalence of dysfibrinogenemia in patients with venous thrombosis is approximately 0.8%. Most patients are asymptomatic (55%); however, it can cause bleeding (25%), venous or arterial thrombosis (20%), or both. Bleeding ranges from minimal bleeding to menorrhagia, postpartum, and intracranial or joint bleeding and often occurs after trauma or surgery. Thrombosis with recurrent VTE and PE appears to be associated with mutations throughout the three fibrinogen genes, causing defective thrombin binding, elevated thrombin, and resistance of the fibrin clot to plasmin degradation. Other clinical manifestations include hereditary fibrinogen amyloidosis characterized by extracellular deposition of amyloid leading to neuropathy and renal failure, abnormal storage of fibrinogen in the hepatic endoplasmic reticulum, cirrhosis, and spontaneous abortion.

Pathologic Features and Laboratory Studies

The initial screening test for dysfibrinogenemia is thrombin time, which can be prolonged in hypofibrinogenemia and dysfibrinogenemia. Another screening test is reptilase time. Reptilase exclusively cleaves the Aα chain and releases fibrinopeptide A, and reptilase time is usually prolonged in dysfibrinogenemia. The most commonly used functional fibrinogen assay is the Clauss method. Dysfibrinogenemia patients can have decreased functional fibrinogen and normal to elevated immunologic fibrinogen. A decreased ratio of functional fibrinogen activity to immunologic fibrinogen antigen will confirm the diagnosis of dysfibrinogenemia. Mutational analysis has identified over 100 mutations throughout the three fibrinogen genes. The most common mutation is substitution of the arginine residue in Aα chain with either histidine or cysteine, accounting for approximately 63% of dysfibrinogenemia. Because this AαArg16 is part of thrombin cleavage site, this substitution can delay or abolish the release of fibrinopeptide A and subsequently delay polymerization. The second most common mutation is substitution at γArg275 with either histidine or cysteine, occurring in 18% of patients with dysfibrinogenemia.

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Differential Diagnosis

Acquired deficiency of fibrinogen can be caused by liver disease, consumptive states such as placental abruption or DIC, or fibrinolytic therapy. As fibrinogen is an acute phase reactant, testing for dysfibrinogenemia should be delayed at least 6 months after an acute thrombotic event.

Prognosis and Therapy

Therapy in dysfibrinogenemia depends on clinical phenotype and personal and family history. Dysfibrinogenemia variants associated with bleeding, usually Aα chain variants, can be managed by fibrinogen replacement therapy with fibrinogen concentrate or cryoprecipitate.

THROMBOTIC THROMBOCYTOPENIC PURPURA

Clinical Features

Thrombotic thrombocytopenic purpura (TTP), a thrombotic microangiopathy (TMA), is a rare life-threatening disease characterized by disseminated microthrombi formation in small vessels. Tissue damage by microthrombi can cause thrombocytopenia and microangiopathic hemolytic anemia accompanied by multiorgan failure, often manifesting as renal failure and mental status changes. The thrombocytopenia is thought to be secondary to a deficiency of or an autoimmune response against a VWF-cleaving metalloproteinase (a disintegrin and metalloproteinase with thrombospondin-1–like domains [ADAMTS13]) in many patients. The ADAMTS13 metalloproteinase cleaves VWF at Tyr842-843Met bonds, creating smaller fragments. In individuals with a deficiency of ADAMTS13, the ultra-large (UL) VWF remains attached to endothelial cells, leading to platelet adhesion, aggregation, and microvascular thrombosis. It is thought that these long VWF–platelet aggregates can detach and embolize, further facilitating microvascular thrombosis. The erythrocyte fragmentation likely occurs as red blood cells are sheared by fluid turbulence in areas of platelet aggregates. Typical TTP presents with the so called classic pentad; however, some patients will not develop the entire pentad. Studies with large series reported the frequency of symptoms in TTP patients; microangiopathic hemolytic anemia (MAHA) and thrombocytopenia in 70% to 100%, neurologic involvement in 50% to 90%, renal dysfunction in approximately 50%, and fever in only 25% of patients. The presence of thrombocytopenia and unexplained MAHA is considered clinically sufficient for high suspicion of TTP.

Rare individuals with a congenital deficiency of ADAMTS13 have a familial form of TTP that often manifests in infancy or childhood and can recur as chronic relapsing TTP or the Upshaw-Schulman syndrome. More than 70 genetic mutations of the *ADAMTS13* gene on chromosome 9q34 have been identified. A more common idiopathic form of TTP that is seen in adulthood is thought to be caused by autoantibodies directed against ADAMTS13. Pathogenic antibodies that have been identified are typically directed against the cysteine-rich–spacer domain of ADAMTS13.

A clinically similar thrombotic microangiopathy can be observed with drugs such as cyclosporine, tacrolimus (FK506), quinine, ticlopidine, and clopidogrel and after allogeneic organ transplantation. Hemolytic-uremic syndrome is a related disorder, typically seen in children with thrombocytopenia, microangiopathic hemolytic anemia, and renal failure. Two types of HUS have been identified: (1) diarrhea-associated HUS caused by a Verotoxin-producing strain of *Escherichia coli* (serotype O157:H7 or O104:H4) and (2) diarrhea-negative familial types of atypical HUS that are related to a defect in complement regulatory proteins.

THROMBOTIC THROMBOCYTOPENIC PURPURA—FACT SHEET

Definition
- Thrombotic microangiopathy owing to a deficiency of or an autoimmune response against a VWF cleaving metalloproteinase (ADAMTS13), leading to diffuse microthrombi formation in small vessels and a thrombocytopenia

Incidence
- 3.7 per 1 million

Morbidity and Mortality
- Death is rare in patients younger than 20 years of age; mortality increases with age older than 20 years

Gender, Race, or Age Distribution
- Typically seen in childhood (<5 years of age) or in adulthood (20–60 years of age)

Clinical Features
- Fever, thrombocytopenia, and microangiopathic hemolytic anemia accompanied by multiorgan failure, such as renal failure, and mental status changes
- Congenital deficiency of ADAMTS13 leads to chronic relapsing TTP

Prognosis and Treatment
- High mortality owing to multiorgan failure
- Supportive therapy employs daily plasma exchange with fresh frozen plasma; newer therapies include rituximab

Pathologic Features and Laboratory Studies

A review of the CBC highlights a normocytic anemia with profound thrombocytopenia; platelet counts are frequently

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less than $20 \times 10^3/\mu L$. The reticulocyte count, red cell distribution width (RDW), and MPV are often increased, indicating the increased turnover of both erythrocytes and platelets. Morphologic evaluation of the peripheral blood smear reveals erythrocyte polychromasia and anisocytosis with prominent schistocytes (Fig. 2.16). BM evaluation is typically not necessary in establishing a diagnosis of TTP, but it may be helpful to exclude other causes of thrombocytopenia. BM morphology will show normal to increased numbers of megakaryocytes. Small vessels may show platelet thrombi, but these are not always observed. Autopsy reflects the pathophysiology of the disease, with platelet-rich thrombi plugging the microvasculature of the brain, heart, pancreas, spleen, adrenal gland, and kidneys but rarely involving the liver or lungs, in distinction from DIC. On immunohistochemical analysis, these thrombi are also rich in VWF, in contrast to the platelet-fibrin thrombin in DIC.

Several different assays for the VWF-cleaving metalloproteinase are available, including assays of ADAMTS13 activity, antigen, neutralizing or nonneutralizing autoantibodies,

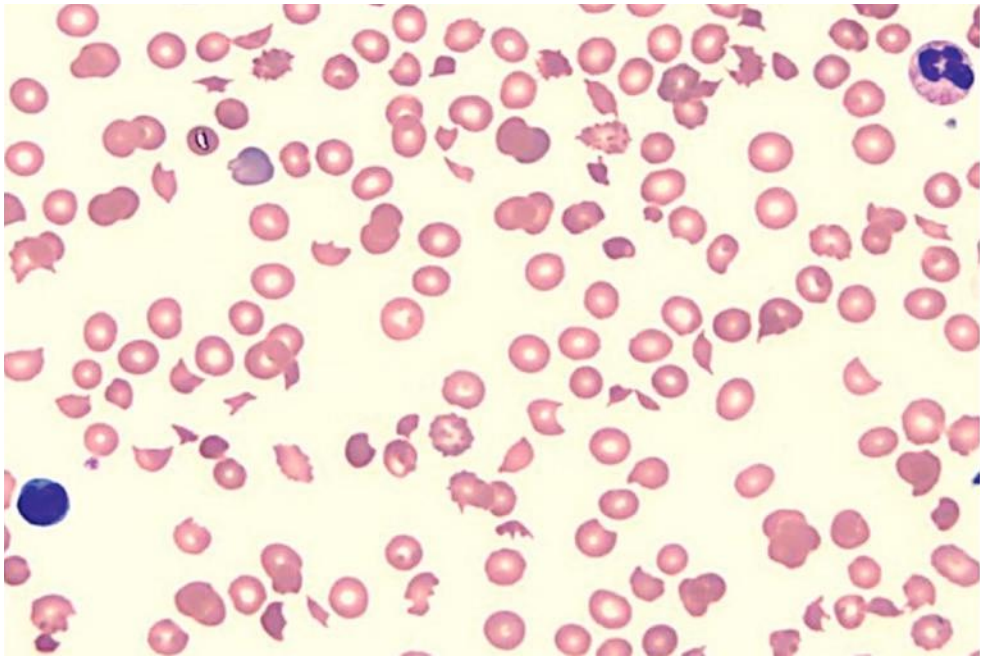


FIG. 2.16

Peripheral blood smear in a patient with thrombotic thrombocytopenic purpura with typical morphologic features of microangiopathic hemolysis; normocytic anemia with polychromasia and anisopoikilocytosis, including numerous schistocytes and thrombocytopenia. (Wright-Giemsa stain, ×400.)

THROMBOTIC THROMBOCYTOPENIC PURPURA—PATHOLOGIC FEATURES

Complete Blood Cell Count and Peripheral Smear Morphology

- Normocytic anemia with profound thrombocytopenia (<20 × $10^3/\mu L$)
- Increased reticulocyte count, RDW, and MPV
- Red blood cell morphology shows anisocytosis and prominent schistocytes

Bone Marrow Findings

- Bone marrow findings are nonspecific, but small vessels may show platelet thrombi
- Autopsy shows obstructive platelet thrombi in the microvasculature of multiple organs

Platelet Function Tests

- Usually unhelpful because of the degree of thrombocytopenia

Ancillary Studies

- ADAMTS13 assays show a decreased amount or decreased function
- ADAMTS13 inhibitors may be detected
- Patients with acute TTP usually have ADAMTS13 activity less than 5%
- VWF multimer analysis may show an increase in UL HMW multimers, but other coagulation assays (PT, APTT, VWF antigen, factor VIII, ristocetin cofactor) are usually normal

Differential Diagnosis

- Exclude other consumptive thrombocytopenic disorders
- DIC may also show schistocytes on the peripheral smear but can be distinguished by characteristic coagulation abnormalities, including elevated PT, APTT, D-dimer, and decreased clotting factor levels
- Hemolytic uremic syndrome is distinguishable by serology for *E. coli* O157:H7 or O104:H4, lack of ADAMTS13 abnormalities, and more severe renal dysfunction
- HIT should have characteristic positive laboratory tests and a history of heparin exposure

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and genetic characterization of the *ADAMTS13* gene for mutations. The functional assays are based on the degradation of VWF or VWF-derived peptides and detection of the cleavage products. The initial assay based on degradation of intact VWF measured the enzyme activity by following a progressive decrease in VWF multimer size, but this relied on a VWF multimer Western blot assay that took as long as 2 days to perform and was not readily available in most laboratories. More recent modifications of the assay based on intact VWF measure the metalloproteinase-induced decrease in VWF multimer size by either immunoradiometric assay in microtiter plates or by tracking the ability of VWF to bind to collagen or ristocetin, an activity that is directly proportional to VWF molecular weight.

Functional assays are based on the degradation of VWF peptides with direct detection of metalloproteinase activity. These assays have used recombinant ADAMTS13 substrate peptides from the VWF A2 domain, such as L1591-R1668 or D1596-R1668. Peptide cleavage products by denaturing agents (i.e., guanidine HCl or urea) are detected by various techniques, including ELISA, Western blot, and FRET. Immunoassays for quantitative measurement of ADAMTS13 antigen are available. The results may be normal in patients with TTP resulting from neutralizing autoantibodies, where immune complexes are formed. Alternatively, the antigen levels may be decreased if non-neutralizing autoantibodies lead to ADAMTS13 clearance. Neutralizing antibodies can be detected using a mixing study of heat-inactivated patient and normal plasma (typically a 1 : 1 mix) combined with either an immunoassay or functional assay. Genetic studies of ADAMTS13 by sequence analysis can be performed. Published studies have shown the majority of mutations to be missense (59%).

Plasma ADAMTS13 activity in healthy individuals typically ranges from 50% to 180%. Patients with acute TTP usually have activity less than 5%. Variably decreased ADAMTS13 activity can also be observed with liver disease, disseminated malignancy, inflammatory disorders, and during pregnancy. Infants may also have a lower activity than adults. Traditional screening coagulation studies, such as PT and APTT, are normal in patients with TTP. Results of assays for VWF, such as VWF antigen, ristocetin cofactor, and factor VIII, are typically normal. However, VWF multimer analysis may show an increase in UL VWF multimers during the acute disease presentation.

Differential Diagnosis

Thrombotic thrombocytopenic purpura can be diagnosed definitively in many patients based on the distinct clinical features and markedly decreased ADAMTS13 activity. However, ADAMTS13 assays are not widely available, and rapid diagnosis often rests in excluding other consumptive thrombocytopenic disorders. In addition, many patients with clinical symptoms of TTP have only modestly decreased levels of ADAMTS13, whereas other individuals with markedly decreased ADAMTS13 levels might not have clinical symptoms of TTP.

TTP and DIC are both thrombotic microangiopathies with schistocytes on the peripheral smear, but these two disorders can be distinguished by the characteristic coagulation test abnormalities found in DIC, as indicated previously. Hemolytic-uremic syndrome is often difficult to differentiate from TTP. HUS shows prominent renal dysfunction by ischemia or tissue injury by microvascular thrombi compared to multiorgan injury in TTP. HUS in children (90% to 95%) is often related to hemorrhagic enterocolitis caused by *E. coli* (O157:H7 or O104:H4) or *Shigella* (Shiga toxin) and preceding diarrhea, typically bloody, but lack abnormalities in ADAMTS13. The atypical HUS, representing 5% to 10% of HUS, is not associated with diarrhea. Often it is caused by genetic mutations involving genes encoding for complement regulator factor H (20% to 30%), CD46 (10%) and other cofactors, or autoantibodies against factor H (10%), which affect the alternate complement pathway and result in chronic excessive complement activation. ITP may also be in the differential, but cases typically lack hemolytic anemia and multiorgan failure and do not have schistocytes on the peripheral smear. TTP can be distinguished from posttransfusion purpura resulting from a lack of association with transfusions or platelet HPA-1a/1b genotype. Distinction of TTP from drug-induced thrombocytopenia can be difficult in patients treated with multiple drugs, but these disorders usually lack microangiopathic thrombosis or organ failure. HIT is a drug-induced thrombocytopenia that is complicated by thrombosis, but HIT is preceded by heparin exposure 5 to 14 days prior and is characterized by large-vessel thrombosis and the presence of anti–platelet factor 4 antibodies. Paroxysmal nocturnal hemoglobinuria occasionally manifests with thrombocytopenia and microangiopathic hemolysis, but it is usually distinguishable by a lack of renal dysfunction and flow cytometric analysis demonstrating decreased expression of glycophosphatidylinositol-linked proteins such as CD55 and CD59 on red blood cells or neutrophils.

Prognosis and Therapy

Thrombotic thrombocytopenic purpura is associated with high mortality because of multiorgan failure. The mainstay of therapy is immediate daily plasma exchange with fresh frozen plasma, which brings 70% to 90% of patients with idiopathic TTP into remission. However, 20% to 50% of patients will experience a relapse. Persistent severely decreased ADAMTS13 activity or the presence of an inhibitor suggests a high risk of relapse in symptomatic TTP. Persistence of autoantibodies during clinical remission or high titers of autoantibodies also suggest increased risk of clinical relapse. Most relapses occurred within the first 2 years after the initial episode with an estimated risk of 43% for relapse at 7.5 years. Patients with TTP experiencing relapse and patients

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refractory to therapeutic plasma exchange (10% to 20%) have been treated with corticosteroids, splenectomy, or immunosuppressive agents. Rituximab demonstrated improvement of response rate and progression-free survival. Newer therapies based on infusion of recombinant ADAMTS13 have shown promise.

PLATELET DISORDERS

Platelet-related bleeding disorders can be caused by both congenital and acquired factors. Although congenital platelet disorders are rare, rapid progress has been made in identifying the underlying genetic causes (see Table 2.4 for a listing of these disorders by functional defect). Acquired platelet disorders are mostly caused by platelet destruction, through immune or consumptive etiologies, but may also be associated with an increased platelet count, as seen in myeloproliferative neoplasms.

The understanding and identification of congenital disorders of platelet production have expanded markedly owing to improved genomic analysis. Recent diagnostic guidance from the ISTH encompasses clinical evaluation followed by platelet function studies and next-generation sequencing to identify many of these rare disorders. Next-generation sequencing is becoming more widely available for specific diagnosis of disorders that were not previously discernible owing to nonspecific platelet function findings.

DEFECTS OF PLATELET PRODUCTION

GATA-1–RELATED DISEASES

The transcription factors GATA-1 and GATA-1, together with a cofactor, FOG-1, are involved in maturation of megakaryocytes. Genetic defects of *GATA-1* that interfere with its binding to FOG-1 have been associated with X-linked thrombocytopenia with dyserythropoiesis, whereas other genetic defects have been associated with X-linked thrombocytopenia with thalassemia (XLTT).

PARIS-TROUSSEAU SYNDROME (*FLI1*)

Paris-Trousseau (Jacobsen) syndrome has been linked to genetic defects of Friend of Leukemia Integration (FLII). It is associated with large alpha granules with a mild bleeding disorder, as well as cardiac and facial defects.

FAMILIAL THROMBOCYTOPENIA–LEUKEMIA (*RUNX1*)

Patients with this autosomal-dominant syndrome have a relatively mild thrombocytopenia (80 to 100 $\times$ 10^3/μL) but often have a clinical platelet dysfunction with bleeding out of proportion to the degree of thrombocytopenia. Platelet function testing usually reveals a platelet storage pool disorder (SPD). Approximately half of the patients develop a malignancy, usually acute myeloid leukemia or solid tumors. Mutations in the transcription factor *RUNX1* (CBFA2, AML1) and decreased expression of myosin light chain (MYL9) have been identified in families with this disorder.

CONGENITAL AMEGAKARYOCYTIC THROMBOCYTOPENIA (CAMT) (*MPL*)

These patients have a genetic defect in the *MPL* (1p34) gene, leading to a deficiency or dysfunction of the thrombopoietin receptor. They have neonatal thrombocytopenia with normal platelet size, lack of skeletal abnormalities associated with marrow megakaryocytic hypoplasia that evolves into marrow aplasia. It should be differentiated from Fanconi anemia, thrombocytopenia with absent radii (TAR), and congenital megakaryocytic thrombocytopenia with radioulnar synostosis (CTRUS) by the lack of other skeletal abnormalities, by hypoplasia of other cell lines, and by the presence of *MPL* mutations and high plasma levels of TPO. It is worth mentioning that the mutations of *MPL* associated with CAMT are distinct from the *MPL* mutations leading to transmembrane abnormalities and persistent receptor activation that are associated with congenital thrombocytosis and some myeloproliferative neoplasms.

CONGENITAL AMEGAKARYOCYTIC THROMBOCYTOPENIA WITH RADIOULNAR SYNOSTOSIS (CTRUS) (*HOXA11*)

Patients with this autosomal-dominant syndrome have a mutation in the *HOXA11* gene (7p15-14), which is involved in the development of both the forelimbs and megakaryocytes. They have thrombocytopenia of normal platelet size with a general aplastic anemia. Other associated abnormalities include radioulnar synostosis and sensorineural hearing loss, together with other skeletal malformations. Bone marrow evaluation reveals reduced to absent megakaryocytes.

THROMBOCYTOPENIA WITH ABSENT RADII (TAR) (*RBM8A*)

This autosomal-recessive disorder is characterized by severe thrombocytopenia of normal platelet size presenting within the first year of life. The thrombocytopenia ameliorates during infancy, and there is lack of marrow failure. The thrombocytopenia progressively improves, with normal platelet count during adulthood. The syndrome is accompanied by bilateral radial aplasia and other abnormalities, including cardiac and renal defects. The genetic defect involves *RBM8A*, which is involved in the TPO signaling pathway.

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DEFECTS OF PLATELET SURFACE RECEPTORS

GLANZMANN THROMBASTHENIA

Clinical Features

Glanzmann thrombasthenia is a congenital deficiency or dysfunction of GP IIb/IIIa (αIIb/β3 integrin), the fibrinogen receptor responsible for mediating platelet aggregation. It manifests in lifelong mucocutaneous bleeding, including purpura, epistaxis, gingival bleeding, and menorrhagia. The GP IIb/IIIa deficiency leads to a virtual absence of platelet aggregation to most agonists. Glanzmann thrombasthenia can be classified according to the amount of GP IIb/IIIa: type 1, 0% to 5% of normal; type 2, 6% to 25%; and variant disease, 50% to 100% with abnormal fibrinogen binding.

The genes for both αIIb and β3 are located at chromosome 17q21-23, with the αIIb gene *(ITGA2B)* having 30 exons and the β3 gene *(ITGB3)* having 15 exons. Mutations and partial deletions of both the GP IIb and GP IIIa have been described; updates can be accessed at http://sinaicentral.mssm.edu/intranet/research/glanzmann. The database currently lists 255 mutations of *ITGA2B* and 164 mutations of *ITGB3* (accessed 21 February 2017). Because of the large number of mutations identified, many patients with Glanzmann thrombasthenia are compound heterozygotes. Often, mutations of one subunit prevent the formation of the entire complex on the platelet surface by inhibiting transport of the αIIbβ3 complexes from the endoplasmic reticulum to the Golgi, and it is common to detect neither GP IIb nor GP IIIa on the surface of the platelet.

Pathologic Features and Laboratory Studies

The CBC in individuals with Glanzmann thrombasthenia is usually normal, with normal platelet morphology on the peripheral smear. This disorder has distinctive laboratory features, so bone marrow analysis is not indicated.

In patients with Glanzmann thrombasthenia, the bleeding time and PFA-100 result will be abnormal. No aggregation response will be seen upon addition of ADP-, collagen-, epinephrine-, and arachidonic acid–aggregating agents, whereas the ristocetin-induced aggregation is normal (Fig. 2.17). This finding is virtually diagnostic of Glanzmann thrombasthenia, but the disorder can be confirmed by platelet flow cytometry or crossed immunoelectrophoresis of platelet membrane proteins (see Table 2.8). Genetic diagnosis of Glanzmann thrombasthenia can be done by direct sequencing.

Differential Diagnosis

Glanzmann thrombasthenia has distinctive laboratory features and usually can be definitively diagnosed. However, afibrinogenemia, a rare deficiency of fibrinogen, can present

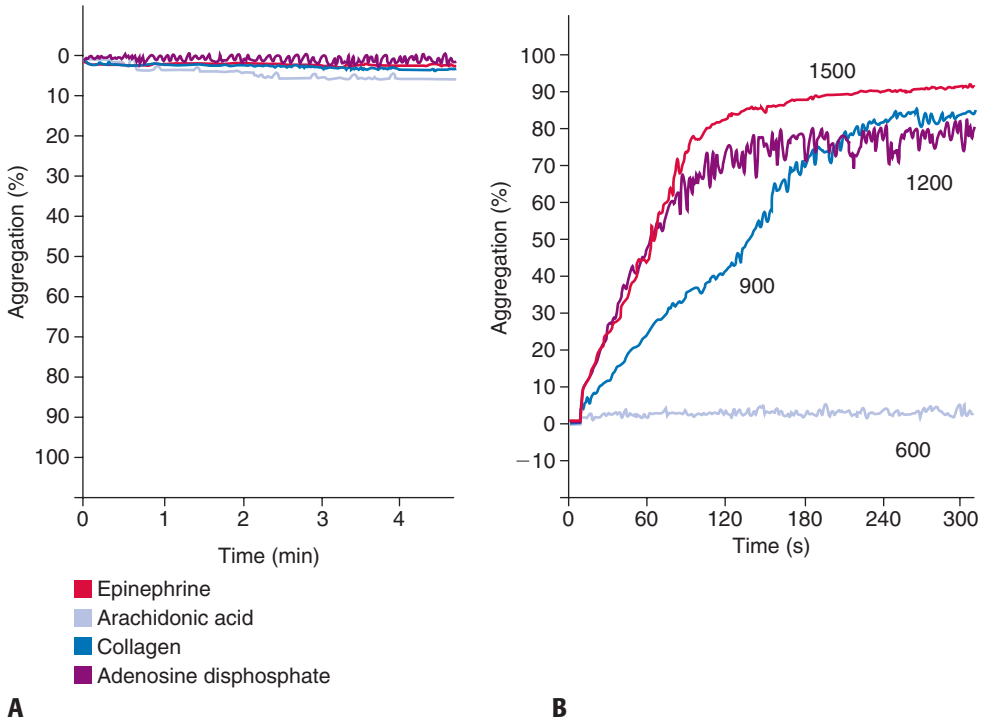


FIG. 2.17

Light transmission platelet aggregation in Glanzmann thrombasthenia. A, Note the absent aggregation response to adenosine diphosphate (ADP), epinephrine (EPI), collagen (COL), and arachidonic acid (AA) (optical platelet aggregation using a Biodata PAPS-4 aggregometer). B, The ristocetin dose response is normal (optical platelet aggregation using a Helena PACKS-4 aggregometer).

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TABLE 2.8
Aggregation Characteristics

Disorder	1°	2°	AA	EPI	Collagen	Ristocetin	Other Studies
SURFACE GLYCOPROTEIN DISORDERS							
Bernard-Soulier syndrome	N	N	N	N	N	↓ or absent	Macrothrombocytopenia, deficiency of GP Ib/IX/V (one or more) by flow cytometry, mutation of *GPIBA*, *GPIBB, GP9*
Glanzmann thrombasthenia	↓ or absent	↓ or absent	↓ or absent	↓ or absent	↓ or absent	N	Deficiency of GP IIb and/or GP IIIa by flow cytometry; mutation of *ITGA2B, ITGB3*
Platelet-type VWD	N	N	N	N	N	↑	FVIII:C, VWF:Ag, VWF:RiCof, VWF multimers
ADP receptor	↓	↓	N	N or ↓	N or ↓	N	Gene defects of *P2Y12*
GP Ia/IIa or GP VI disorder	N	N	N	N	↓	N	Mutations of GP Ia/IIa or GP VI genes *(ITGA2, GP6)*, decreased adhesion to collagen
STORAGE POOL DISORDERS							
Dense granule platelet storage pool disorder (δ-SPD)	N	↓	N	N or ↓	N or ↓	N	Decreased ATP release by lumiaggregometry ↓ dense granule mepacrine uptake and release by flow cytometry Decreased dense granules by TEM Abnormally high ATP/ADP ratio Acquired SPD "exhausted platelets" (CPB, DIC, TTP, HUS, MPD) Albinism in Hermansky-Pudlak and Chédiak-Higashi syndrome Infections, small platelets seen with Wiskott-Aldrich syndrome
Gray platelet disorder	Var	Var	N	N	Var ↓	N	Pale platelets on smear, ↓ a-granules by TEM, ↓ P-selectin
ACTIVATION DISORDERS							
Signal transduction disorders	N	↓	Var ↓	Var ↓	Var ↓	N	Decreased granule release with normal numbers of granules ↓ G-protein activation, phospholipase C activation, calcium mobilization, pleckstrin or tyrosine phosphorylation
Scott syndrome	N	N	N	N	N	N	Defective platelet procoagulant activity Defective microparticle formation
Defects of thromboxane synthesis or aspirin-like drug	N	↓↓	↓ or absent	↓	↓ or absent	N	Normal aggregation with prostaglandin G2 seen with aspirin or cyclooxygenase deficiency Decreased prostaglandin G2 aggregation with thromboxane synthetase deficiency

Continued

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TABLE 2.8
Aggregation Characteristics—cont'd

Disorder	1°	2°	AA	EPI	Collagen	Ristocetin	Other Studies
OTHER DISORDERS WITH PLATELET DYSFUNCTION							
Myeloproliferative disorder	N	N	N	↓ or absent	N	N	Other abnormalities: α- or δ-SPD, cyclooxygenase abnormality, other surface GP derangements, ↓ or ↑ aggregation to ADP, collagen, spontaneous aggregation
Uremia	N	↓	↓	N /↓	N /↓	N	Abnormal creatinine, BUN Decreased PF3
DRUG EFFECTS							
Thienopyridines	↓	Absent	N/↓	N or ↓	N or ↓	N	Clopidogrel or prasugrel therapy
GP IIb/IIIa antagonists	↓ or absent	↓ or absent	↓ or absent	↓ or absent	↓ or absent	N	History of abciximab, tirofiban, or eptifibatide
Aspirin	N/↓	↓↓	↓↓↓ or Absent	↓	↓	N	

AA, Arachidonic acid; *ADP,* adenosine diphosphate; *ATP,* adenosine triphosphate; *BUN,* blood urea nitrogen; *CPB,* cardiopulmonary bypass; *DIC,* disseminated intravascular coagulation; *EPI,* epinephrine; *GP,* glycoprotein; *HUS,* hemolytic uremic syndrome; *MPD,* myeloproliferative disorder; *N,* normal; *SPD,* storage pool disease; *TEM,* transmission electron microscopy; *TTP,* thrombotic thrombocytopenic purpura; *VWD,* von Willebrand disease; *VWF,* von Willebrand factor.

with similar initial platelet aggregation results with no response to ADP, epinephrine, collagen, and arachidonic acid. However, the aggregation defect in afibrinogenemia is restored with the addition of fibrinogen to the specimen. Additionally, patients with afibrinogenemia have unmeasurably long fibrinogen-dependent clotting tests, such as PT and APTT, and normal platelet flow cytometry. The GP IIb/IIIa antagonist drugs induce a Glanzmann-like inhibition of platelet function as part of their potent anti-platelet mechanism, but these are easily distinguished by the history of intravenous medication therapy.

Prognosis and Therapy

The bleeding symptoms in Glanzmann thrombasthenia are significant, and prophylactic therapy with platelet transfusions or DDAVP is recommended prior to major surgical procedures. DDAVP leads to increased blood levels of VWF and does not directly affect the platelets, but it has been shown to lessen bleeding complications in Glanzmann thrombasthenia.

GLANZMANN THROMBASTHENIA—FACT SHEET

Definition
- Congenital deficiency or dysfunction of GP IIb/IIIa (αIIb/β3 integrin), the fibrinogen receptor responsible for mediating platelet aggregation
- Type 1: 0% to 5% GP IIb/IIIa; type II, 6% to 25%; variant, 50% to 100% with abnormal fibrinogen binding
- More than 400 mutations/deletions of *ITGA2B* and *ITGB3* have been described

Incidence and Location
- Rare

Morbidity and Mortality
- Moderately severe bleeding disorder

Gender, Race, or Age Distribution
- Autosomal-recessive congenital disorder; many patients are compound heterozygotes

Clinical Features
- Lifelong mucocutaneous bleeding disorder

Prognosis and Treatment
- Routine treatment is not usually necessary
- Prophylactic therapy with DDAVP or platelet transfusions usually required prior to surgical procedures

BERNARD-SOULIER SYNDROME

Clinical Features

Bernard-Soulier syndrome is a rare congenital deficiency of the platelet GP Ibα/Ibβ/IX/V receptor, the surface

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GLANZMANN THROMBASTHENIA—PATHOLOGIC FEATURES

Complete Blood Count and Peripheral Smear Morphology
- Usually normal
- Normal platelet count and morphology

Bone Marrow Findings
- Not helpful

Platelet Screening Tests
- Abnormal PFA-100

Platelet Aggregation
- Characteristic abnormality; no aggregation response to ADP, collagen, epinephrine, and arachidonic acid with normal dose response to ristocetin

Ancillary Studies
- Flow cytometry or crossed immunoelectrophoresis show lack of expression of both GP IIb and GP IIIa
- Genetic diagnosis can be done by sequencing

Differential Diagnosis
- Distinctive laboratory features usually allow definitive diagnosis
- Afibrinogenemia can give similar aggregation findings, has undetectable fibrinogen levels and normal expression of GP IIb/IIIa

receptor for VWF-mediated platelet aggregation. It is usually classified as a macrothrombocytopenia disorder, with clinical findings of early-onset bleeding symptoms, thrombocytopenia, decreased platelet adhesion, reduced platelet survival, and giant platelets. Most of the Bernard-Soulier genetic defects are caused by mutations of *GPIBA,* the GP Ibα gene, but they may also be caused by defects of *GPIBB* and *GP9*, the GP Ibβ and GP IX genes, respectively. The large platelets are thought to be caused by the loss of interaction between actin-binding proteins in the platelet cytoskeleton and the GP Ibα cytoplasmic domain.

Pathologic Features and Laboratory Studies

Individuals with Bernard-Soulier syndrome typically have moderately severe thrombocytopenia (30 to 200 × 10^3/μL), with uniformly large, granulated platelets. Platelets occasionally are the size of erythrocytes or lymphocytes, so automated platelet counts are often inaccurate. This disorder has diagnostic surface GP abnormalities, which can be confirmed with flow cytometry or crossed immunoelectrophoresis.

Normal platelet aggregation is noted with exposure to ADP, collagen, epinephrine, and arachidonic acid, but aggregation is characteristically absent with the addition of ristocetin or botrocetin (Fig. 2.18; see Table 2.8). Patients

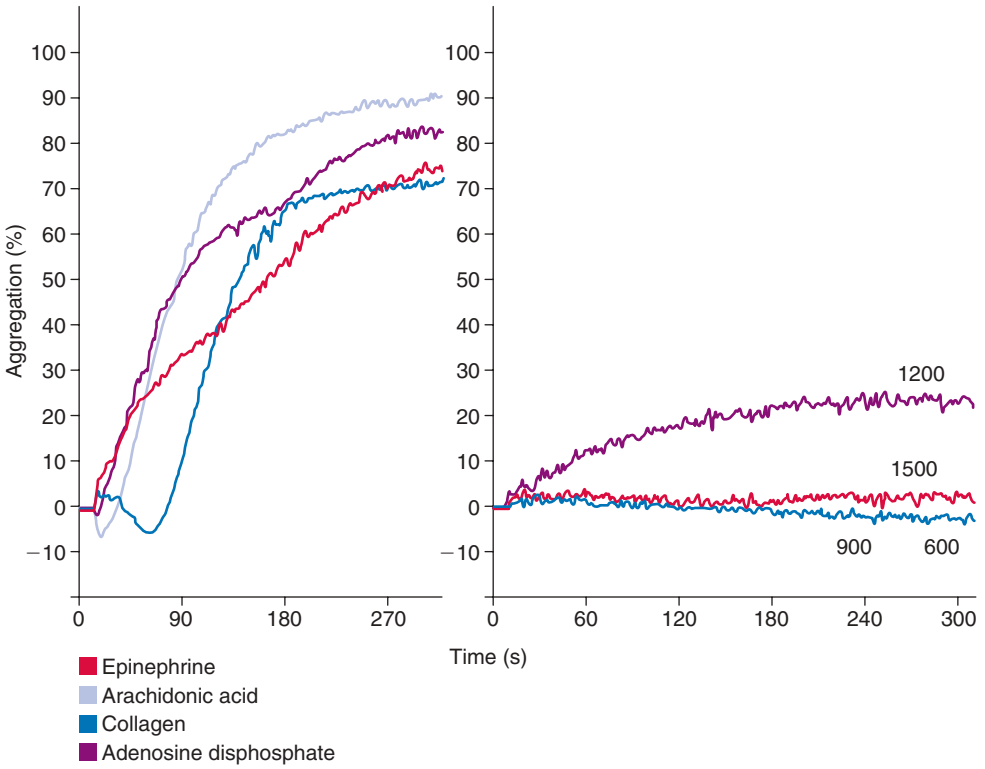


FIG. 2.18

Platelet aggregation in Bernard-Soulier syndrome A. The aggregation response to adenosine diphosphate (ADP), epinephrine (EPI), collagen (COL), and arachidonic acid (AA) is preserved. However, the aggregation response to ristocetin, which facilitates aggregation through von Willebrand factor binding to glycoprotein Ib, is nearly absent (optical platelet aggregation using a Helena PACKS-4 aggregometer).

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with Bernard-Soulier syndrome will also have abnormal PFA-100 results.

The genes *GPIBA* and *GPIBB* map to chromosomes 17 (17q12) and 22 (22q11.2), respectively. The genes *GP9* and *GP5* are both on chromosome 3, 3q21 and 3q29, respectively. Over 100 genetic defects in Bernard-Soulier syndrome have been described; most are caused by mutations of *GPIBA* and *GPIBB*. More rarely, mutations of *GP9* have been described. Although the genetic defect usually affects only one protein, the entire GP Ib/IX/V complex is not expressed on the platelet surface if there is a deficiency of the GP Ibα, GP Ibβ, or GP IX chains.

Differential Diagnosis

Bernard-Soulier syndrome, although rare, has characteristic surface GP abnormalities and is not often confused with other platelet disorders. However, the thrombocytopenia in Bernard-Soulier syndrome presenting in childhood can often be mistaken for ITP. The lack of neutrophil inclusions excludes MYH9 disorders.

Prognosis and Therapy

Patients with Bernard-Soulier syndrome are subject to a lifelong bleeding diathesis and can be at increased risk of bleeding during surgical procedures. Patients do not usually require therapy on a routine basis, but preoperative therapy is prudent.

BERNARD-SOULIER SYNDROME—FACT SHEET

Definition
- Macrothrombocytopenia with a congenital deficiency of the platelet glycoprotein Ibα/Ibβ/IX/V receptor
- Incidence and Location
- Rare, <1 per 1,000,000

Morbidity and Mortality
- Moderate to severe bleeding disorder

Gender, Race, or Age Distribution
- Autosomal-recessive congenital disorder

Clinical Features
- Moderate to severe mucocutaneous bleeding

Prognosis and Therapy
- Routine therapy is not required
- Increased bleeding risk during surgical procedures
- DDAVP and/or platelet transfusions may help prevent operative bleeding

BERNARD-SOULIER SYNDROME—PATHOLOGIC FEATURES

Complete Blood Count and Peripheral Smear Morphology
- Thrombocytopenia (30 to 100 × 10^3/μL) with large, normally granulated platelets

Bone Marrow Findings
- Not indicated for diagnosis; normal megakaryocyte morphology

Platelet Screening Tests
- Markedly abnormal PFA-100

Platelet Aggregation
- Absent aggregation response to ristocetin and botrocetin
- Normal aggregation to ADP, epinephrine, collagen, and arachidonic acid

Ancillary Studies
- Flow cytometry shows lack of platelet expression of GP Ib/IX
- Genetic mutations of *GPIBA, GPIBB*, and/or *GP9*

Differential Diagnosis
- Characteristic glycoprotein abnormalities are usually diagnostic
- Lack of neutrophil inclusions distinguishes Bernard-Soulier syndrome from *MYH9* disorders

There is no uniform therapeutic approach to these patients, but DDAVP and platelet transfusions are the most common. Owing to the risk of development of platelet refractoriness, the use of leukocyte-reduced platelets is advised. There is little experience with the use of recombinant factor VIIa.

PLATELET-TYPE VON WILLEBRAND DISEASE

Most types of VWD are caused by abnormalities of the VWF protein, but platelet-type VWD (also called pseudo-VWD) is caused by *GPIBA* mutations leading to a molecular abnormality of the GP Ib/IX/V VWF receptor on platelet surfaces. This is an autosomal-dominant inherited disorder in which an unusual gain-of-function abnormality of GP Ib leads to increased binding of platelet GP Ib/IX/V to VWF, with removal of high-molecular-weight VWF multimers from the plasma, resulting in a bleeding diathesis. It is covered above under von Willebrand disease.

GLYCOPROTEIN IV DISORDERS

Glycoprotein IV, also known as CD36, is a membrane glycoprotein expressed on platelets, monocytes, macrophages, and several other cell types that was recently demonstrated to be involved in platelet activation in response to oxidized phospholipids, including oxidized LDL. It also may have a role as an adhesive receptor for collagen and thrombospondin. It is deficient in approximately 4% to 7% of healthy donors

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in the Japanese population and 7% to 10% of healthy individuals in a sub-Saharan African population. A clinical association with GP IV deficiency has not been elucidated specifically.

COLLAGEN RECEPTOR DISORDERS

Glycoproteins Ia/IIa

The integrin $\alpha_2\beta_1$ (GP Ia/IIa) is a platelet surface receptor for collagen. Rare patients with deficiency of this receptor and selective absence of platelet aggregation to collagen have been described. These patients have decreased adhesion to vascular subendothelium, poor activation, and decreased spreading.

Glycoprotein VI

Glycoprotein VI is a member of the immunoglobulin superfamily of cell membrane receptors that participates with $\alpha_2\beta_1$ and FcRγ in platelet adhesion under shear. Deficiency of GP VI has been described in Japanese subjects in conjunction with a mild bleeding diathesis and lack of collagen-induced platelet aggregation. Defects in GP VI may be acquired owing to receptor loss by proteolysis involving the ADAM10 metalloproteinase.

ADENOSINE DIPHOSPHATE RECEPTOR ABNORMALITIES

Rare patients with a congenital platelet defect characterized by abnormalities of *P2Y12* and severe impairment of platelet response to ADP have been reported. A rare mutation of the *P2X1* gene has been identified that is associated with bleeding symptoms.

OTHER SURFACE GLYCOPROTEIN DEFECTS

Rare defects of the platelet receptors for thromboxane A2 and epinephrine have been described. These defects can be distinguished as they demonstrate a selective defect in aggregation to a single agonist.

PLATELET PROCOAGULANT DISORDERS

Platelets play an important procoagulant role with assembly of coagulation complexes on activated platelet membranes that are rich in phosphatidyl serine. A rare congenital platelet functional disorder is Scott syndrome, caused by defective a "flip" of phosphatidyl serine to the outer table of the platelet membrane and a bleeding disorder caused by a lack of thrombin generation. These patients will have normal platelet aggregation study results but have abnormal platelet procoagulant activity (platelet factor 3) and decreased microparticle formation. Mutations of *ANO6*, which encodes a phospholipid scrambling protein, and the ATP-binding cassette transporter A1 gene, *ABCA1*, leading to abnormal regulation of phosphatidyl serine translocase regulation, have been ascribed to Scott syndrome.

DEFECTS OF SECRETION AND ORGANELLE BIOGENESIS

Abnormalities of platelet secretion can be caused by either deficiency of platelet granules or defects in the signal transduction events that regulate secretion or aggregation. Platelet storage pool disorders (SPDs) are the result of either a deficiency of granules (α- and/or dense granules) or defective granule release upon platelet activation.

GRAY PLATELET SYNDROME

Clinical Features

Gray platelet syndrome (GPS) is a congenital α-granule SPD (α-SPD) that is also considered a macrothrombocytopenia disorder. Some patients may have marrow fibrosis, pulmonary fibrosis, and splenomegaly. The disorder is characterized by mild, lifelong bleeding symptoms.

Pathologic Features and Laboratory Studies

Individuals with GPS have a variable thrombocytopenia and large (mean 13 fL), gray-appearing platelets on the peripheral blood smear owing to decreased α-granules. Platelets may appear vacuolated. Bone marrow examination often shows reticulin fibrosis of the bone marrow, thought to be caused by an inability of megakaryocytes to store platelet-derived growth factor.

Platelet aggregation studies may be normal for ADP and epinephrine, but they are often abnormal for thrombin and collagen (see Table 2.8). Gray platelets are principally deficient in soluble proteins normally contained in α-granules, such as platelet factor 4, β-thromboglobulin, VWF, thrombospondin, and platelet-derived growth factor. In contrast, the α-granule membrane proteins are normal, suggesting that the disorder is primarily one of defective targeting and packaging of proteins into platelet α-granules. By electron microscopy, platelets are enlarged and α-granules are not present; however, dense granules, mitochondria, and lysosomes appear normal. Gray platelet syndrome has been associated with genetic variants in both *NBEAL2* (neurobeachin-like 2) and *GFI1B* (growth factor independence 1b).

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Differential Diagnosis

Pale platelets can also be seen with ongoing platelet activation and circulating "exhausted" platelets in patients with disseminated intravascular coagulation or with implanted cardiac devices, but in these patients, there will be a mixture of normal and pale platelets, and routine coagulation test results will be abnormal. A related disorder is the Quebec platelet syndrome, caused by tandem duplication of *PLAU*, the urokinase plasminogen activator gene, leading to degradation of α-granule proteins. In Quebec platelet syndrome platelets have normal α-granule ultrastructural morphology but demonstrate protease-mediated degradation of α-granule proteins.

QUEBEC PLATELET SYNDROME

Quebec platelet syndrome, an α-granule disorder, is caused by duplication of the *PLAU* gene, leading to increased urokinase plasminogen activator in α-granules. This results in degradation of α-granule proteins.

DENSE GRANULE STORAGE POOL DISORDERS

Dense granule SPDs (δ-SPDs) can be seen as a singular clinical entity or as part of other hereditary disorders, such as Chédiak-Higashi syndrome, Hermansky-Pudlak syndrome, thrombocytopenia with absent radii (TAR syndrome), or Wiskott-Aldrich syndrome (WAS). Patients with δ-SPD have a bleeding diathesis of variable severity, usually characterized by mucocutaneous bleeding. In more severely affected patients, postsurgical bleeding is also observed.

A decreased aggregation response to ADP, epinephrine, and collagen, with normal aggregation to arachidonic acid and ristocetin, is often seen with δ-SPD. Aggregation studies with ADP and epinephrine characteristically show only a primary wave of aggregation with an absent to decreased secondary wave, but aggregation studies with these agonists may be normal in as many as 25% of patients. The PFA-100 result may be abnormal in some, but not all, patients. Decreased lumiaggregometry ATP release and decreased mepacrine dense granule uptake are observed by flow cytometry. Agonist-stimulated mepacrine release from dense granules is usually not impaired. Ultrastructural abnormalities in these disorders show decreased dense granules, although dense granules are not usually absent. Often the dense granules lack their characteristic electron-dense core and appear empty. Two syndromic δ-SPD, Chediak-Higashi syndrome and Hermansky-Pudlak syndrome, have defined genetic causes, whereas most of the more common non-syndromic δ-SPDs remain genomically elusive.

Chediak-Higashi Syndrome

In patients with Chédiak-Higashi syndrome, there is neutropenia with recurrent infections in addition to both the SPD and oculocutaneous albinism. Neutrophils in patients with Chédiak-Higashi syndrome have characteristic

GRAY PLATELET SYNDROME—FACT SHEET

Definition
- Macrothrombocytopenia syndrome and a deficiency of platelet α-granules

Incidence and Location
- Rare

Morbidity and Mortality
- Mild bleeding disorder

Gender, Race, or Age Distribution
Autosomal-recessive or dominant congenital disorder

Clinical Features
- Mild, lifelong mucocutaneous bleeding disorder
- Bone marrow fibrosis

Prognosis and Therapy
- Routine treatment not necessary

GRAY PLATELET SYNDROME—PATHOLOGIC FEATURES

Complete Blood Count and Peripheral Smear Morphology
- Variable thrombocytopenia (30 to 100 × 10^3/μL)
- Uniformly large, pale, agranular platelets

Bone Marrow Findings
- Reticulin fibrosis

Platelet Screening Tests
- PFA-100 may be abnormal

Platelet Aggregation
- Sometimes normal; may be decreased with thrombin and collagen

Ancillary Studies
- Variants with mutations of *NBEAL2* or *GFI1B*

Differential Diagnosis
- In DIC there is usually a mixture of normal and degranulated platelets, as well as red blood cell fragments and coagulation abnormalities

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peroxidase-positive cytoplasmic granules. Chédiak-Higashi syndrome is distinguishable by the presence of large peroxidase-positive cytoplasmic granules in neutrophils and recurrent infections. The *LYST* gene responsible for Chédiak-Higashi syndrome codes a scaffolding protein involved in vesicle fusion and trafficking. It is located at 1q42-44, with more severe phenotypes associated with nonsense and frameshift mutations.

Hermansky-Pudlak Syndrome

Hermansky-Pudlak syndrome (HPS) is a well-defined autosomal-recessive syndrome with oculocutaneous albinism and variable hypopigmentation of hair, skin, and irises. The syndrome is prevalent in Puerto Rico. HPS is a disorder of subcellular organelles in many tissues, including dense granules of platelets. The HPS proteins interact in complexes called BLOCS, which, when disrupted by genetic mutations, leads to defective trafficking and sorting of dense granule membrane proteins. Seven different subtypes of the syndrome owing to genetic mutations of nine genes (*HPS-1* through *HPS-9*) have been described. Type 1 Hermansky-Pudlak syndrome is the most severe form; it is caused by a 16-bp duplication mutation of the *HPS-1* gene located at 10q23.1-13.3. This is the form of the syndrome most commonly observed in northwest Puerto Rico, where it has been described in 1 in 1800 individuals and 5 of 6 albinos and is associated with granulomatous colitis and pulmonary fibrosis. The other subtypes of HPS have been described in relatively few individuals, but the gene defects in these disorders have recently been elucidated owing to studies of associated SPDs in mice.

Hermansky-Pudlak syndrome can be distinguished from isolated δ-SPD by the associated oculocutaneous albinism and its prevalence in the Puerto Rican population. Platelets in HPS have very low levels of the α-granule membrane protein granulophysin, in contrast with normal levels in isolated δ-SPD. However, this assay is done only in the research setting.

SIGNAL TRANSDUCTION DISORDERS

In addition to the SPDs, platelet release defects can be seen with disorders of platelet signal transduction. In general, these are a poorly defined group of disorders, but they may constitute a significant percentage of patients with an abnormal secondary wave of aggregation and decreased granule release in which α- and δ-granules are not deficient. These disorders include defects of the platelet cyclooxygenase and signaling pathways, including G-protein activation, phospholipase C activation, calcium mobilization, pleckstrin phosphorylation, and tyrosine phosphorylation. Abnormalities of the guanidine triphosphate (GTP)-binding proteins that link surface receptors and intracellular enzymes, such as Gaq, Gai2, and Gas, have been described.

CYTOSKELETAL DISORDERS

MYH9 DISORDERS

Clinical Features

Mutations in the *MYH9* gene encoding for the nonmuscle myosin heavy chain IIA result in a spectrum of macrothrombocytopenia disorders with neutrophil inclusions, known as MYH9 disorders. Clinically, these disorders are characterized by a mild bleeding diathesis with large platelets and neutrophils with Dohle-like body inclusions, a ribonucleoprotein complex composed of aggregates of myosin IIA protein MYH9 mRNA, and clusters of ribosomes. There are variable clinical findings of nephritis, sensorineural hearing loss, and cataracts. There is a genotype-phenotype correlation, with *MYH9* mutations in the motor head domain of myosin IIA having severe macrothrombocytopenia and deafness, nephritis, and cataracts, whereas mutations in the tail domain have mild macrothrombocytopenia and low risk of non-hematologic complications.

Pathologic Features and Laboratory Studies

MYH9-related macrothrombocytopenia disorders are characterized by a decreased platelet count with large, granulated platelets and the presence of neutrophilic inclusions (Fig. 2.19). The Dohle-like bodies are blue, spindle-shaped inclusions in the periphery of the neutrophil cytoplasm. The thrombocytopenia is usually moderate, with typical platelet counts of 60 to 100 $\times 10^3/\mu L$, and the mean MPV is elevated at 12.5 fL or larger. Some caution should be exercised in interpreting CBCs performed on automated instruments in these patients, as some of the platelets are so large they are outside of the "platelet gate" and may be counted as leukocytes. Immunostains of the peripheral smear with an antibody against human nonmuscle myosin heavy chain IIA will show abnormal subcellular localization in leukocytes. Three patterns of inclusions have been described: type 1, 2, and 3. In type 1, there are one or two large, intensely stained granules. In type 2, there are up to 20 small granules. In type 3, there is speckled staining.

Platelet aggregation studies are normal, attesting to the increased functionality of the larger platelets. Owing to the large platelet size, centrifugation speeds for the preparation of platelet-rich plasma may have to be modified or many platelets may sediment with the leukocytes or erythrocytes. Over 40 distinct *MYH9* mutations have been described.

Differential Diagnosis

The MYH9 disorders can be distinguished from other macrothrombocytopenias by *MYH9* mutations, the presence

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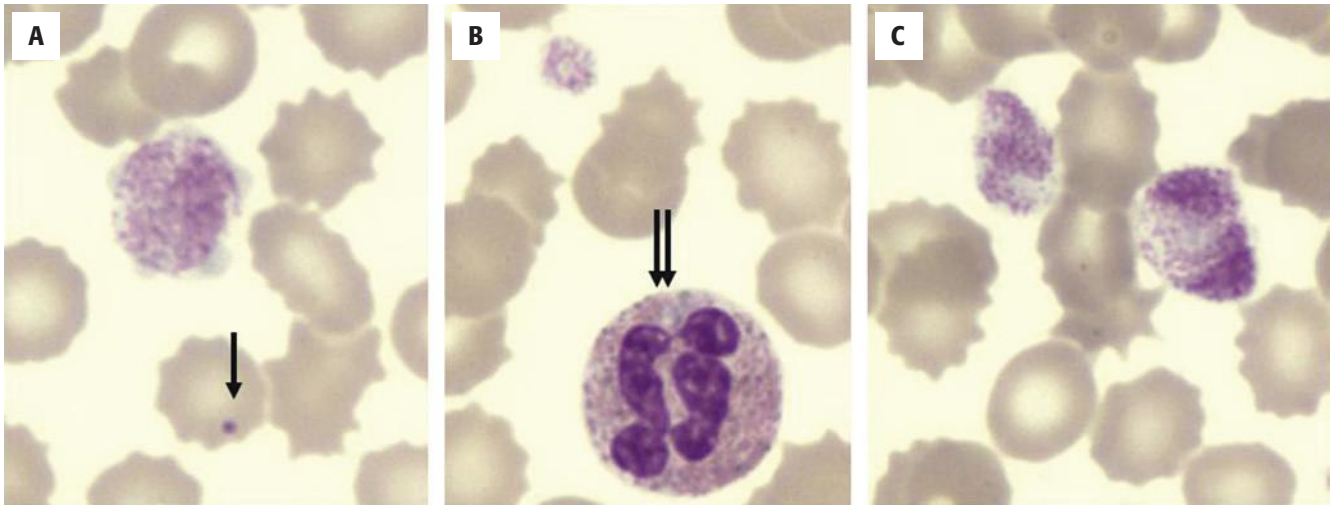


FIG. 2.19

Peripheral smear in MYH9-associated macrothrombocytopenia. A–C, Note the large granular platelets that are nearly the size of erythrocytes. In this patient, Howell-Jolly bodies were observed in the erythrocytes, a consequence of a splenectomy at age 4 years for suspected immune thrombocytopenic purpura (ITP; *arrow*). Patients with these rare macrothrombocytopenias, associated with a genetic disorder of the myosin heavy chain gene *(MYH9)*, may be thought to have ITP if the familial nature of the thrombocytopenia is not detected. The neutrophils show faint blue Dohle bodies *(double arrow)*. (A–C, Wright stain, original magnification ×100.)

MYH9 DISORDERS—FACT SHEET

Definition
- Macrothrombocytopenia owing to *MYH9* mutation
- 1 in 500,000

Morbidity and Mortality
- Associated with mild bleeding diathesis and variable nephritis, deafness, cataracts

Gender, Race, or Age Distribution
- Autosomal-dominant congenital disorder

Clinical Features
- Mutations in motor head region of myosin heavy chain II associated with more severe thrombocytopenia, nephritis, sensorineural hearing loss, ocular abnormalities

Prognosis and Therapy
- The mild bleeding disorder is not often associated with significant morbidity

MYH9 DISORDERS—PATHOLOGIC FEATURES

Complete Blood Count and Peripheral Smear Morphology
- Moderate thrombocytopenia (60 to 100 × $10^3/\mu L$) with giant, normally granulated platelets
- Cytoplasmic inclusions in neutrophils

Bone Marrow Findings
- Increased megakaryocytes with otherwise normal hematopoiesis
- Megakaryocytes are surrounded by production of large platelets

Platelet Screening and Platelet Aggregation Tests
- Usually normal

Ancillary Studies
- Abnormal subcellular localization of nonmuscle myosin heavy chain IIA in neutrophils. Type 1 (few large granules), type 2 (up to 20 smaller granules), and type 3 (speckled) immunofluorescent staining patterns
- Over 40 *MYH9* mutations have been described

Differential Diagnosis
- *MYH9* disorders can be distinguished from other macrothrombocytopenias by the presence of mutations in *MYH9*, neutrophilic inclusions, and abnormal localization of nonmuscle myosin heavy chain IIA in polymorphonuclear neutrophils

of neutrophilic inclusions, and abnormal neutrophil localization of nonmuscle myosin heavy chain IIA.

Prognosis and Therapy

The bleeding symptoms in the MYH9 disorders are usually mild and are not often a source of significant morbidity or mortality; the morbidity in these disorders is more related to the severity of the renal abnormalities.

WISKOTT-ALDRICH SYNDROME AND X-LINKED THROMBOCYTOPENIA

Clinical Features

These are related X-linked recessive disorders caused by a defect in the Wiskott-Aldrich syndrome gene characterized

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by immunodeficiency, recurrent infections, eczema, and thrombocytopenia with small platelets and a platelet SPD. The WAS protein (WASp) is involved in signal transduction and also is known to regulate actin filament assembly. Deficiency of WASp induces premature protoplatelet formation. These individuals also will have absent immunologic responses to polysaccharide antigens, and a subset of patients will develop malignancies, primarily lymphomas and leukemias. More than 300 mutations of the WAS gene on the X chromosome have been identified, with the majority of missense mutations in exons 1–4. The XLT form involves defects in exon 2 of the WAS gene and is accompanied by a minimal immunodeficiency. These patients have an increased risk of bleeding, even at modestly low platelet counts, owing to small platelet mass and platelet dysfunction. The thrombocytopenia often responds to splenectomy with a return in both platelet count and size, but postsplenectomy sepsis is a risk. Female carriers are asymptomatic.

Pathologic Features and Laboratory Studies

The peripheral smear shows a decreased number of uniformly small platelets, and the MPV is often low (approximately half normal size). Bone marrow evaluation shows normal megakaryocyte number and morphology.

Platelet dysfunction is severe; the platelets are unable to aggregate to ADP, epinephrine, and collagen. Platelets have decreased dense granules and a storage pool pattern.

Differential Diagnosis

Patients with thrombocytopenia caused by marrow aplasia may also have small platelets, but the MPV is usually low normal, not decreased. Patients with TORCH (*to*xoplasma-*r*ubella-*c*ytomegalovirus-*h*erpes) infection may also have small platelets, but clinical and serologic features are distinctive. The mild thrombocytopenia seen in XLT may be misdiagnosed as ITP.

ACQUIRED PLATELET DISORDERS

PLATELET DISORDERS WITH THROMBOCYTOSIS

Thrombocytosis is most typically acquired and may be seen in the setting of myeloproliferative neoplasms (essential thrombocythemia [ET], chronic myelogenous leukemia [CML], polycythemia vera [PV], and primary myelofibrosis [PM]), or some myelodysplastic processes (5q-). There are also reactive thrombocytoses associated with an underlying disorder, such as iron deficiency.

Patients with elevated platelet counts may have clinical bleeding but may also be asymptomatic or have thrombosis. Laboratory evaluation should be aimed at elucidating the cause of the thrombocytosis and should include a CBC, peripheral blood smear, bone marrow evaluation, cytogenetic study, and platelet aggregation study.

Myeloproliferative neoplasms (MPNs) associated with thrombocytosis include CML, ET, PM, and PV. A large proportion of patients with non-CML MPNs have a mutation of the Jak2 tyrosine kinase, JAK2-V617F, through which the Epo-R, Mpl, and Gcf-R receptors signal. MPNs are associated with thrombocytosis, accompanied by thrombosis and/or bleeding, with progression to acute leukemia and/or marrow failure and fibrosis. The reader is directed to Chapter 17 for further discussion of the clinical and diagnostic features of these disorders. Platelet testing is not needed for diagnosis, which is based on bone marrow and cytogenetic studies. Aggregation studies in patients with MPNs are often abnormal, but the patterns observed are neither specific nor diagnostic.

A reactive, or secondary, thrombocytosis is not a single disease entity and can be associated with many clinical disorders, such as iron deficiency and inflammatory and infectious diseases; malignancies, such as carcinomas or lymphomas; smoking; or exercise. It can also be observed as a rebound thrombocytosis after splenectomy or during treatment for immune thrombocytopenic purpura or pernicious anemia, or after cessation of myelosuppressive drugs. Reactive thrombocytoses are usually associated with normal platelet aggregation studies. In contrast to myeloproliferative disorders, cytogenetic studies do not show a clonal process; there is absence of the BCR-ABL translocation and JAK2 V617F, MPL, and TPO mutations.

ACQUIRED THROMBOCYTOPENIAS WITH DECREASED PLATELET PRODUCTION

These disorders are caused by ineffective megakaryopoiesis and are usually associated with many disorders of bone marrow failure, such as aplastic anemia, drug-induced aplasia, toxic chemicals, viral infection, radiation, myelophthisic disorders, paroxysmal nocturnal hemoglobinuria, and myelodysplasia. A few rare disorders, such as acquired amegakaryocytic thrombocytopenic purpura and cyclic thrombocytopenia, affect platelet production only.

ACQUIRED THROMBOCYTOPENIAS WITH INCREASED PLATELET TURNOVER

The finding of adequate or increased megakaryocytes on the bone marrow or increased reticulated platelets suggests peripheral platelet destruction. Platelet function tests are usually not helpful in differentiating among the entities in this class of disorders because most functional studies will give abnormal results simply owing to the low platelet number. The overall MPV is usually normal with destructive thrombocytopenia, but there is typically a range of platelet size with increased platelet distribution width (PDW) and many

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large platelets, indicating the rapid platelet turnover. These disorders are invariably acquired and an underlying abnormality should be sought. In general, the clinical scenario is most helpful in classifying these disorders.

IMMUNE THROMBOCYTOPENIC PURPURA

Clinical Features

ITP is a disorder characterized by thrombocytopenia caused by platelet sensitization with autoantibodies leading to platelet destruction in the reticuloendothelial system. However, the recent success of treatment with thrombopoietin receptor agonists also suggests that platelet production may also be insufficient in ITP. ITP is a diagnosis of exclusion defined clinically as thrombocytopenia in the presence of a normal hemoglobin level and a normal white blood cell count and differential and in the absence of hepatosplenomegaly, lymphadenopathy, and abnormalities of the radii or other underlying disease. Patients usually present with an acute onset of bleeding symptoms and marked thrombocytopenia. Chronic ITP refers to patients who have had the illness for 12 months or more, and refractory ITP is defined as patients with severe symptoms who have failed previous therapies, including splenectomy.

Adult ITP has an incidence of 1 in 100,000 with a 2:1 to 3:1 female-to-male predominance. Childhood ITP has a peak age incidence of 2 to 4 years, with girls and boys affected equally. ITP is often associated with viral infections and may have a genetic predisposition.

Pathologic Features and Laboratory Studies

The platelet count is decreased and may be markedly decreased in cases of acute ITP. The pathologic features of ITP on the peripheral smear are nondiagnostic, showing a mixture of normal and larger platelets, with the MPV being normal to slightly increased. Erythrocyte and granulocyte counts and indices should be normal. In uncomplicated ITP, the bone marrow usually demonstrates normal to increased numbers of morphologically normal megakaryocytes, with occasional megakaryocyte clustering. Autoantibodies to surface GPs can be detected by flow cytometry or immunoassay, although these findings may be neither sensitive nor specific. Platelet functional tests are usually not helpful in ITP because the low platelet count alone can give abnormal functional test results.

Differential Diagnosis

The diagnostic approach usually involves excluding other causes of thrombocytopenia, such as congenital thrombocytopenias, and other causes of destructive thrombocytopenia, such as hypersplenism, HIT, TTP, and DIC.

Prognosis and Therapy

Patient prognosis in ITP is usually good, but severe bleeding complications can be seen when the platelet count is less than 10 to $20 \times 10^3/\mu L$. Acute treatment is aimed at increasing the platelet count through immunoglobulin therapy (intravenous immunoglobulin or anti-D), steroids, vincristine, or plasmapheresis. Approximately 50% of children and 10% to 30% of adults respond to initial therapy. Long-term therapy, if necessary, may entail splenectomy. There has been therapeutic success with rituximab, a CD20 monoclonal antibody, and thrombopoietin (TPO) receptor agonists, such as romiplostim and eltrombopag.

Alloimmune Thrombocytopenias

Alloimmune thrombocytopenias include posttransfusion purpura and neonatal alloimmune thrombocytopenia. Posttransfusion purpura is a rare alloimmune disorder that occurs in HPA-1a–negative individuals characterized by posttransfusion thrombocytopenia owing to immune destruction of both

IMMUNE THROMBOCYTOPENIC PURPURA—FACT SHEET

Definition
- Immune destructive thrombocytopenia owing to platelet autoantibodies

Incidence and Location
- 1:100,000

Morbidity and Mortality
- Mild to severe mucocutaneous bleeding disorder

Gender, Race, or Age Distribution
- 2: to 3:1 female-to-male predominance
- Childhood ITP has a peak age incidence of 2 to 4 years with 1:1 female-to-male ratio

Clinical Features
- Acquired bleeding disorder

Prognosis and Therapy
- Prognosis is usually good, but severe bleeding can be seen when the platelet count is less than $10 \times 10^3/\mu L$
- Acute treatment is usually aimed at increasing the platelet count by immunoglobulin therapy (intravenous immunoglobulin or anti-D), steroids, vincristine, or plasmapheresis. Some success with rituximab (monoclonal antibody against CD20) and thrombopoietin receptor agonists
- Long-term therapy, if necessary, may entail splenectomy

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IMMUNE THROMBOCYTOPENIC PURPURA—PATHOLOGIC FEATURES

Complete Blood Count and Peripheral Smear Morphology
- Thrombocytopenia, often severe, with an otherwise normal CBC
- Mixture of normal and large platelets; the MPV normal to slightly increased

Bone Marrow Findings
- Normal to increased megakaryocytes with occasional megakaryocyte clustering

Platelet Screening Tests
- Abnormal PFA-100, usually in proportion to the degree of thrombocytopenia

Platelet Aggregation
- Not usually helpful because the degree of thrombocytopenia alone may result in aggregation abnormalities

Ancillary Studies
- Autoantibodies to GPs are present, but this finding is neither sensitive nor specific
- Elevated thrombopoietin and increased reticulated platelets

Differential Diagnosis
- ITP is a diagnosis of exclusion
- Exclude congenital thrombocytopenias and other destructive thrombocytopenias, such as hypersplenism, HIT, TTP, and DIC

transfused and recipient platelets. Neonatal alloimmune thrombocytopenia (NAIT) is a severe thrombocytopenia caused by maternal immunization against fetal platelet antigens. It is the platelet equivalent of hemolytic disease of the newborn. Maternal antibodies cross the placenta and cause the severe thrombocytopenia that can result in intracranial hemorrhage. Sixteen HPAs have been described with NAIT, but 95% of cases in white populations are caused by mutations in HPA-1a, HPA-5b, or GHP-15b, with 75% of cases attributable to HPA-1a.

Drug-Induced Thrombocytopenias

Drug-induced thrombocytopenia caused by immunologic and/or nonimmunologic platelet destruction is most commonly associated with quinidine, quinine, heparin, sulfonamide drugs, and gold salts. Various mechanisms are involved, with the drug acting as either a direct or an indirect hapten. The development of immune complex formation and thrombocytopenia in response to heparin is characteristic of HIT (discussed previously).

Other Causes of Thrombocytopenia With Increased Platelet Turnover

Other causes of consumptive or destructive thrombocytopenia include thrombocytopenia associated with hypertensive pregnancy disorders, the Kasabach-Merritt syndrome, hypersplenism, human immunodeficiency virus–associated thrombocytopenia, malignancy, and connective tissue disorders. Kasabach-Merritt syndrome is an infrequent disorder with cavernous hemangiomas associated with a microangiopathic hemolytic anemia, consumptive coagulopathy, and thrombocytopenia. Hypersplenism is associated with a destructive thrombocytopenia owing to platelet sequestration in an enlarged spleen. Hypertensive disorders of pregnancy account for approximately 20% of pregnancy-associated thrombocytopenia, and include preeclampsia, eclampsia, and HELLP (*h*emolysis, *e*levated *l*iver enzymes, and *l*ow *p*latelets), which may be associated with a thrombotic microangiopathy.

PLATELET DYSFUNCTION ASSOCIATED WITH OTHER ILLNESSES

Other significant disorders of platelet function are usually acquired with the presence of another disease or drug therapy. These are, by far, more common than the disorders described previously. Platelet dysfunction is often observed with chronic renal failure or liver disease and in patients with a variety of myeloproliferative and lymphoproliferative disorders. Platelet dysfunction also may be associated with a variety of clinical scenarios, such as cardiopulmonary bypass, implantation of vascular grafts, prosthetic heart valves, and ventricular assistive devices. Platelet dysfunction in these disorders is usually difficult to characterize, as nonspecific defects of platelet aggregation are usually observed.

SUMMARY

Multiple causes exist for platelet-derived disorders, including the hereditary disorders of platelet production, cell membrane, cytoplasmic granules, signaling, and cytoskeleton and the acquired disorders of platelet destruction and myeloproliferative neoplasms. The laboratory evaluation of these disorders can range from simple to complex. However, the laboratory evaluation of all these disorders should start with a clinical history, followed by the measurement of the platelet count and evaluation of platelet morphology on a well-prepared blood smear. This should be followed by platelet function tests, ultrastructural morphology, and increasingly available genomic studies.

The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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3

Non-Neoplastic Morphologic Abnormalities of White Blood Cells and Macrophages

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■ INTRODUCTION

A variety of morphologic changes can occur in non-neoplastic white blood cells (WBCs). Some reflect nonspecific reactive changes, whereas others are specific and are associated with clinically significant disorders. In this chapter we review non-neoplastic changes representing a spectrum of morphologic abnormalities of leukocytes and macrophages seen in congenital or acquired disorders. The standard organization seen in other chapters will not be followed here, because these disorders may not necessarily represent distinct biological entities and do not lend themselves easily to such a format.

CONGENITAL MORPHOLOGIC ABNORMALITIES OF WHITE BLOOD CELLS

Congenital morphologic changes in WBCs are rare. In many cases, there is a known history of a genetic disorder, and the corresponding WBC changes are identified subsequent to an established diagnosis. On rare occasions, an astute morphologist may suggest a diagnosis, based on the morphologic changes seen in either the peripheral blood smear or in a bone marrow sample.

PELGER-HUET ANOMALY

The Pelger-Huet anomaly is characterized by granulocytes that are either bilobed or completely unsegmented (Fig. 3.1). The bilobed neutrophils are referred to as *pince-nez* ("nose pinch") *forms,* referring to an old style of eyeglasses that sat on the bridge of the nose. Eosinophils typically have a unilobated nucleus. The chromatin is mature with heavy condensation, which is the same as seen in normal mature neutrophils. All cells in a peripheral blood smear will be affected. The cells are typically found only on manual review of peripheral blood smears, because automated hematology analyzers do not detect the neutrophils as abnormal in patients with Pelger-Huet anomaly. Inheritance of Pelger-Huet anomaly is usually autosomal dominant, although it is likely that new mutations are common. The prevalence is estimated at 0.01%. The unilobed forms of Pelger-Huet cells are occasionally called *Stodtmeister forms.* These cells can be the predominant cell type in the exceedingly rare homozygous form of Pelger-Huet anomaly. Homozygous patients may have varying degrees of psychomotor retardation, abnormal body stature, macrocephalus, ventricular septal defect, polydactyly, developmental delay, epilepsy, and skeletal abnormalities. In heterozygous Pelger-Huet anomaly, neutrophil function is normal, and there are no other clinical abnormalities. The Pelger-Huet anomaly is due to mutations in the laminin beta-receptor (LBR) gene that have been discovered in several kindreds. The gene maps to chromosome 1q41-q43.

Morphologic mimics of Pelger-Huet anomaly are more common than the true disorder (Table 3.1). The changes of hypolobation can be seen primarily in three circumstances: as a reactive condition in association with infections (typically severe), in association with medications or drugs such as mycophenolate or valproate, or in cases of myelodysplastic syndromes or other myeloid stem cell disorders. When this hypolobation is seen in these circumstances, it is referred to as *pseudo–Pelger-Huet* or *Pelgeroid change*. Distinguishing the mimics and true Pelger-Huet anomaly is usually straightforward. In the case of myelodysplastic syndrome (MDS) or other myeloid neoplasms, the hypolobation is often accompanied by hypogranulation of neutrophils, as well as other dysplastic changes seen in erythroid elements, platelets, and megakaryocytes. In particular, isolated isochromosome 17q is a cytogenetic abnormality specifically associated with these changes. Patients with a myeloid neoplasm with isolated i17q have been characterized as having MDS/myeloproliferative features (including Pelgeroid changes),

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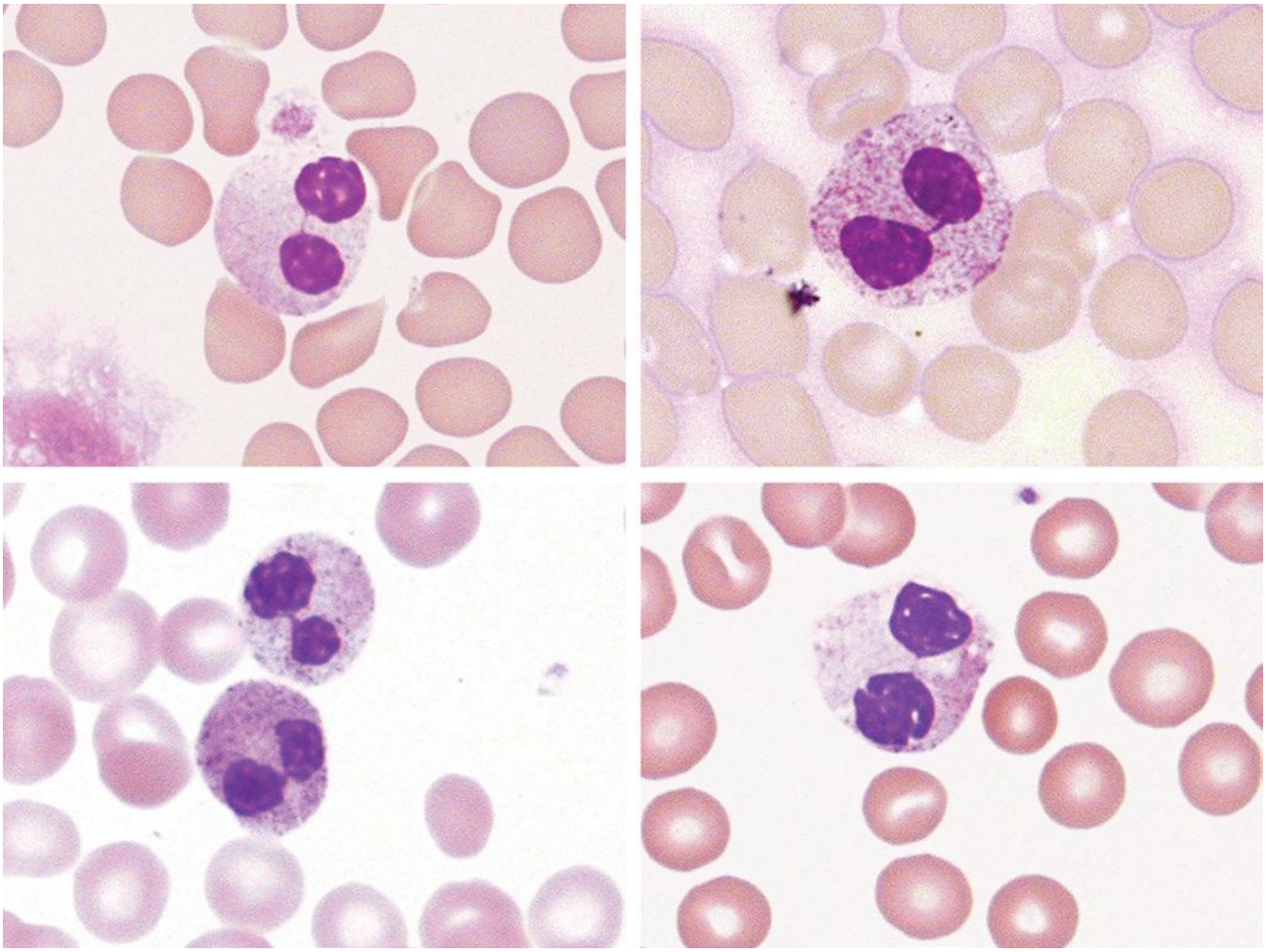


FIG. 3.1

Multiple neutrophils with Pelger-Huet anomaly showing the classic, bilobed appearance of the nuclei. Note the presence of normal granulation of the cytoplasm. In myelodysplastic syndrome, the neutrophils are often hypogranular and hypolobated.

TABLE 3.1

Potential Causes of Pelger-Huet–Like Changes in Granulocytes

Medications	Chemicals	Hematologic Disorders	Systemic Disorders	Infectious Disorders
Colchicine	Benzene	Acute myeloid leukemia	Enteritis	Influenza
Docetaxel	Urethane	Chronic myelogenous leukemia	Muscular dystrophy	HIV
Fluconazole		Erythroleukemia	Myxedema	Malaria
Ganciclovir		Fanconi anemia	Systemic lupus	Mycoplasma pneumonia
Ibuprofen		Granulocytosis	Trisomy 21	Tuberculosis
Melphalan		Hodgkin lymphoma		
Mycophenolate mofetil		Immune thrombocytopenic purpura		
Paclitaxel		Leukemoid reaction		
Sulfisoxazole		Megaloblastic anemia		
Sulfonamide		Myelodysplastic syndrome		
Tacrolimus		Myelofibrosis		
Valproic acid		Non-Hodgkin lymphoma		
		Plasma cell myeloma		
		Polycythemia vera		

Data from Speeckaert MM, Verhelst C, Koch A, Speeckaert R, Lacquet F: Pelger-Huët. anomaly: a critical review of the literature, *Acta Haematol* 121:202–206, 2009.

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wild-type TP53, but frequent recurrent mutations in *SRSF2, SETBP1, ASXL1*, and *NRAS* (30% to 60% reported incidence for each gene). In both reactive conditions and dysplastic-type changes, only a subset of neutrophils is usually affected, in contrast to the entire population in Pelger-Huet anomaly. Toxic changes of granulocytes may also be seen in cases associated with infection. Identification of drug-related cases requires knowledge of the clinical history and a high index of suspicion.

MYELOKATHEXIS

Myelokathexis is an exceedingly rare disorder of neutrophil development and function. Patients exhibit severe neutropenia, evident in infancy, which results in recurrent bacterial or fungal infections. The inheritance pattern is typically autosomal dominant, but recessive and sporadic cases have been described. Neutrophil precursors and especially mature neutrophils are retained in the bone marrow (*kathexis* = retention), leading to hypercellularity and increased myeloid-to-erythroid (M:E) ratio. Morphologically, the neutrophil and neutrophilic marrow precursors have marked abnormalities of nuclear shape and lobation (Fig. 3.2). Often, the chromatin appears fragmented and broken, with pyknotic chromatin lobes connected by a long strand of chromatin. Cytoplasmic vacuoles may be present. There are significant functional abnormalities of neutrophils and other marrow lineages, leading to abnormal innate immunity. Other abnormalities that have been associated with myelokathexis include skeletal abnormalities and growth retardation. Treatment with growth factors has been successful in some patients. Mutations in the chemokine receptor type-4 gene (*CXCR4*; *CD184*) appear to be responsible for WHIM syndrome, which includes myelokathexis as a key component. *CXCR4* signaling plays a critical role in bone marrow cellular homing.

WHIM syndrome is an autosomal-dominant immunodeficiency disease characterized by *w*arts, *h*ypogammaglobulinemia, *i*mmunodeficiency, and *m*yelokathexis. These patients have increased susceptibility to human papillomavirus infection and increased upper respiratory infections. *CXCR4* also plays a role in lymphocyte trafficking and B-cell development and affinity maturation. A small molecule antagonist of *CXCR4* (plerixafor) improves mobilization of neutrophils from bone marrow, and this and similar drugs may significantly improve quality of life in patients with WHIM syndrome.

CHÉDIAK-HIGASHI SYNDROME

Chédiak-Higashi syndrome (CHS) is an autosomal-recessive disorder that manifests in early childhood. It is characterized by immunodeficiency, recurrent infection, oculocutaneous albinism, bleeding tendency, and multiple neurologic abnormalities. The molecular defect has been defined as mutation in the *CHS1 (LYST)* gene located on chromosome 1q42.1-1q42.2. This gene product is involved in lysosomal trafficking. Abnormal endosomal-lysosomal fusion occurs in these patients and results in large, abnormal granules within granulocytes, monocytes, and lymphocytes (Fig. 3.3). These abnormal granules result from fusion of normal secondary granules of neutrophils, eosinophils, basophils, or the cytotoxic granules

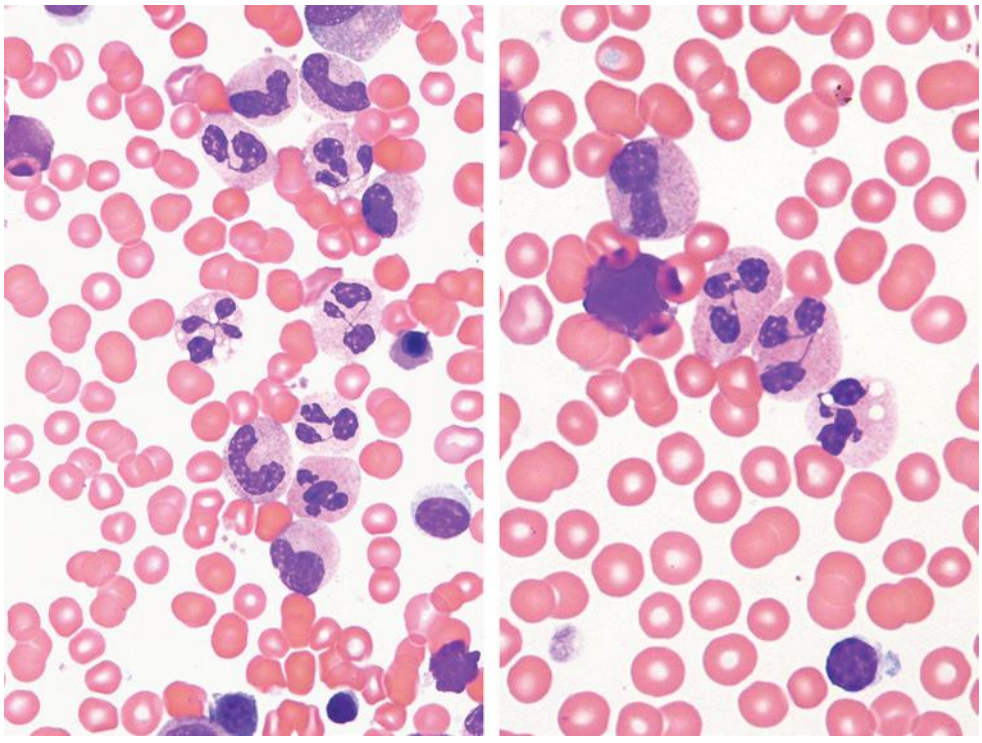


FIG. 3.2

Bone marrow aspirate in myelokathexis. Note the abnormal nuclear segmentation and connecting strands of nuclear material. *(Courtesy S. Kroft, Medical College of Wisconsin.)*

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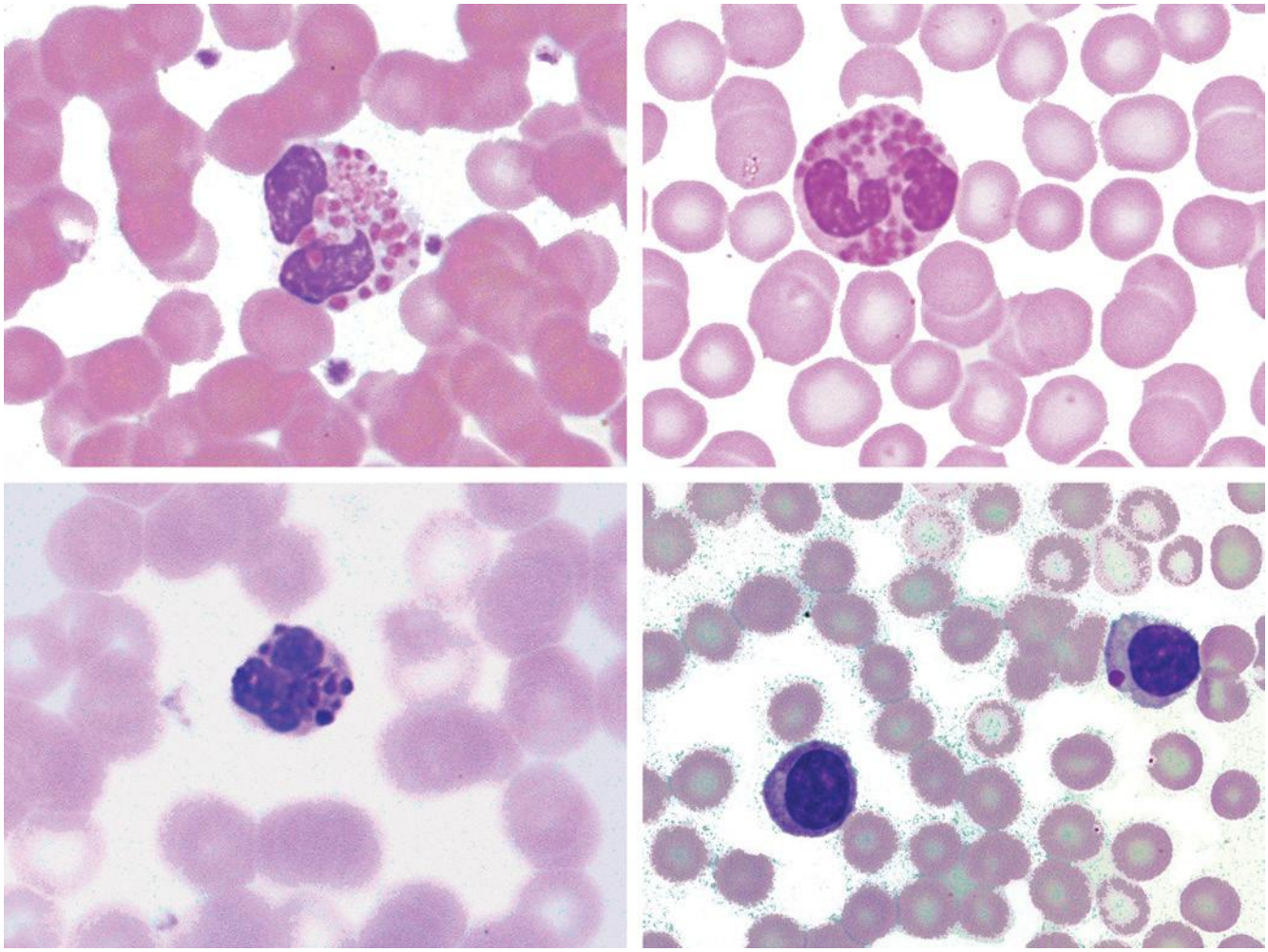


FIG. 3.3
Abnormal large granules of Chédiak-Higashi syndrome seen in a neutrophil, eosinophil, basophil, and lymphocyte.

of monocytes or lymphocytes. Peripheral blood smears show abnormal giant cytoplasmic granules in leukocytes.

The neutrophils do not function appropriately, leading to increased susceptibility to bacterial infections. Natural killer (NK) cell function is also impaired. Deficiencies of pigmentation are due to abnormal, giant melanosomes.

Patients who do not succumb to infection may eventually develop an unusual lymphoproliferative syndrome, the so-called accelerated phase of the disorder, characterized by generalized lymphohistiocytic infiltrates, fever, jaundice, hepatosplenomegaly, lymphadenopathy, pancytopenia, and bleeding. Allogeneic bone marrow transplantation has been successful in some cases and improves the hematologic and immunologic defects but does not affect the neurologic complications.

Morphologic changes similar to CHS occur rarely in acute myeloid leukemia (AML) or MDS, including acute promyelocytic leukemia. In these instances, there may be abnormal large granules; however, they are not found in the context of a pediatric patient with other genetic anomalies, but usually in adult patients with underlying myeloid disorders. Furthermore, the abnormal granules are usually limited to a subset of cells; they are most commonly seen in a population of myeloid blasts and are not seen in other cell lines such as granular lymphocytes.

ALDER-REILLY ANOMALY

The morphologic changes in myeloid cells termed *Alder-Reilly anomaly* are seen in patients with mucopolysaccharidoses (MPS). Seen predominantly in granulocytes, although also variably present in monocytes, there are large numbers of relatively coarse, azurophilic granules in the cytoplasm resembling toxic granulation (Fig. 3.4). Patients with MPS have either X-linked or autosomal-recessive abnormalities in the metabolism of mucopolysaccharides. Historically, eponymous names (e.g., Hunter syndrome, Hurler syndrome, Morquio syndrome, Sanfilippo syndrome, Scheie syndrome) and the term *gargoylism,* referring to the coarse facies seen in these patients, have been used to refer to this group of diseases; currently they are classified according to the

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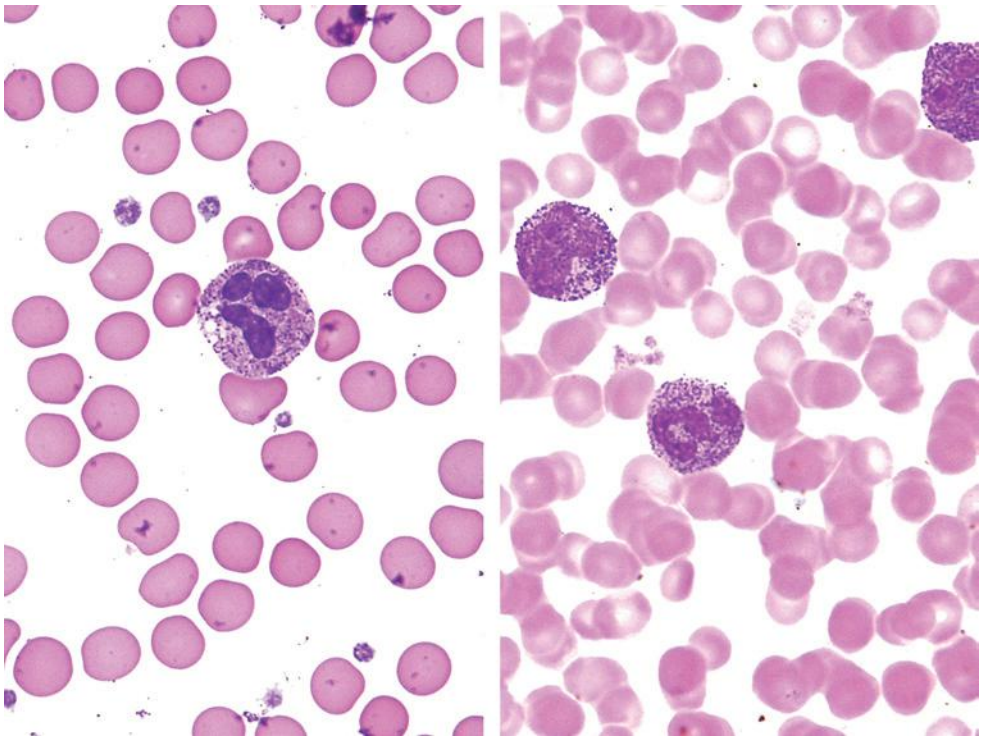


FIG. 3.4

Two examples of Alder-Reilly anomaly. The degree of granulation can vary in individual cases. The coarse, azurophilic granules most resemble toxic changes in neutrophils.

TABLE 3.2

Features of *MYH9* Mutation Disorders

Clinical Feature	May-Hegglin Anomaly	Sebastian Syndrome	Fechtner Syndrome	Epstein Syndrome
Macrothrombocytopenia	+	+	+	+
Döhle-like bodies	+	+	+	–
Hearing impairment	–	–	+	+
Cataracts	–	–	+	–
Nephritis	–	–	+	+

underlying enzymatic defects, and then named according to type (e.g., MPS type I, type II, etc.). All these disorders are associated with numerous other physical abnormalities with varying degrees of severity, which are beyond the scope of this chapter, and the interested reader is referred to the reference list. The Alder-Reilly anomaly should not be mistaken for typical toxic granulation. A mimic has also been described in association with myelodysplasia.

MAY-HEGGLIN ANOMALY

The group of May-Hegglin, Fechtner, Sebastian, and Epstein syndromes are collectively referred to as *MYH9-related disorders.* Most cases are of autosomal-dominant inheritance, but up to 30% of cases are sporadic. Characteristics are shown in Table 3.2. All these disorders have mutations in the gene encoding nonmuscle myosin heavy chain-9 *(MYH9)* located at chromosome 22q12.3-q13.2. Numerous different mutations have been identified. May-Hegglin is characterized by the triad of thrombocytopenia, giant platelets, and inclusion bodies in leukocytes (Fig. 3.5). Most patients are asymptomatic, although some may have a mild bleeding tendency. Patients are discovered because of thrombocytopenia. The main complications are misdiagnosis as another disorder, such as idiopathic thrombocytopenic purpura, and undue therapy.

The characteristic white blood cell abnormality in May-Hegglin anomaly is the presence of large blue cytoplasmic inclusions that resemble Döhle bodies. The Döhle-like bodies are composed of paracrystalline arrays of ribosomes and RNA and are larger (2 to 4 μm) than true Döhle bodies. Furthermore, Döhle-like bodies are seen in the absence of toxic granulation and are not limited to neutrophils. In May-Hegglin anomaly, the inclusions can be seen within all types of granulocytes (neutrophils, basophils, eosinophils) and monocytes. Large platelets are also present and are a characteristic feature.

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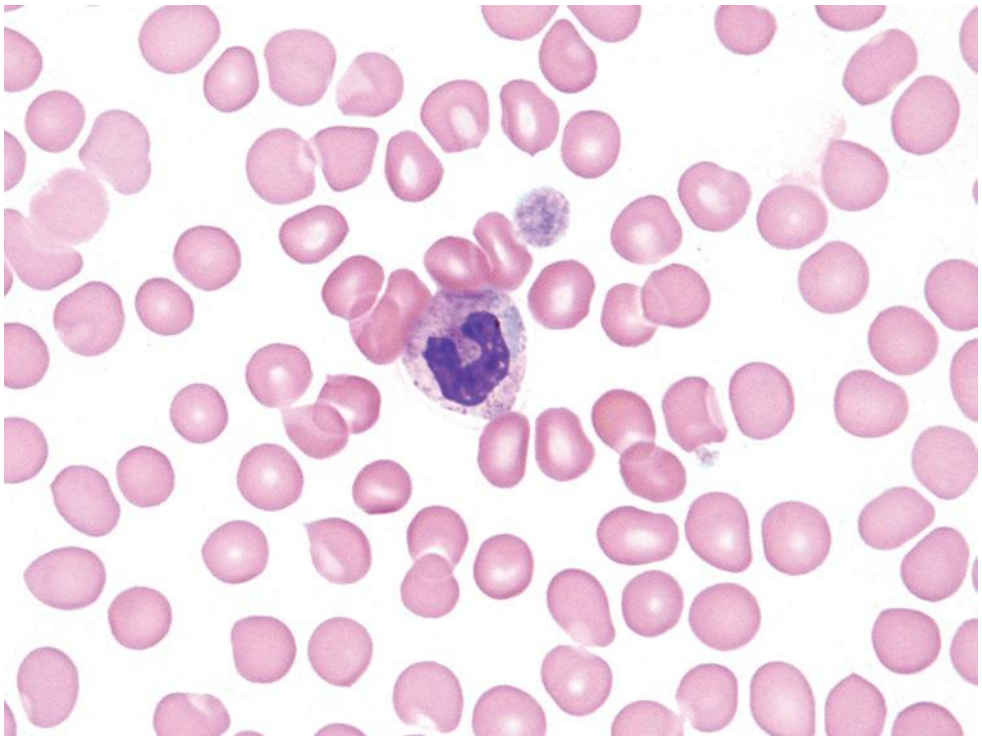


FIG. 3.5
Peripheral blood smear in May-Hegglin anomaly. Note the pale blue inclusion in the neutrophil and the large platelet, which are typical findings in May-Hegglin anomaly.

Mean platelet volume is increased but often underestimated because of inclusion as erythrocytes or leukocytes by automated cell counters.

The inclusions are composed of aggregates of abnormal myosin IIA protein, MYH9 mRNA, and clusters of ribosomes. The granulocytes appear to have normal function. The results of platelet function studies are typically normal, but there still may be an increased tendency for bleeding. Defects in expression of GP Ib/IX/V complex have been reported in May-Hegglin anomaly, which may account for bleeding tendencies.

VACUOLATED LYMPHOCYTES

Lymphocyte vacuoles are relatively rare in reactive conditions. In a wide variety of inherited genetic disorders, otherwise normal peripheral blood lymphocytes may be vacuolated. In some cases, the vacuoles will stain positively with the periodic acid-Schiff (PAS) stain. The disorders in which these vacuoles are seen are shown in Box 3.1. Although rarely seen in reactive conditions or infections, the vacuoles are present in more than 20% of lymphocytes when seen in association with genetic disorders.

Box 3.1 Disorders With Vacuolated Lymphocytes

Mannosidosis
Batten disease (Spielmyer-Vogt)
GM1 gangliosidosis
Neuraminidase deficiency
Galactosidosis
Mucolipidosis II
Aspartylglycosaminuria
Salla disease
Infantile free sialic acid storage
Wolman disease
Niemann-Pick disease type A
Aspartylglycosaminuria
Glycogenosis type II, Pompe disease
Fucosidosis
Mucolipidosis III

ACQUIRED ABNORMALITIES OF WHITE BLOOD CELLS

Several abnormalities are seen in normal leukocytes in response to acquired conditions. These abnormalities can occasionally mimic the findings seen in genetic disorders or may be similar to those seen in neoplastic cells. Familiarity with these changes can prevent misinterpretation.

"TOXIC" CHANGES IN LEUKOCYTES

Increased WBC counts (e.g., leukocytosis) can be seen in a wide variety of conditions. One common cause is as a response to infections, particularly bacterial. In these cases, a left shift of granulocytes is also seen. The term *toxic changes* refers to a constellation of alterations that occur in the cytoplasm of granulocytes, including excessive granulation (toxic granules), vacuolation, or Döhle bodies (Fig. 3.6). These changes can be seen either individually or in combination.

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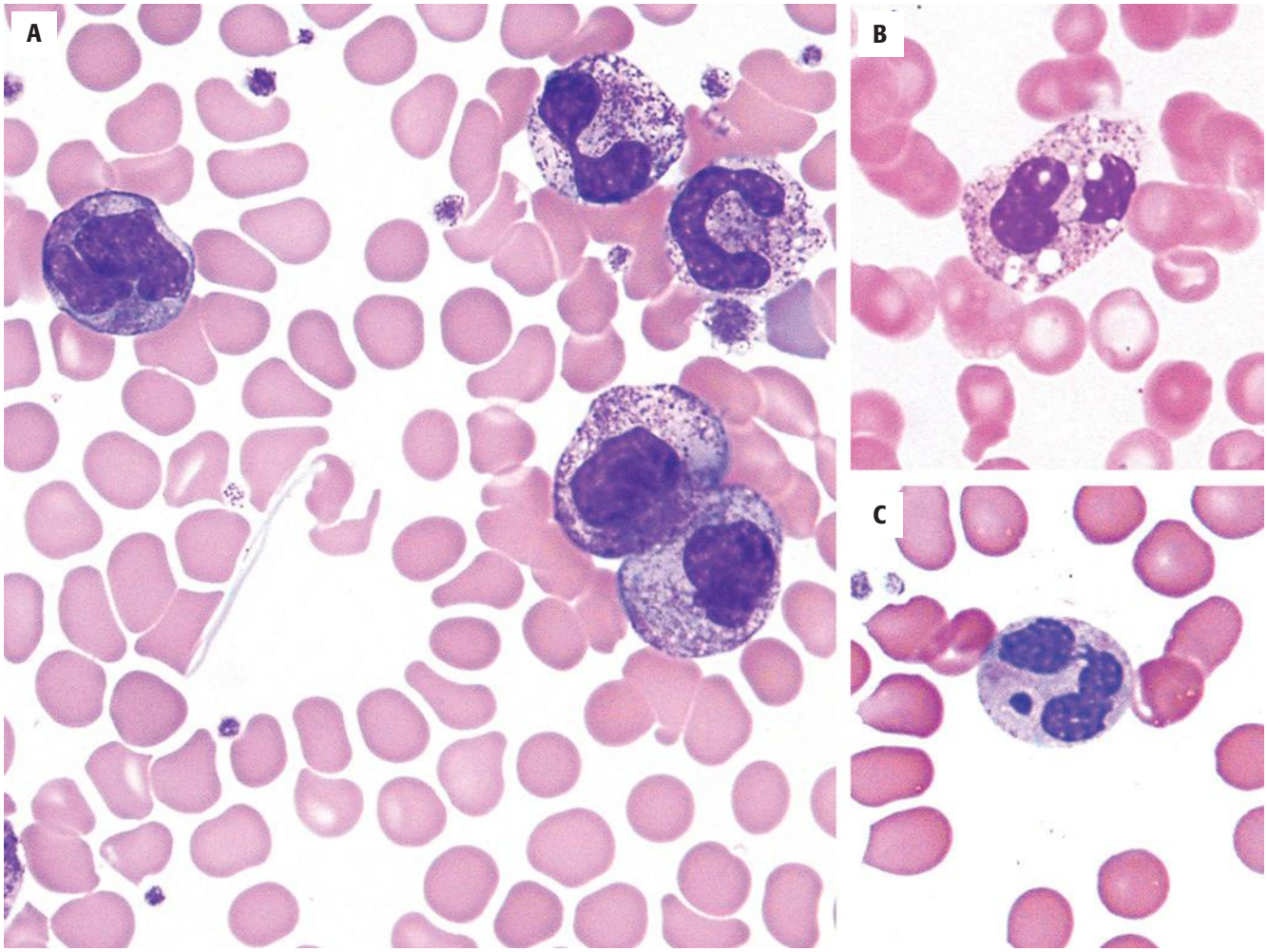


FIG. 3.6

A, Peripheral blood changes in a case of neonatal sepsis, which include prominent toxic granulation and a left shift in the neutrophils. B, Toxic vacuoles in a neutrophil. C, A Howell-Jolly–like body (e.g., nuclear fragment) in the neutrophils of a patient with human immunodeficiency virus or acquired immunodeficiency syndrome (HIV/AIDS).

Toxic granulation is a persistence of the deep blue-purple color of the primary granules of the neutrophil; they are azurophilic and appear most prominent in neutrophils and band forms but are also present in myelocytes and metamyelocytes. These granules are thought to be due to abnormal, accelerated maturation and exit from the marrow space. Toxic vacuoles may be seen as circular, clear areas in the cytoplasm of neutrophils. They are typically less than 1 to 2 µm in diameter and represent sites of phagocytized material. They are most often seen in septicemia but can also be seen in cases of acute ethanolism and certain medications (e.g., chloramphenicol). Döhle bodies are pale blue or blue-gray inclusions, 1 to 3 µm in diameter, and are seen in the cytoplasm of neutrophils and precursors; they are composed of aggregates of denatured ribosomes.

In the neutrophils of patients being treated for human immunodeficiency virus or acquired immunodeficiency syndrome (HIV/AIDS), intracytoplasmic inclusions of DNA material can be seen (termed *Howell-Jolly body–like inclusions;* see Fig. 3.6). Like true Howell-Jolly bodies, these bodies appear to be fragments of nuclear chromatin separated from the main body of the nucleus. They have the same dark purple color of nuclear material and are round in shape. These inclusions have also been described in patients without HIV but being treated with immunosuppressive drugs (e.g., organ transplant patients) and chemotherapy. The most common differential diagnosis of these inclusions are intracytoplasmic infectious agents, such as bacteria or parasites.

Apoptotic neutrophils, also termed *necrobiosis,* appear as several round hyperchromatic nodules of nuclear chromatin material. These cells can be seen in a variety of conditions, including infections and medication-related conditions. Comparable changes can be seen in MDS or AML. Apoptosis of neutrophils is also a common finding in aged samples and should not be overinterpreted as evidence of dysplasia.

Refractile, bright green cytoplasmic inclusions have been rarely reported within neutrophils and, less commonly, monocytes. These appear to represent lipofuscin pigment and are seen in patients with ischemic liver damage. When seen,

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they may suggest that the patient is critically ill, and many reported patients have died shortly after discovery of the inclusions.

CYTOKINE EFFECTS

Cytokine therapy has become commonplace. Some of these cytokines can produce significant bone marrow and peripheral blood changes in WBCs. The most commonly seen effect is that of granulocyte-colony stimulating factor (G-CSF). Commonly G-CSF is used to increase the number of neutrophils in the peripheral blood, or as part of therapy to increase the number of peripheral blood stem cells. The effects of G-CSF on peripheral blood neutrophils include "toxic" granulation, a left shift, an overall increase in granulocytes and precursors, a transient increase in circulating blasts, vacuolation, Döhle bodies, variable nuclear-cytoplasmic dyssynchrony, and abnormalities of nuclear segmentation. Marrow changes include similar findings, with prominent granulation being especially frequent, and an increase in myeloid elements (e.g., increased M-to-E ratio; Fig. 3.7). Other less common features include binucleate promyelocytes and myelocytes, giant myeloid precursors, and rarely the development of bone marrow fibrosis (typically mild). Granulocyte-macrophage colony-stimulating factor (GM-CSF) has similar changes to G-CSF, with the addition of significant monocytosis and eosinophilia.

Cytokine effects may occasionally simulate the findings seen in acute leukemias, especially acute promyelocytic leukemia. Rare cases of myeloid stem cell disorders (acute myeloid leukemia, myelodysplastic syndromes) may respond to G-CSF, leading to dramatic increases in circulating blasts. Careful attention to the morphologic appearance of the cells, evaluation of the clinical history, and in some circumstances flow cytometry or bone marrow examination may aid in distinguishing cytokine effect from leukemia. Presence of a spectrum of maturing granulocytic precursors in addition to blasts favors a regenerative response over acute leukemia. Reassessment after waiting several days to allow cytokine effects to subside is often a prudent course of action.

MEGALOBLASTIC AND DYSPLASTIC CHANGES

Megaloblastic changes in WBCs are typically described in bone marrow as a result of B_{12} or folate deficiencies, although the changes may be seen as a result of medications or other medical disorders. These changes manifest as nuclear and cytoplasmic dyssynchrony, with nuclear development lagging behind that of the cytoplasm (Fig. 3.8). As a result, WBCs in the bone marrow may show giant precursors (giant myelocytes, giant bands) and hypersegmentation of granulocytes. In the peripheral blood, hypersegmented neutrophils (more than four lobes, with six lobes or more being diagnostic) suggest megaloblastic anemia but should be correlated with red blood cell indices (especially mean corpuscular volume [MCV]), medication history, and clinical findings (see Fig. 3.8).

One common issue that hematopathologists face is distinguishing secondary causes of dyspoiesis from primary myelodysplastic syndromes. This may not be possible by evaluating only the morphology. Familiarity with potential clinical situations in which dysplastic features might arise,

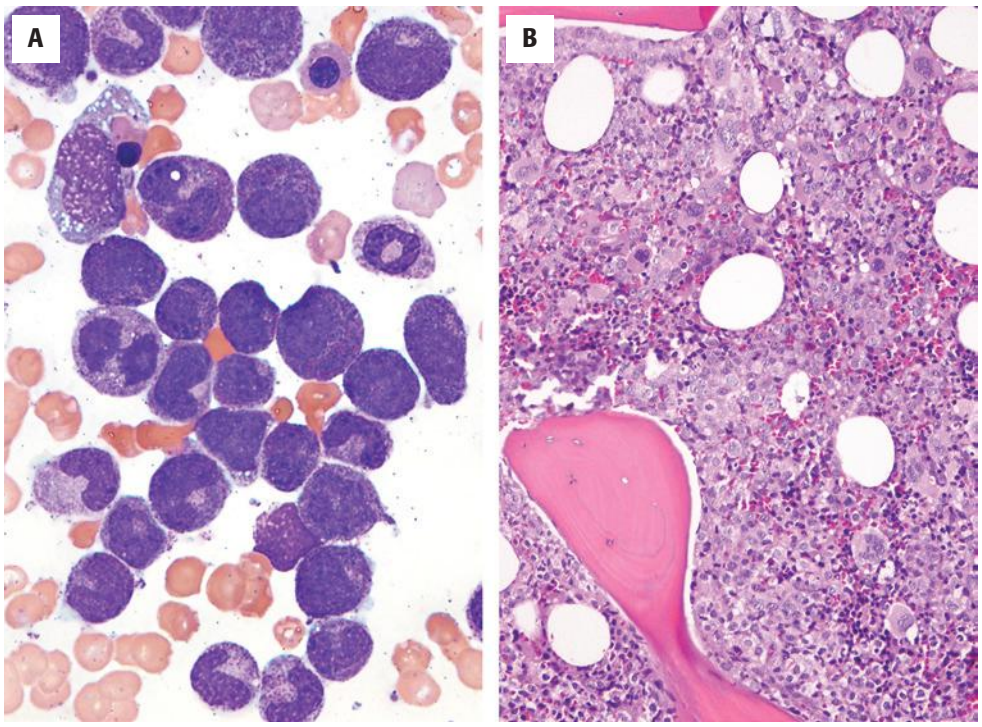


FIG. 3.7

Bone marrow changes associated with granulocyte colony-stimulating factor administration. The aspirate smear (A) may show extensive, heavy toxic granulation. In the core biopsy (B), there is an increase in immature myeloid elements, expanding away from the bone trabeculae into the intertrabecular space.

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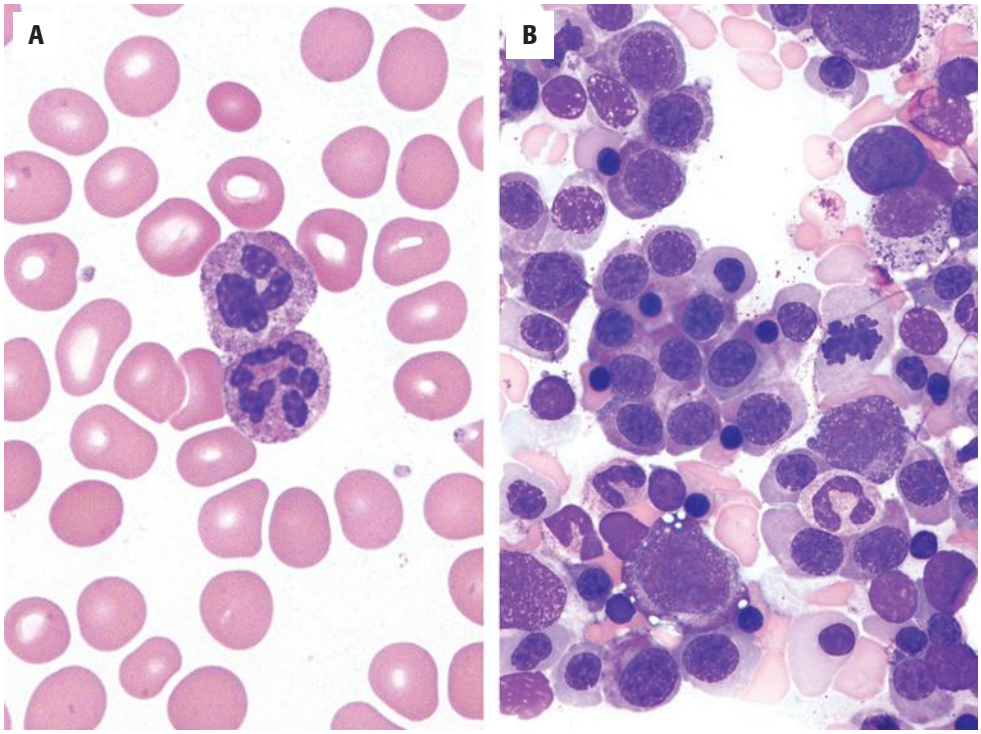


FIG. 3.8

Megaloblastic anemia caused by folate deficiency. A hypersegmented neutrophil with a normal neutrophil (A) and a bone marrow aspirate showing megaloblastic changes, including erythroid hyperplasia, nuclear/cytoplasmic dyssynchrony, and dyserythropoiesis (B).

including those not only limited to leukocytes, can be invaluable. Drug or toxin exposures represent one of the most significant challenges. Common scenarios that could produce cellular dyspoiesis include recent cytotoxic therapy with any number of chemotherapeutic agents, arsenic (either as a toxic or therapeutic) exposure, heavy metal exposure, copper deficiency (zinc induced), alcohol toxicity, antituberculous drugs (isoniazid) therapy, trimethoprim therapy, valproic acid therapy, and HIV/AIDS.

EOSINOPHILS

Eosinophils can occasionally be associated with the formation of Charcot-Leyden crystals, which are large, orange-red hexagonal or diamond-shaped forms within the cytoplasm of eosinophils, within macrophages, or in tissues infiltrated by eosinophils. The crystals are formed by fusion of eosinophil granules that contain large amounts of Charcot-Leyden protein or lysophospholipase. Charcot-Leyden crystals are seen in conditions associated with eosinophilia. Vacuolated eosinophils can be seen in reactive conditions with eosinophilia. This change has been associated with hypereosinophilic syndrome, but in our experience it is nonspecific.

REACTIVE LYMPHOCYTES AND LYMPHOCYTOSIS

Smudge Cells

Smudge cells, sometimes referred to as *basket* or *Gumprecht cells,* are fragments of cells that are damaged and broken apart during the process of preparing a blood smear. It is presumed that the cells are more fragile than their intact counterparts. Most commonly, these cells are lymphocytes, but monocytes, blasts, or any cell type can fragment during the process of smear preparation. In the appropriate clinical context (i.e., an older patient), the presence of numerous smudge cells with lymphocytosis is suggestive of a diagnosis of a lymphoproliferative disorder, most commonly chronic lymphocytic leukemia (Fig. 3.9). However, smudge cells can also be seen in acute lymphoblastic leukemia and in infectious mononucleosis. The addition of albumin to the blood droplet before smearing usually stabilizes the cell membranes and prevents the formation of smudge cells. Careful morphologic examination of the intact cells on the smear usually provides enough information to infer the identification of the smudge cell population.

Viral Lymphocytosis

Infectious mononucleosis resulting from Epstein-Barr virus infection is the prototypical condition for transient reactive (viral) lymphocytosis. Morphologic heterogeneity of lymphocyte appearance is the rule. Compared to normal lymphocytes, reactive lymphocytes contain abundant basophilic cytoplasm. Cytotoxic granules may be present. The nuclei are slightly enlarged (1.5 to 2 times normal), have loosely clumped nuclear chromatin, and have one or two nucleoli. These cells are mostly cytotoxic T cells responding to the infection. Occasional immunoblasts and plasma cells may be seen. Depending on the changes seen, reactive lymphocytes can mimic a variety of lymphoproliferative disorders, including both acute and chronic lymphoid leukemias. Careful morphologic examination (particularly noting the heterogeneity

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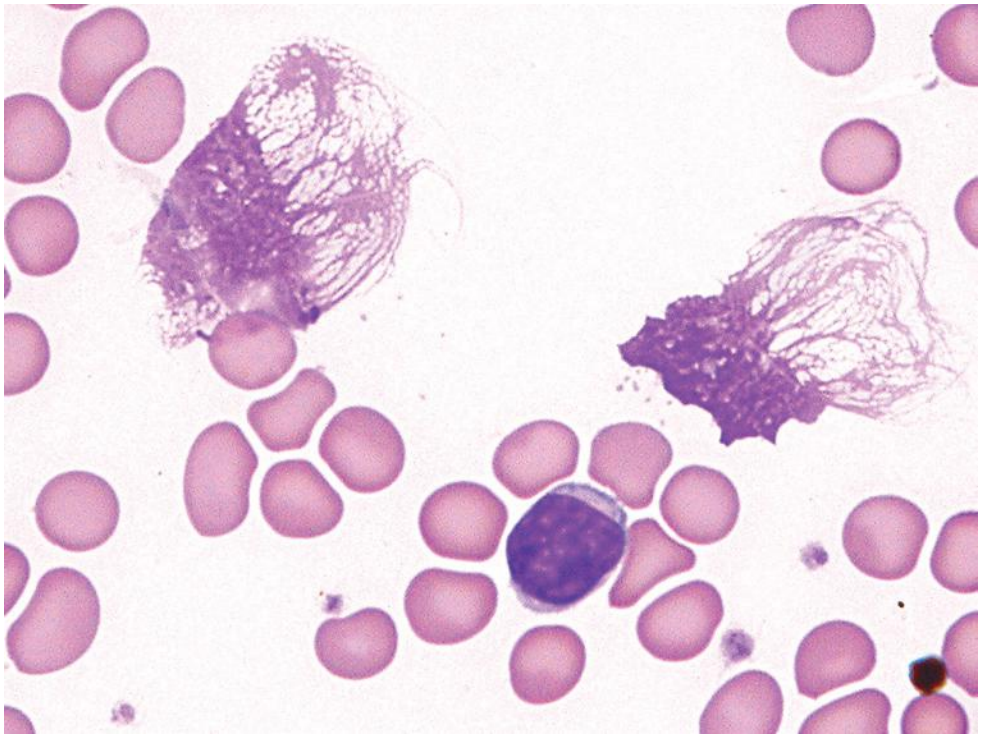


FIG. 3.9
A peripheral blood smear with two smudge cells and an intact lymphocyte.

of lymphocyte morphology), knowledge of the clinical findings, and occasionally immunophenotypic or molecular testing may be necessary to appropriately identify the process. Other viral infections including but not limited to cytomegalovirus, hepatitis, HIV, and hantavirus can also cause reactive lymphocytosis.

Other Reactive Lymphocyte Changes

Bordetella pertussis infection can result in a peculiar lymphocytosis composed of cleaved or notched lymphocytes mimicking the buttock cells of leukemic follicular lymphoma (Fig. 3.10). Infection occurs in children, and the situation should be monitored closely to avoid a costly workup.

An underrecognized lymphocytosis in adults (so-called stress lymphocytosis) can also be seen in patients in the emergency department. Lymphocytosis is usually mild and does not persist. Thus, a reevaluation in 2 to 4 weeks' time will allow distinction from a neoplastic condition since the transient stress lymphocytosis resolves.

Persistent polyclonal B cell lymphocytosis is an intriguing reactive cause of lymphocytosis and should be noted as a mimic of lymphoid leukemia. Affected individuals are usually women who smoke and have an HLA-DR7 phenotype. There is a persistent lymphocytosis that, when investigated by immunophenotyping, is polyclonal B-cells with a memory B-cell phenotype. The lymphocytes are small and often have deep nuclear clefts. Interestingly, some studies have shown *BCL2/IGH* rearrangements (polyclonal) in lymphocytes from these patients. Isochromosome 3q has also been reported in 34% of patients. The vast majority of patients have a stable lymphocyte count and an indolent course. A small number of patients have been reported with slowly progressive splenomegaly composed of polyclonal marginal zone B cells and sinusoidal bone marrow infiltrate. One large series of 111 patients reported 89% of patients to be free of complications at a median of 53 months. B-cell lymphomas have been reported in rare patients, but whether persistent polyclonal B-cell lymphocytosis can progress to lymphoma is an open question, and patients should receive clinical follow-up.

MORPHOLOGIC ABNORMALITIES IN BONE MARROW MACROPHAGES

Macrophages, also referred to as *histiocytes,* are phagocytic cells of the marrow that are derived from monocytes. When the cytoplasmic contents of macrophages have a distinctive appearance, they may be useful in suggesting a diagnosis or may mimic other disorders. One distinctive macrophage change is that seen in Gaucher disease, an inherited (autosomal-recessive) enzyme defect of β-glucocerebrosidase. Macrophages in Gaucher disease characteristically have increased amounts of blue cytoplasm on Wright stain, with a fibrillar or "folded tissue paper" appearance (Fig. 3.11).

Pseudo-Gaucher cells are difficult to distinguish from true Gaucher cells by morphology. Pseudo-Gaucher cells are caused by the rapid turnover of cells and ingestion of the cellular/membrane products by macrophages. Pseudo-Gaucher cells are seen most frequently in chronic myeloproliferative disorders, especially chronic myelogenous leukemia, and less commonly in other marrow neoplasms. True Gaucher disease

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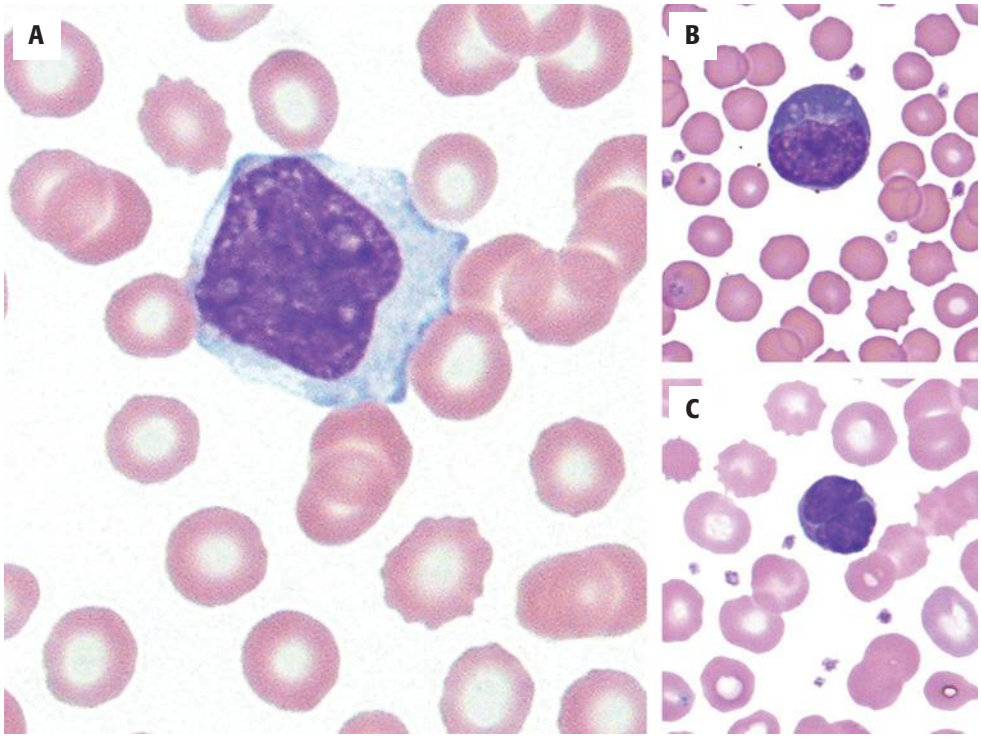


FIG. 3.10

A, A classic, atypical or reactive lymphocyte seen in a case of infectious mononucleosis. B, An atypical lymphocyte seen in nonspecific viral infection. C, A cleaved lymphocyte in the peripheral blood of a patient with pertussis infection. *(C, Courtesy M. Drachenberg, Long Beach Memorial Hospital. Long Beach, CA.)*

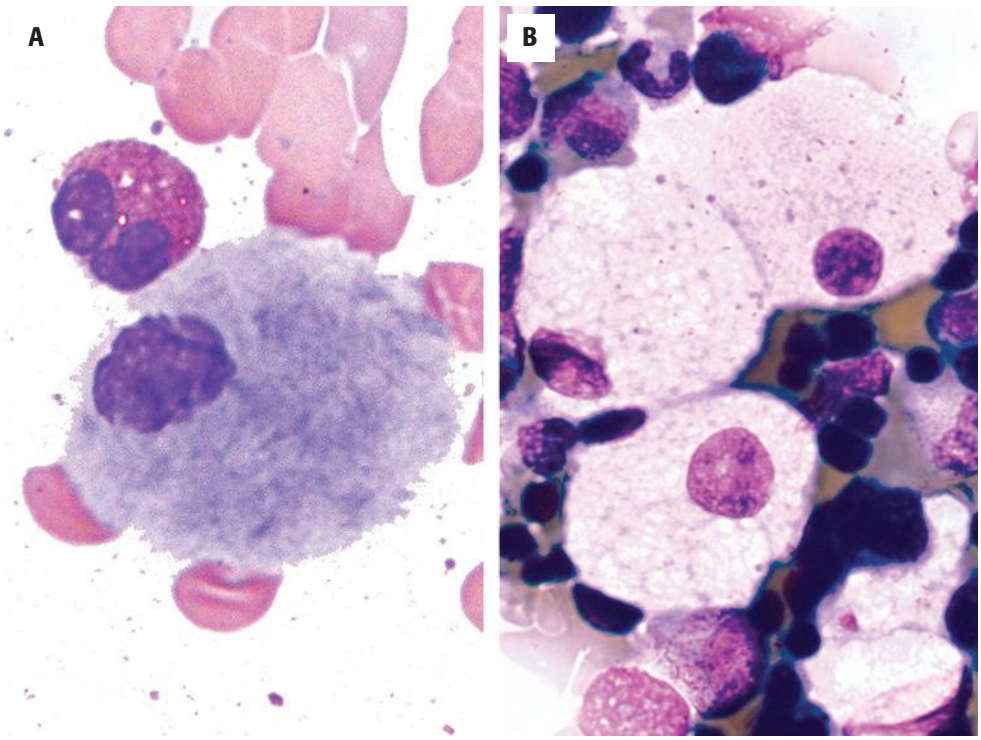


FIG. 3.11

A, A classic Gaucher cell, with pale blue, "folded tissue paper" cytoplasm. B, A Niemann-Pick cell with numerous cytoplasmic vacuoles filled with lipid.

is suggested when numerous Gaucher cells are present and other clinical features, such as hepatosplenomegaly, are present. Confirmation of Gaucher disease should be done in all suspected cases by appropriate biochemical/molecular testing.

Foamy macrophages have multiple clear cytoplasmic vacuoles and can be seen in a variety of conditions, including genetic disorders of lipid metabolism or excess marrow accumulation of lipids. One specific type of foamy macrophage is the Niemann-Pick cell. Niemann-Pick disorder is an autosomal-recessive inherited disorder; a lack of sphingomyelinase leads to accumulation of the abnormal metabolic product in a variety of tissues. The macrophages in this disorder are typically large with numerous small, uniform

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lipid-filled vacuoles (see Fig. 3.11) and can be found in large numbers. The macrophages are typically seen in the bone marrow, liver, and spleen. As in any suspected storage or metabolic disorder, confirmatory testing (enzyme activity or molecular/genetic) should be performed.

Sea-blue histiocytes are macrophages with cytoplasm that appears blue or blue-green in a standard Wright stain. The staining pattern is due to accumulation of ceroid, a metabolic product of cell membrane digestion. As an acquired phenomenon, sea-blue histiocytes can be found in disorders with rapid cell turnover, such as chronic myelogenous leukemia, where they are also commonly found, albeit in small numbers. They can also be seen in other hematologic disorders such as myelodysplastic syndromes. An unusual scenario in which sea-blue histiocytes are seen is in the setting of prolonged total parenteral nutrition. Bone marrow smears from these patients may demonstrate large numbers of sea-blue histiocytes, believed to be related to the lipid emulsion component of the total parenteral nutrition. Idiopathic sea-blue histiocytosis was described in 1970; it is a rare syndrome of sea-blue histiocytes in bone marrow, hepatosplenomegaly, thrombocytopenia, purpura, and pulmonary infiltrates; it has a benign clinical course. Some authors consider this sea-blue histiocytosis to be part of the spectrum of Niemann-Pick disease, because a partial sphingomyelinase deficiency may be the cause. A rare genetic disorder, mutation in the *APOE* (apolipoprotein E) gene (19q13.2) may also be associated with splenomegaly, sea-blue histiocytes, thrombocytopenia, and hypertriglyceridemia.



The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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4

Reactive Lymph Nodes and Castleman Disease

Judith A. Ferry, MD

NORMAL LYMPH NODE ANATOMY

Normal lymph nodes are typically bean shaped. They have, beginning at their periphery and moving centrally, a fibrous capsule, a cortex, a paracortex, a medulla, and a hilus. The cortex, a B-cell–predominant area, contains follicles. When inactive, follicles are composed of a uniform population of small lymphoid cells (primary follicles). Hyperplastic follicles have a germinal center surrounded by a mantle of small cells (secondary follicle). Germinal centers are composed of centrocytes (small cleaved cells) and centroblasts (large noncleaved cells) of B-cell lineage. There are also T cells admixed with follicle center cells, and a narrow layer of T cells is typically seen around the periphery of a hyperplastic germinal center. In peripheral lymph nodes, a marginal zone is not usually conspicuous, but in certain sites, such as Peyer's patches, mesenteric lymph nodes, and the spleen, follicles often have a distinct marginal zone composed of small lymphoid cells with a moderate quantity of clear cytoplasm surrounding the mantle zone.

The paracortex, primarily a T-cell area, contains lymphocytes, antigen-presenting cells, and varying numbers of immunoblasts depending on the degree of activation of the lymph node. High endothelial venules lined by cuboidal ("high") endothelial cells are found in the paracortex; they play an important role in lymphocyte trafficking.

The medulla contains cords occupied by a varying admixture of lymphocytes, plasma cells, and immunoblasts. A mixture of B and T cells is usually found in medullary cords. A network of sinuses traverses the lymph node. The network begins with the subcapsular sinus, which then feeds into sinuses that cross the node from cortex to medulla. The sinuses in the medulla delineate the medullary cords (Fig. 4.1).

A number of nonspecific changes may be found in reactive lymph nodes. One of these is follicle lysis, in which follicles and their underlying dendritic networks are disrupted, imparting a fragmented appearance to the follicle center. This phenomenon is usually found in the setting of florid follicular hyperplasia.

Progressive transformation of germinal centers is characterized by infiltration of germinal centers by small lymphocytes of mantle zone type, with enlargement of the follicles. The follicles eventually acquire a dark, monotonous appearance. When multiple follicles show progressive transformation of germinal centers, the appearance can raise the question of nodular lymphocyte-predominant Hodgkin lymphoma.

Monocytoid B cells are not usually a conspicuous feature of nonspecific reactive hyperplasia but are prominent in certain reactive conditions, including cytomegaloviral lymphadenitis, and toxoplasma lymphadenitis. Monocytoid B cells are lymphoid cells with small to medium-sized oval or indented nuclei and abundant pale cytoplasm. They are found in bands along sinuses.

VIRAL INFECTIONS

EPSTEIN-BARR VIRUS: INFECTIOUS MONONUCLEOSIS

Clinical Features

The diagnosis of symptomatic Epstein-Barr virus (EBV) infection, infectious mononucleosis (IM), is usually established on clinical grounds, but when involved lymph nodes or tonsils are biopsied, they frequently cause problems in differential diagnosis. IM most often affects adolescents and young adults, although cases of IM in young children and in adults as old as 80 years have been reported. Manifestations include fever, pharyngitis, cervical lymphadenopathy, splenomegaly, rash, atypical peripheral blood lymphocytosis, and a positive heterophile antibody (Monospot) test. However, the heterophile antibody test may not be positive throughout the illness, sometimes requiring repeated monospots or serologic studies to establish a diagnosis. In some cases, particularly among young children, a positive heterophile antibody test may never be obtained. Evaluating plasma EBV viral load by molecular methods may also be useful in establishing a diagnosis. In more severe cases, there may be generalized lymphadenopathy, upper airway obstruction related to lymphoid hyperplasia, hepatomegaly with hepatic dysfunction, peripheral blood cytopenias, neurologic complications, such as aseptic

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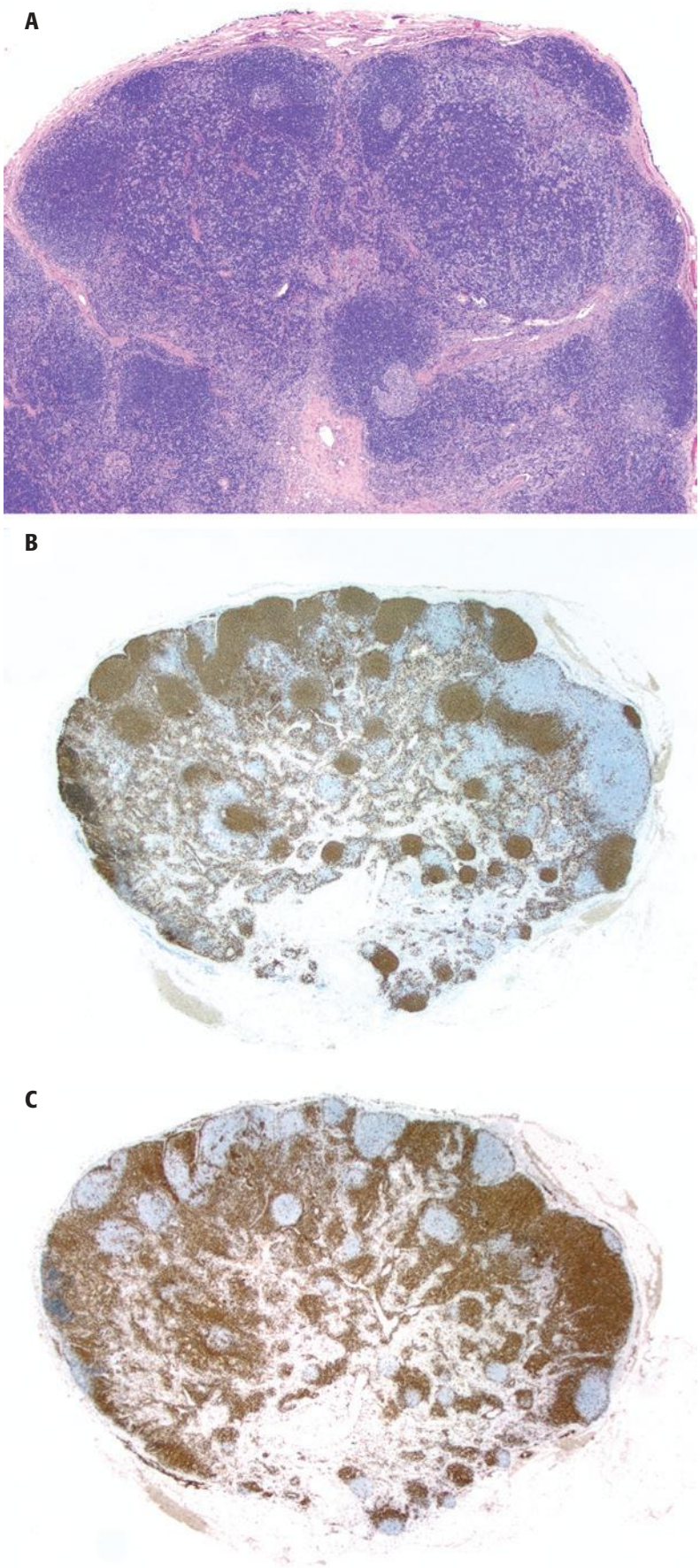


FIG. 4.1

Lymph node with nonspecific reactive hyperplasia. A, Low power shows a lymph node with intact architecture, with several reactive follicles and a hyperplastic paracortex. B, B cells (CD20+) are found mainly in follicles and distributed in a thin layer along sinuses. C, T cells (CD3+) occupy the paracortex and are mixed with B cells in medullary cords.

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INFECTIOUS MONONUCLEOSIS—FACT SHEET

Definition
- Symptomatic infection by EBV

Age, Race, and Gender Distribution
- Most patients are teenagers or young adults, but individuals of any age may be affected.
- No known gender or racial predisposition

Risk Factors
- Close contact with an EBV-infected person

Clinical Features
- Fever, pharyngitis, cervical lymphadenopathy, splenomegaly, fatigue, and an atypical lymphocytosis in blood are common.
- Some patients have more widespread lymphadenopathy, hepatomegaly, hepatic dysfunction, or splenic rupture.

Morphology
- Lymph nodes, tonsils: Interfollicular or diffuse polymorphous infiltrate of EBV-positive immunoblasts that are sometimes Reed-Sternberg–like in a mixed background of plasma cells, histiocytes, and lymphocytes, with apoptotic debris, ± zonal necrosis
- Peripheral blood: Lymphocytosis, usually with >50% lymphocytes and >10% atypical lymphocytes

Diagnosis
- Monospot (heterophile antibody), serology, biopsy of affected tissue, assay for EBV viral load

Prognosis and Therapy
- Supportive therapy is sufficient, and spontaneous recovery is the rule in nearly all cases, except for those with serious complications.

meningitis and meningoencephalitis, and even a hemophagocytic syndrome/hemophagocytic lymphohistiocytosis. Most patients with infectious mononucleosis who receive a beta-lactam antibiotic develop a morbilliform rash. In most cases, the illness is self-limited, but rarely intercurrent infection, Guillain-Barré syndrome, or splenic rupture with hemorrhage results in death. Young children with genetic defects, such as perforin deficiency, are at increased risk for hemophagocytic syndrome/hemophagocytic lymphohistiocytosis. Boys with the X-linked lymphoproliferative disorder are at risk for severe infectious mononucleosis; those who survive the acute infection often develop lymphoma and/or have persistent immunologic abnormalities.

Pathologic Features

The lymph nodal architecture is typically distorted but not effaced by an expanded paracortex containing a polymorphous population of lymphoid cells, including small lymphocytes, intermediate-sized lymphoid cells, immunoblasts, tingible body macrophages, and mature and immature plasma cells. The EBV-infected immunoblasts may form aggregates and may be atypical, with pleomorphic or lobated nuclei; binucleated cells resembling Reed-Sternberg cells may be identified. Mitotic figures may be numerous. Apoptosis is common, and zonal necrosis may be present. EBV+ cells may be most numerous surrounding the foci of necrosis. Reactive follicles may be present, but follicular hyperplasia is usually inconspicuous. Sinuses are patent in at least some areas, and frequently they are dilated. Sinuses contain histiocytes and a polymorphous population of lymphoid cells, including immunoblasts. Lymphoid cells sometimes infiltrate the capsule and extend into perinodal fat. Similar histologic features are found in the tonsils of patients with IM. In tonsils, crypts are usually present, although the epithelium lining them may be necrotic (Fig. 4.2A through C).

The small and intermediate-sized cells in the paracortex are predominantly T cells, including many that are activated in response to the presence of the virus. The CD4-to-CD8 ratio is typically decreased. The EBV+ immunoblasts are B cells, typically CD20+, with a non-germinal center immunophenotype (CD10-, BCL6-/weakly positive, MUM1+). They are in addition CD30+/-, CD15-, Oct2+, Bob1+. Polytypic light chain expression by plasma cells and sometimes by immunoblasts is seen. Using in situ hybridization with probes for EBV-encoded RNA (EBER), EBV can be detected in the immunoblasts and in some smaller cells (see Fig. 4.2D and E).

Differential Diagnosis

The differential diagnosis of IM includes a variety of reactive and neoplastic conditions. Obtaining adequate clinical information is helpful in making the correct diagnosis, whether the differential is with lymphoma or other types of reactive hyperplasias. Since the early years of the twentieth century the tendency of IM to mimic lymphoid malignancies has been recognized. If a patient is known to have clinical or laboratory evidence of IM, a diagnosis of Hodgkin lymphoma or non-Hodgkin lymphoma should be made with great caution.

When B immunoblasts and intermediate-sized reactive T cells are numerous, the possibility of B-cell or T-cell lymphoma may be a consideration. In favor of IM are lack of architectural effacement, presence of areas readily recognizable as reactive hyperplasia, a polymorphous background of lymphoid cells, patent sinuses containing lymphoid cells including immunoblasts, and lack of monotypic immunoglobulin (Ig) expression on immunophenotyping. The reactive T cells are usually predominantly CD8+, whereas most lymph nodal peripheral T-cell lymphomas (PTCLs) are CD4+. PTCLs are uncommon in the young patients who most often develop IM. Of note, IM can be associated with oligoclonal and rarely clonal T-cell populations directed against the virus, further potentially mimicking a PTCL.

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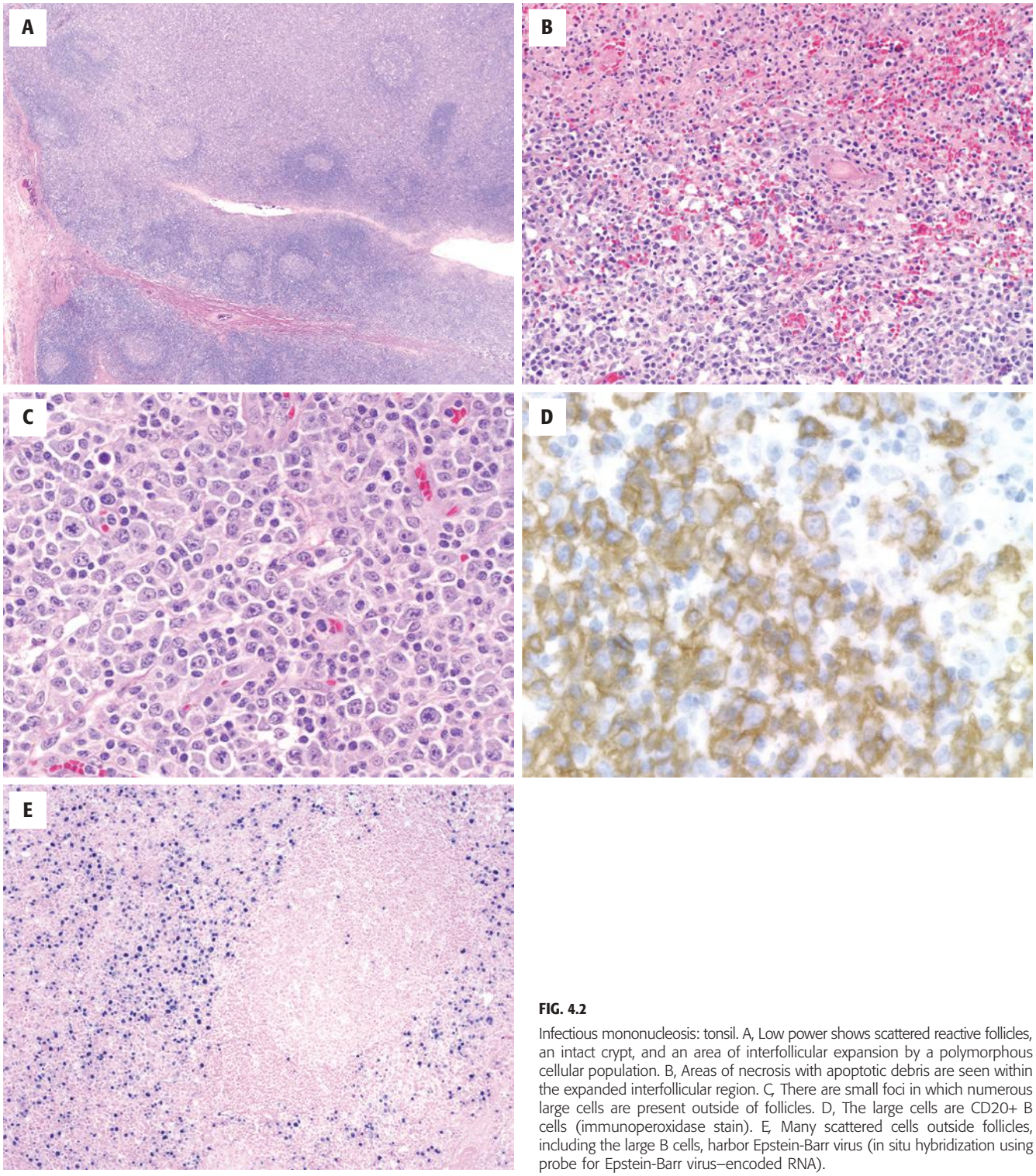


FIG. 4.2

Infectious mononucleosis: tonsil. A, Low power shows scattered reactive follicles, an intact crypt, and an area of interfollicular expansion by a polymorphous cellular population. B, Areas of necrosis with apoptotic debris are seen within the expanded interfollicular region. C, There are small foci in which numerous large cells are present outside of follicles. D, The large cells are CD20+ B cells (immunoperoxidase stain). E, Many scattered cells outside follicles, including the large B cells, harbor Epstein-Barr virus (in situ hybridization using probe for Epstein-Barr virus–encoded RNA).

Hodgkin lymphoma is often included in the differential diagnosis because Reed-Sternberg–like cells are often seen in IM; however, most of the large, EBV-infected cells resemble immunoblasts rather than Reed-Sternberg cells and variants. Hodgkin lymphoma is more often associated with obliteration of the lymph nodal architecture. The polymorphous background of lymphoid cells ranging from small to intermediate and large in the paracortex and sinuses seen in IM is very helpful in excluding Hodgkin lymphoma, in which the reactive background population includes small lymphocytes and, usually, granulocytes. Eosinophils are uncommon in IM. Immunoblasts in IM are CD15−, in contrast to the CD15 expression by Reed-Sternberg cells found in most cases of Hodgkin lymphoma.

Other viral infections, vaccination, certain drugs, and acute reaction to severe necrotizing processes can produce

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lymphadenopathy with histologic features similar to or indistinguishable from those of IM. Clinical information can be helpful in investigating the etiology of the lymphadenopathy. In addition, in IM, EBER-positive immunoblasts are typically present in large numbers, whereas in other conditions, EBER-positive cells are absent or rare.

CYTOMEGALOVIRAL LYMPHADENITIS

Clinical Features

Cytomegalovirus (CMV) can cause localized or generalized lymphadenopathy. Lymph node enlargement due to CMV infection may be found in patients who are otherwise asymptomatic. It may also be seen in the setting of a heterophile-negative, IM-like illness. CMV can infect lymphoid tissues in patients who have or have had Hodgkin lymphoma, non-Hodgkin lymphoma, or acquired or inherited immunodeficiency syndromes or in patients with a normal immune system. CMV infection is common in patients with common variable immunodeficiency.

CYTOMEGALOVIRAL LYMPHADENITIS—FACT SHEET

Definition
- Lymphadenopathy due to CMV infection

Incidence
- Uncommon

Gender, Race, and Age Distribution
- Can occur in patients of any age and either gender
- No known racial predisposition

Risk Factors
- Increased risk with congenital or acquired immunodeficiency
- Possible increased risk in lymphoma patients
- Also occurs in immunologically normal individuals

Clinical Features
- Patients may be asymptomatic or have an IM-like illness.
- Lymphadenopathy may be localized or generalized.

Morphology
- Florid follicular hyperplasia and monocytoid B-cell hyperplasia, with or without paracortical hyperplasia
- CMV-infected cells are most often found within monocytoid B-cell aggregates.
- Inclusions occasionally seen in cells recognizable as endothelial cells
- Infected cells harbor large red nuclear inclusions and sometimes also finely granular red cytoplasmic inclusions.

Diagnosis
- Recognition of CMV-infected cells on routinely stained slides; immunostaining for CMV is confirmatory.
- Perform immunostaining for CMV in cases with suspicious histology but with no definite inclusions identified.

Prognosis and Therapy
- Most patients have a self-limited illness and require no specific therapy.
- Patients with associated lymphoproliferative disorder or immunodeficiency may have a more severe course.

Pathologic Features

On microscopic examination, involved lymph nodes most often show florid follicular hyperplasia and monocytoid B-cell hyperplasia. Paracortical hyperplasia may also be prominent. Infected cells contain a large eosinophilic intranuclear inclusion (mean size, 9 μm) and often also contain multiple tiny eosinophilic to amphophilic cytoplasmic inclusions. Cells with inclusions are usually present focally in relatively small numbers, but occasionally they are numerous. Neutrophils and histiocytes are often scattered around the infected cells. Inclusions are typically found among monocytoid B cells, but the infected cells are most likely endothelial cells or histiocytes rather than lymphocytes; finding inclusions in the paracortex has also been described. Immunoperoxidase stains for CMV-associated antigens can be used to confirm the diagnosis when inclusions are found on routine sections and can help identify CMV-infected cells when inclusions are difficult to find. Cells containing inclusions may express CD15, usually with a Golgi region or diffuse cytoplasmic pattern of staining, but membrane staining is uncommon (Fig. 4.3).

Differential Diagnosis

The differential diagnosis of CMV lymphadenitis includes reactive hyperplasia due to some cause other than CMV, Hodgkin lymphoma, and certain non-Hodgkin lymphomas. The overall appearance of the lymph node in CMV lymphadenitis is very similar to that of toxoplasma lymphadenitis, although in most cases, the characteristic epithelioid cell aggregates of toxoplasmosis are not seen. Cells with nuclear viral inclusions may resemble Reed-Sternberg cells and variants on routinely stained sections, raising the question of Hodgkin lymphoma. In addition, CD15 expression by the virally infected cells heightens the resemblance to Hodgkin lymphoma. However, CMV lymphadenitis is much less likely to cause obliteration of the nodal architecture. Cells harboring virus may contain numerous granular cytoplasmic inclusions, in contrast to the agranular cytoplasm of Reed-Sternberg cells. Membrane staining by CD15 is more common in Reed-Sternberg cells than in CMV-infected cells. On occasion, the follicular and monocytoid B-cell hyperplasia of CMV lymphadenitis can be so florid as to distort the nodal architecture, potentially suggesting follicular lymphoma or nodal marginal

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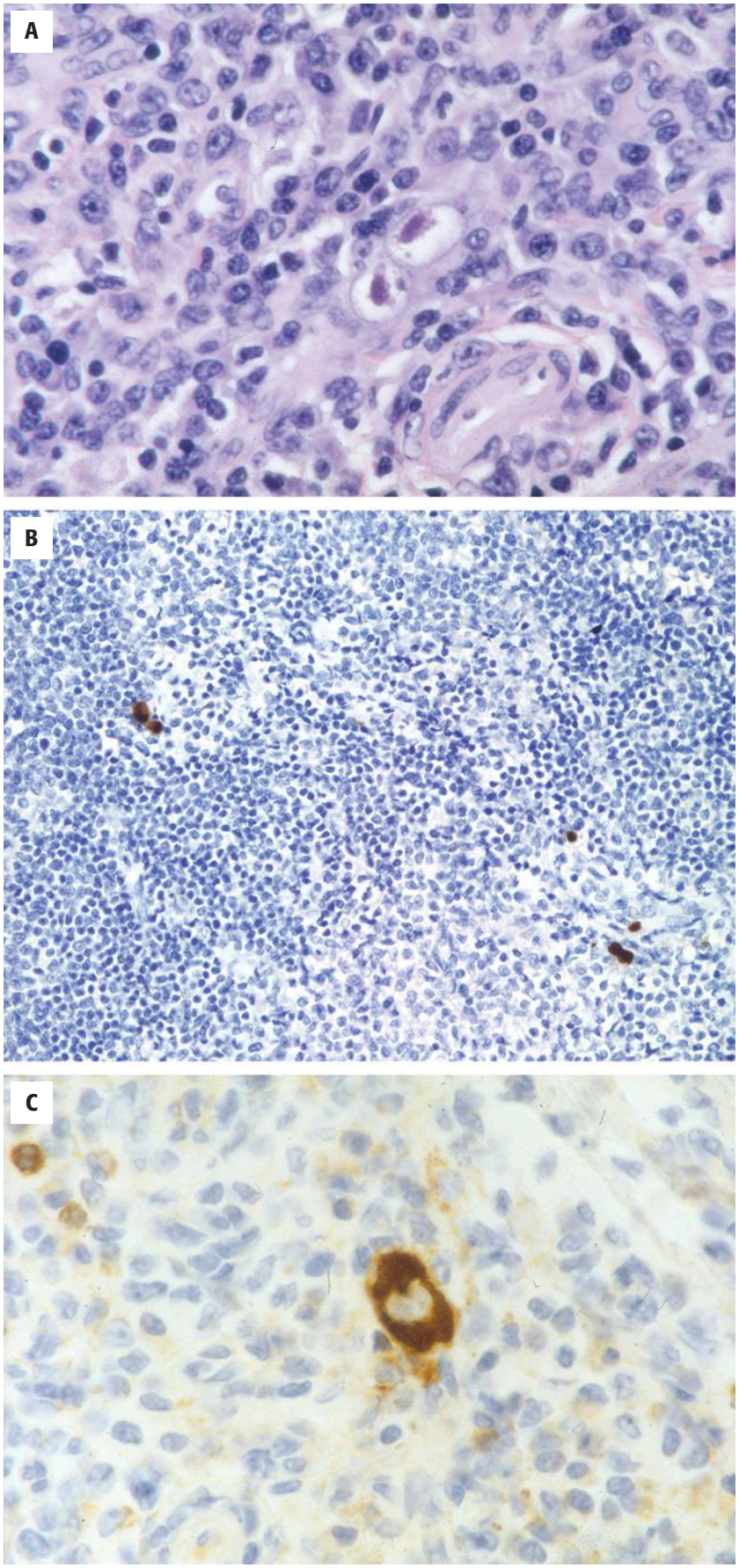


FIG. 4.3

Cytomegaloviral lymphadenitis in an individual with human immunodeficiency virus. A, The large red inclusions give an appearance closely mimicking a binucleated Reed-Sternberg cell. B, Scattered cells are intensely stained using immunoperoxidase staining for cytomegalovirus. C, A cell infected by cytomegalovirus shows diffuse cytoplasmic staining for CD15, a finding that heightens the resemblance to a Reed-Sternberg cell.

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zone lymphoma. Immunostaining with CMV-specific antibodies provides a definitive diagnosis of CMV lymphadenitis.

HERPES SIMPLEX VIRAL LYMPHADENITIS

Clinical Features

Infection by herpes simplex virus (type 1 or 2) can cause localized or generalized lymphadenopathy or involve lymph nodes in the setting of widespread visceral infection. When localized, the lymphadenopathy most often affects inguinal nodes, with cervical nodes next most often affected. Lymphadenopathy is usually painful. Typical mucocutaneous herpetic lesions are found in some patients, but they may be inconspicuous or may not appear until after a lymph node biopsy has been performed. Many patients who present with herpes simplex lymphadenitis have an associated hematologic malignancy or an underlying immunodeficiency. The most common associated disorder is chronic lymphocytic leukemia, but B-cell lymphomas and myeloid leukemias are also occasionally found. Although disseminated herpes simplex viral infection has a poor prognosis, isolated herpes simplex lymphadenitis is self-limited in most cases.

HERPES SIMPLEX VIRAL LYMPHADENITIS—FACT SHEET

Definition
- Lymphadenitis due to infection by herpes simplex virus type 1 or 2

Incidence
- Rare

Gender, Race, and Age Distribution
- Adults over a wide age range have been affected.
- No gender predilection
- No known racial predisposition

Risk Factors
- In many reported cases, patients also have a hematologic malignancy or other cause of immunodeficiency.

Clinical Features
- Tender lymphadenopathy, which may be localized, multifocal, or associated with widespread visceral involvement
- Skin or mucosal lesions may or may not be present or may be overlooked.

Diagnosis
- Biopsy of infected tissue, identification of cells with inclusions on routine sections, and confirmation with immunostains for herpes simplex viral antigens
- Diagnosis can also be made using electron microscopy or viral culture.

Prognosis and Therapy
- Some patients have had no specific therapy, some have received acyclovir, and some have received therapy for the associated hematologic disorder.
- Prognosis is related to extent of infection and to the prognosis of any underlying hematologic disorder or immunodeficiency.

Pathologic Features

Lymph nodes show prominent paracortical hyperplasia with areas of necrosis often with prominent extension into perinodal soft tissue. Paracortical immunoblasts may be numerous. The necrotic areas contain neutrophils, karyorrhectic or amorphous eosinophilic debris, and a variable number of cells with nuclear viral inclusions, ranging from rare to abundant. Most of them are uninucleate, although a few multinucleated cells are seen. Intact neutrophils are most abundant in early lesions and may be absent in long-standing lymphadenitis. Histiocytes often surround the necrotic areas, but granulomas are absent. Varicella zoster virus rarely causes lymphadenitis; the histologic features are similar to those of herpes simplex lymphadenitis. The diagnosis can be confirmed using immunohistochemical stains, in situ hybridization, electron microscopy, or viral culture (Fig. 4.4).

Differential Diagnosis

The differential diagnosis based on histologic features includes other types of necrotizing lymphadenitis and, in some cases, lymphoma. The lack of granulomatous inflammation with epithelioid or palisading histiocytes provides evidence against infections caused by mycobacteria, fungi, yersinia, cat-scratch bacilli, and lymphogranuloma venereum. When there is pronounced paracortical expansion with numerous immunoblasts, the differential includes lymphoma. In herpes simplex lymphadenitis, the nodal architecture may be distorted, although it is not obliterated, in contrast to most nodes involved by lymphoma. Discrete foci of necrosis are less common in lymphoma, and herpes viral inclusions are absent in lymphoma, except in cases in which both herpes simplex virus and lymphoma involve the same lymph node. In instances in which patients have an established diagnosis of lymphoma and then develop rapidly enlarging lymphadenopathy related to viral infection, the clinical differential can include relapse or progression of the lymphoma.

HUMAN IMMUNODEFICIENCY VIRUS–ASSOCIATED LYMPHADENOPATHY

Clinical Features

Acute symptomatic human immunodeficiency virus (HIV) infection presents as an IM-like illness with fever, pharyngitis,

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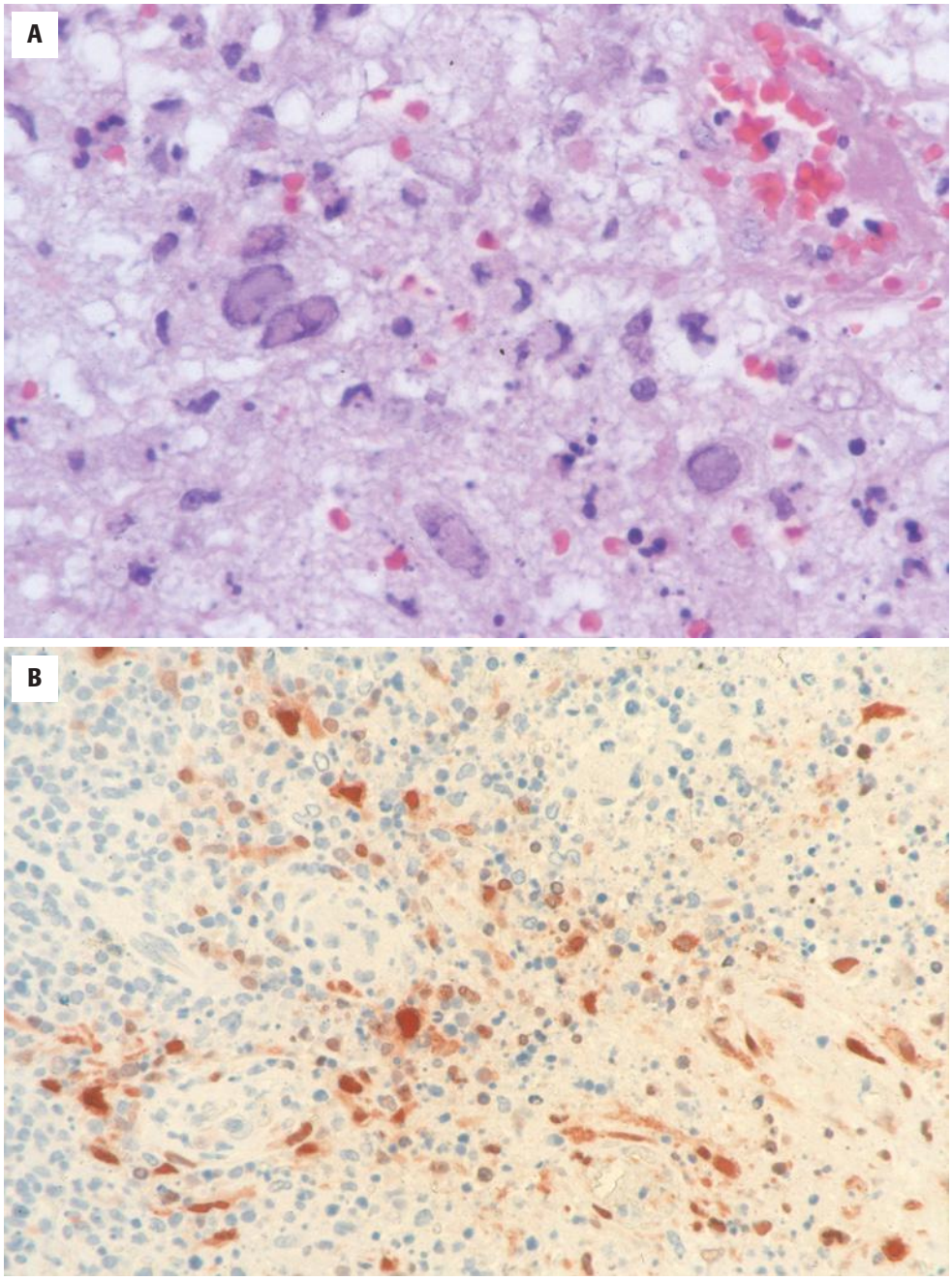


FIG. 4.4

Herpes simplex viral lymphadenitis. A, Scattered virus-infected cells are present in a background of debris. The infected cells are usually uninucleate and contain glassy inclusions, with peripheral margination of chromatin. B, Many cells are stained using an immunostain for herpes simplex virus.

and cervical lymphadenopathy in approximately 16% of cases. Persistent generalized lymphadenopathy, defined as extrainguinal lymphadenopathy persisting for at least 3 months, involving at least two noncontiguous node groups, is common among HIV+ patients. Persistent generalized lymphadenopathy mainly affects adult males and is often accompanied by fever, weight loss, headaches, and malaise. A range of histologic and immunohistologic changes is found in lymphadenopathy associated with HIV infection; similar changes are found in organized extranodal lymphoid tissue. HIV+ patients with opportunistic infections (mycobacterial, fungal) who initiate antiretroviral therapy (ART) may develop "immune reconstitution inflammatory syndrome" (IRIS), an exaggerated inflammatory reaction to an infection that has already been diagnosed and treated (paradoxical IRIS) or to a previously unrecognized, untreated infection (unmasking IRIS). The most common manifestations of IRIS are lymphadenopathy, fever, and lung infiltrates.

Pathologic Features

Early stages of immunodeficiency are characterized by florid follicular hyperplasia, with large, irregular germinal centers with a high mitotic rate, numerous blast cells, many tingible

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HUMAN IMMUNODEFICIENCY VIRUS–ASSOCIATED LYMPHADENOPATHY—FACT SHEET

Definition
- Reactive lymphoid changes related to HIV infection, not due to another specific cause

Incidence
- Common among HIV+ patients; in one study 4% of cases of unexplained follicular hyperplasia were from HIV+ individuals not known to be HIV+ prior to the lymph node biopsy.

Gender, Race, or Age Distribution
- Most patients are young to middle-aged adults (males > females), but smaller numbers of older adults and children of either gender may be affected.

Clinical Features
- A number of different clinical settings in which HIV-associated lymphoid hyperplasia can be found:
  - Lymphadenopathy, usually in the cervical area, occurring in the setting of an IM-like illness, precipitated by initial HIV infection
  - Persistent generalized or isolated lymphadenopathy that may be accompanied by constitutional symptoms
  - Cystic lymphoid hyperplasia, involving periparotid lymph nodes and causing multicystic, often bilateral, masses in the area of the parotid glands
  - Hyperplasia of organized extranodal lymphoid tissue, such as Waldeyer's ring, with symptoms related to mass effect

Morphology
- In early stages, lymph nodes show florid follicular hyperplasia, often with mantle zone attenuation and follicle lysis. Some follicles are highly irregular with geographic shapes.
- In advanced stages of immunodeficiency, there is lymphoid depletion. Follicles are small and burned out and sometimes difficult to identify, and the paracortex appears hypocellular, often with prominent vascularity.
- Plasma cells are often abundant in lymphoid tissues in early and late stages of disease.

Immunophenotype
- B cells in follicles are polytypic; T cells show a decreased CD4-to-CD8 ratio.
- Few to many scattered cells infected by EBV may be found.

Diagnosis
- The morphologic changes are not completely specific, but if an individual has lymphadenopathy with the features described, and the patient is not known to be HIV+, performing a test for HIV is warranted.

Prognosis and Therapy
- Therapy is directed against the HIV infection itself.
- Outcome is related to the prognosis of the underlying HIV infection, but with the availability of highly active antiretroviral therapy, the prognosis has significantly improved.

body macrophages, and ill defined, attenuated, or effaced mantle zones. There is often follicle lysis. The interfollicular region contains a mixture of immunoblasts, plasma cells, lymphocytes, and histiocytes. Monocytoid B cells are often prominent. Sinus histiocytosis, sometimes with erythrophagocytosis, epithelioid histiocytes, and polykaryocytes (Warthin-Finkeldey–type giant cells) may be seen.

More advanced stages of immunodeficiency are associated with lymphoid depletion. Reactive follicles are decreased in number or absent. Residual follicle centers are "burned out" or regressively transformed and contain a decreased number of B cells. Over time, follicles may become inconspicuous and difficult to identify. The interfollicular region contains scattered lymphocytes, immunoblasts, plasma cells, and many blood vessels. Amorphous eosinophilic material may be present, or there may be fibrosis, and the node may have a pale, depleted appearance. In some cases, lymph nodes show changes intermediate between florid follicular hyperplasia and lymphoid depletion. Plasmacytosis is frequent. Occasional polykaryocytes may be found. Cutaneous rashes are common among HIV+ patients; these individuals often have a component of dermatopathic lymphadenopathy. The interfollicular region contains decreased numbers of CD4+ cells, and the CD4-to-CD8 ratio is usually reversed. It is common to find scattered cells harboring Epstein-Barr virus (EBER+) consistent with poor T-cell control of EBV-infected B cells.

Most patients who have been treated with highly active antiretroviral therapy show improvement in lymphoid architecture, as well as an increase in CD4+ T cells and a decrease in CD8+ T cells, within lymphoid tissue. However, HIV often is detectable in follicular dendritic cells in lymphoid tissue, even after a prolonged course of highly active antiretroviral therapy, and even in the absence of detectable virus in the peripheral blood.

MEASLES LYMPHADENITIS

Lymphadenitis due to measles virus, an RNA paramyxovirus, is now seen only rarely in the United States because of inoculation programs. Patients typically present with fever, rash, malaise, and cough and usually also with headache and sore throat. A minority of patients have lymphadenopathy. On pathologic examination of lymphoid tissue, the most distinctive feature is the presence of Warthin-Finkeldey giant cells within reactive germinal centers. Warthin-Finkeldey cells are found in the prodromal stage of infection and generally disappear as a rash develops (Fig. 4.5).

KAWASAKI DISEASE

Although Kawasaki disease is of uncertain cause, evidence strongly suggests that it is of infectious etiology. For this reason, it is included in the section on infectious lymphadenitis. Pathologic findings are not specific but include multifocal necrosis and fibrin thrombi in the microvasculature.

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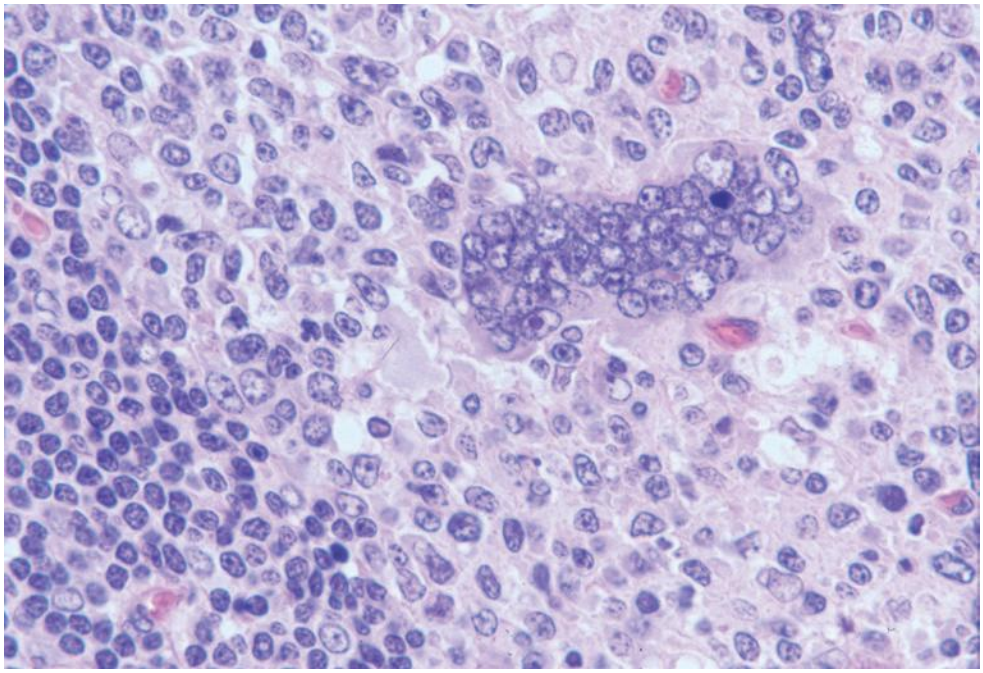


FIG. 4.5

Measles: appendix. The lymphoid follicles in this appendix contain multinucleated Warthin-Finkeldey giant cells. The appendix was removed because of symptoms suggesting acute appendicitis; the patient developed a rash typical of measles immediately after appendectomy.

KAWASAKI DISEASE—FACT SHEET

Definition

- An acute febrile disease of young children; cause is uncertain, but most evidence suggests an infectious, possibly viral, cause.
- Synonyms: mucocutaneous lymph node syndrome; infantile polyarteritis

Incidence and Location

- Approximately 17 cases per 100,000 children younger than 5 years of age per year in the United States
- Incidence is higher in Japan and in other Asian countries.

Gender, Race, and Age Distribution

- Of patients, 85% are younger than 5 years of age.
- Boys are more often affected than girls (male-to-female ratio, approximately 2:1).
- Prevalence is higher among Asians.

Risk Factors

- Higher risk among family members of patients with Kawasaki disease.

Diagnosis

- The Centers for Disease Control and Prevention requirements for the diagnosis of Kawasaki disease include finding fever of 5 or more days, unresponsive to antibiotics, and at least four of the following five features:
  - Bilateral conjunctival congestion
  - Abnormalities of lips and oral cavity including diffuse erythema, dry fissured lips, and prominent lingual papillae (strawberry tongue)
  - Abnormalities of the skin of the distal extremities, including erythema of palms and soles with edema early on and desquamation of fingertips later in the course of the disease
  - Polymorphous, nonvesicular, primarily truncal rash
  - Acute, nonsuppurative cervical lymphadenopathy not due to any other identifiable cause

Clinical Features

- In addition to the diagnostic criteria noted, patients may have cardiac abnormalities (electrocardiographic changes, cardiomegaly, murmurs), diarrhea, arthritis or arthralgia, proteinuria, sterile pyuria, neutrophilic leukocytosis with a leftward shift, anemia, thrombocytosis, elevated sedimentation rate, aseptic meningitis, mild jaundice, and elevated transaminases.
- Most patients recover after an illness of 3 to 4 weeks' duration, but without therapy, 20% to 25% develop complications of coronary arteritis (aneurysm, thrombosis), which may be fatal, sometimes years later.
- Among patients with cervical lymphadenopathy the male-to-female ratio tends to be higher than among those without adenopathy. Patients with cervical lymphadenopathy are on average older. They tend to have more pronounced changes in markers of systemic inflammation, such as an elevated white blood cell count and C-reactive protein, and to include a higher proportion of patients with coronary artery disease.

Pathologic Features

- Lymphadenopathy is typically cervical, but nodes in other sites may be affected.
- Findings are variable from case to case; they include:
  - Nonspecific reactive hyperplasia with dilated sinuses and paracortical expansion by lymphocytes and a variable number of immunoblasts, plasma cells, and histiocytes.
  - A subset of cases shows small to large areas of necrosis beginning beneath the capsule, containing karyorrhectic debris.
  - Increased numbers of blood vessels lined by swollen endothelial cells and containing fibrin thrombi around the necrotic areas. Inflammation with mononuclear cells often extends to involve the capsule and perinodal soft tissue, with phlebitis.
  - Absence of abscess formation and granulomas; neutrophils and eosinophils are typically sparse.
  - Underlying the changes is activation of the immune response, with important components being IgA, cytotoxic T cells, and monocytes/macrophages.

Prognosis and Therapy

- Intravenous Ig and aspirin; steroids and other anti-inflammatory agents can be used if symptoms persist.
- Prompt administration of intravenous Ig reduces the risk of coronary arterial changes and thus the risk of serious or fatal complications of this disease.

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BACTERIAL LYMPHADENITIS

PYOGENIC BACTERIAL LYMPHADENITIS

Bacterial infections are a common cause of cervical lymphadenopathy. Lymphadenitis due to streptococcal or staphylococcal infection is probably the most common cause of acute cervical lymphadenopathy in children in the United States, although a biopsy is not often performed. Microscopic examination usually shows follicular hyperplasia and a variably dense paracortical neutrophilic infiltrate.

CAT-SCRATCH DISEASE

Clinical Features

Cat-scratch disease is among the most common causes of subacute or chronic benign lymphadenopathy in the United States, with about 24,000 cases occurring per year. For reasons that may be related to the breeding patterns of cats, it is more common between July and December than between January and June. The principal causal agent for cat-scratch disease is *Bartonella henselae*. Although cat-scratch disease can affect patients of any age, the vast majority are younger than 30. A history of exposure to a cat (typically a kitten with fleas) can be found in most cases. The infectious agent has been found within the gastrointestinal tract of fleas, and it is possible that fleas act as vectors in the transmission of the disease. Three to 10 days after exposure, a papule develops in the skin at the inoculation site; the papule typically becomes vesiculated and then crusted over the next several days. Regional lymphadenopathy is usually found 1 to 2 weeks after the papule appears.

The lymphadenopathy, which is often tender, usually involves only one node or one group of nodes. Noncontiguous lymphadenopathy is occasionally found, probably because of more than one inoculation site or because the inoculation site is midline, allowing organisms to drain to lymph nodes bilaterally. Axillary lymph nodes are most commonly involved, followed by cervical and then by inguinal lymph nodes. When the inoculation site is the eye, patients may develop the oculoglandular syndrome of Parinaud (granulomatous conjunctivitis and preauricular lymphadenopathy). As many as half of patients have fever, but it is usually low grade (lower than 39° C). Patients may also experience malaise, anorexia, or, rarely, nausea or abdominal pain. The disease is usually mild and self-limited, and some cases probably go unrecognized. Severe complications, occurring in as many as 2%, include involvement of the nervous system, bone, lung, liver, or spleen. Neurologic manifestations include encephalopathy, encephalitis, meningitis, and involvement of cranial or peripheral nerves. The liver and spleen may be the sites of abscess formation. The skeleton is rarely involved by a necrotizing granulomatous osteomyelitis, sometimes via direct extension from an affected lymph node. Patients with lung involvement may have pneumonia or pleural effusions. Immunocompromised patients are more likely to develop severe manifestation of infection, including disseminated disease.

CAT-SCRATCH DISEASE—FACT SHEET

Definition
- Lymphadenitis typically transmitted by cats, caused in nearly all cases by *Bartonella henselae*, occasionally by *B. quintana*.

Incidence and Location
- Relatively common
- Approximately 24,000 cases/year in the United States

Gender, Race, and Age Distribution
- Most patients under 30 years of age
- No known gender or racial predilection

Risk Factors
- Contact with a cat, especially a kitten with fleas

Clinical Features
- A cutaneous lesion appears at the inoculation site (usually the site of a scratch) after 3 to 10 days.
- Tender regional lymphadenopathy appears 1 to 2 weeks later.
- Patients often have low-grade fever and malaise and infrequently have evidence of involvement of other sites, such as lungs, liver, spleen, bones, or central nervous system.

Morphology
- Early stage: Lymph nodes show follicular and monocytoid B-cell hyperplasia, with small foci of acute inflammation and necrosis usually beginning within aggregates of monocytoid B cells.
- Later stage: Large stellate microabscesses and necrotizing granulomas with palisading histiocytes

Diagnosis
- Identification of cat-scratch bacilli with a silver stain or an immunostain
- PCR on affected tissues
- Serologic studies

Prognosis and Therapy
- Most patients, including those with localized lymphadenopathy, have a mild, self-limited course and do not require specific therapy. Patients with severe manifestations of disease require antibiotic therapy.

Pathologic Features

The appearance of the infected lymph nodes changes over time. The earliest changes are follicular hyperplasia and a prominent proliferation of monocytoid B cells. Subsequently, within aggregates of monocytoid B cells, small foci of necrosis

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with a few neutrophils and scant fibrin and cellular debris appear. These foci are usually close to, or encroach on, germinal centers, or they may be adjacent to the subcapsular sinus. In some cases, they are found in the vicinity of a small blood vessel. The paracortex is hyperplastic. Sinuses contain immunoblasts, neutrophils, and histiocytes. With time, the necrotic foci enlarge, extending deeper into the node. They contain pus, fibrin, and abundant cellular debris and acquire a rim of macrophages. The foci continue to enlarge and coalesce and are surrounded by palisading histiocytes to produce the classic stellate microabscess or granuloma. At later stages, the necrosis can become amorphous and eosinophilic without recognizable neutrophils, resembling caseation necrosis. Multinucleated giant cells are occasionally seen. Plasmacytosis and extension to involve the capsule and perinodal soft tissue are common. The cat-scratch bacillus is a small, slender, pleomorphic, weakly gram-negative rod as long as 3 μm. Its small size makes it very difficult to see, but using a Warthin-Starry stain or Steiner stain, the bacilli are coated with the black reaction product, making them appear larger and more readily visualized. Bacilli are present singly, in chains, or large clumps. They may be found along the walls of blood vessels, in macrophages in necrotic areas, in sinus histiocytes, or admixed with necrotic debris. Bacilli are most numerous in the stages of early necrosis within clusters of monocytoid B cells. When well-developed stellate abscesses have formed, the organisms can be difficult to detect (Fig. 4.6). *Bartonella henselae* is a fastidious organism that is difficult to culture.

Serologic studies may be helpful in establishing a diagnosis, although serology may yield false-negative results and also may not distinguish between recent and past infections. An antibody to *B. henselae* has been developed that may help to identify the microorganisms. In addition, probes for *Bartonella*-specific genetic sequences are available that can be used to detect cat-scratch disease using the polymerase chain reaction (PCR). However, establishing a diagnosis is often difficult because microorganisms may be present only focally and in small numbers and because prior antibiotics may decrease the sensitivity of some of these assays.

Differential Diagnosis

A variety of microorganisms cause necrotizing lymphadenitis that can be considered in the differential diagnosis of cat-scratch disease. Clinical features, including age, risk factors for different types of infections, anatomic distribution of lymphadenopathy, and severity of disease; special stains on tissue sections; serologic studies; and culture are helpful in establishing a definite diagnosis. Infection caused by certain bacteria, including *Chlamydia trachomatis* (lymphogranuloma venereum), *Francisella tularensis* (tularemia), *Hemophilus ducreyi* (chancroid), *Yersinia enterocolitica* (pseudotuberculous mesenteric lymphadenitis), *Listeria monocytogenes* (listeriosis), *Pseudomonas mallei* (glanders), and *Pseudomonas pseudomallei* (melioidosis), can be associated with a necrotizing lymphadenitis with histologic features that may be indistinguishable from those of cat-scratch disease. In nearly all cases, the diseases listed here are associated with significantly greater morbidity than cat-scratch disease. Early involvement by cat-scratch disease may also resemble toxoplasma lymphadenitis (see later section on this topic).

As noted previously, infection by pyogenic cocci may be associated with an acute suppurative lymphadenitis with follicular hyperplasia that can suggest cat-scratch disease. Unlike cat-scratch disease, however, the pyogenic cocci typically do not elicit a rim of palisading histiocytes around areas of suppurative necrosis. One exception is in cases of chronic granulomatous disease of childhood, in which palisading granulomas with suppurative necrosis may be found in association with infection due to *Staphylococcus aureus* and a variety of gram-negative bacteria (see later section on this topic). The appearance can closely resemble that of cat-scratch disease.

Mycobacteria (*Mycobacterium tuberculosis* and non-tuberculous mycobacteria) and fungi can produce lymphadenitis with granulomatous microabscesses that may resemble those of cat-scratch disease. The most important differential diagnosis may be between cat-scratch disease and non-tuberculous mycobacterial infection of cervical lymph nodes in children. As noted above, in some late-stage cases of cat-scratch disease, the necrotic material acquires a pink amorphous quality closely resembling the caseation necrosis characteristic of tuberculosis. Special stains for microorganisms, culture, and other special techniques may be helpful in establishing a diagnosis. The distinction is clinically relevant, as cat-scratch disease often does not require treatment, whereas infection by *M. tuberculosis* should be treated with antibiotics. In immunocompromised hosts, *B. henselae* infection rarely takes the form of localized granulomatous lymphadenitis. Instead, it may result in widespread granulomatous inflammation, bacillary angiomatosis, bacillary peliosis, or bacteremia.

SYPHILITIC LYMPHADENITIS

The first manifestation of syphilis, primary syphilis, takes the form of a red-brown, painless, indurated, ulcerated chancre, which is usually 1 to 2 cm in diameter, appearing within 21 days of exposure on exposed skin or mucosa. Lymphadenopathy in the region draining the chancre then develops. In secondary syphilis, disease disseminates, with widespread distribution of red-brown, scaly plaques on the skin, especially the palms and soles. Multifocal lymphadenopathy is also found. Untreated, the infection may progress to tertiary syphilis, with gummas involving skin, bone, and liver, and occasionally cardiovascular structures and the central nervous system. During recent years, there has been an increase in the number of cases of syphilis among men in the United States, particularly among men having sex with men.

In primary and secondary syphilis, lymph nodes show marked follicular hyperplasia with numerous plasma cells.

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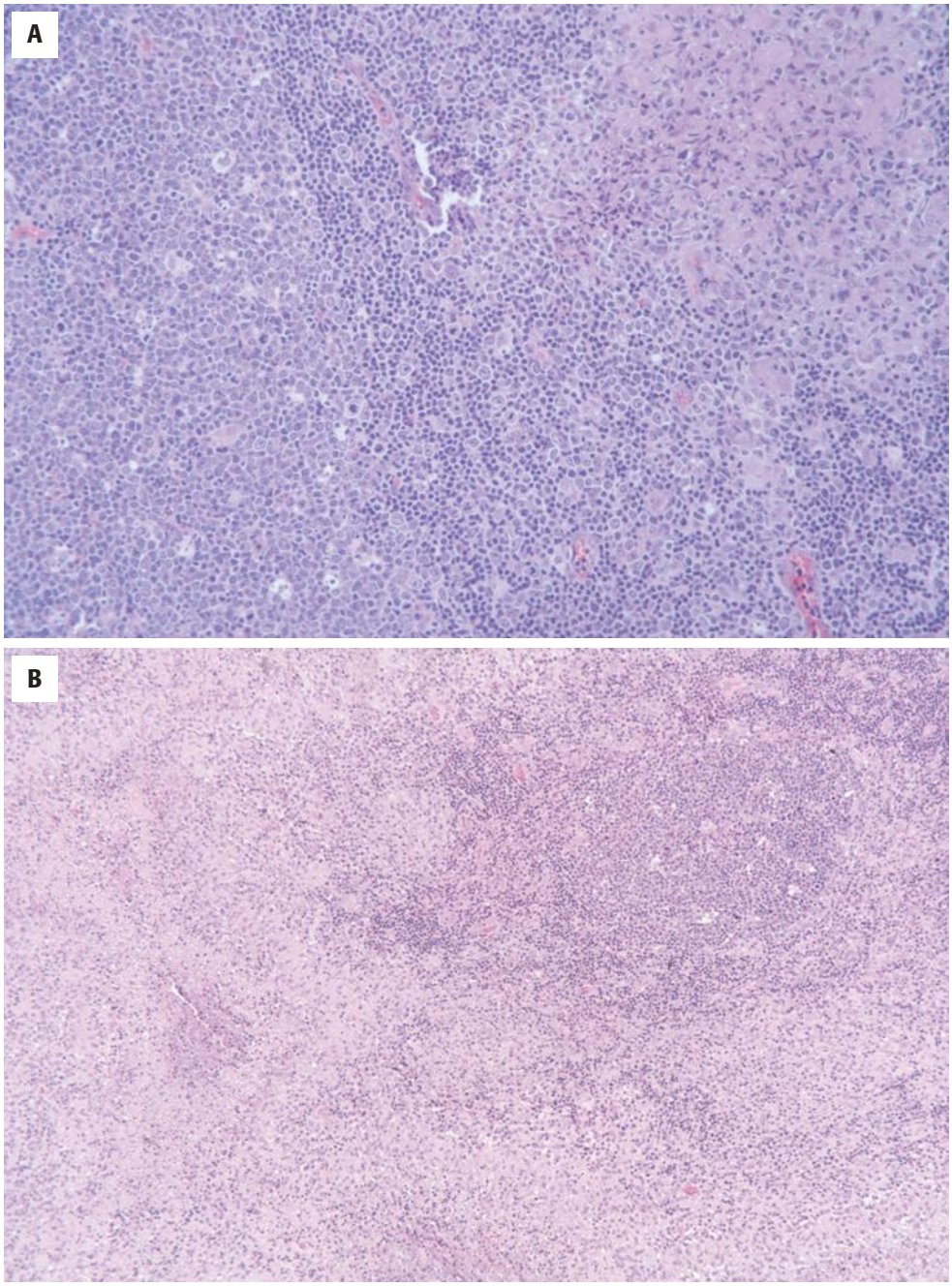


FIG. 4.6

Cat-scratch disease (*Bartonella henselae*–associated lymphadenitis). A, In an example of an early stage of infection, there is a large reactive follicle; adjacent to it is an aggregate of histiocytes, fibrin, and debris. B, In this case, the inflammatory process is more advanced. One reactive follicle is seen, but most of the rest of the node is replaced by necrotizing granulomatous inflammation with palisading histiocytes.

They may also contain poorly formed, non-necrotizing or suppurative granulomas and show marked capsular and perinodal fibrosis and prominent vascular changes (endarteritis and phlebitis). Spirochetes can be found in blood vessels, germinal centers, and granulomas and are often within the cytoplasm of histiocytes. Involved lymph nodes may have the appearance of an inflammatory pseudotumor on routinely stained sections. The changes characteristic of syphilis are best seen in inguinal nodes. Although secondary syphilis may be associated with diffuse lymphadenopathy, nodes away from the inguinal region may show only nonspecific follicular hyperplasia.

BRUCELLOSIS

Brucellosis is a zoonotic infection that may be acquired directly or indirectly from animals.

MYCOBACTERIAL LYMPHADENITIS

Mycobacterial lymphadenitis can occur in isolation or in conjunction with pulmonary tuberculosis or disseminated infection. Tuberculous lymphadenitis is the most common form of tuberculosis except for pulmonary tuberculosis. The

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BRUCELLOSIS—FACT SHEET

Definition
- An acute, intermittent, or chronic febrile zoonotic illness due to infection by *Brucella* species
- Typically, *Brucella melitensis* affects goats and sheep, *B. abortus* affects cattle, and *B. suis* affects pigs.
- *Brucella melitensis* is the most common cause of brucellosis in humans and is especially prevalent in developing countries.
- Brucella are gram-negative, intracellular coccobacilli.
- Synonym: undulant fever

Incidence and Location
- Highly variable
- Approximately 3.5 cases per 100,000 persons per year in an endemic area
- Rare in the United States

Gender, Race, and Age Distribution
- Children and adults, both males and females, are affected, depending on exposure.
- No known racial predisposition

Risk Factors
- Close animal contact (shepherds, goatherds), ingestion of unpasteurized milk, consumption of uncooked meat

Clinical Features
- The severity and types of symptoms present are highly variable from case to case.
- Fever is virtually always present, and nearly all patients complain of malaise and night sweats.
- Arthralgia, myalgia, back pain, headache, abdominal pain, and gastrointestinal symptoms (diarrhea, nausea, vomiting) are common.
- Splenomegaly is common.
- Hepatomegaly and lymphadenopathy are slightly less common.
- Lymphadenopathy appears to be more common in children than in adults; it is more conspicuous early in the course of the disease. Cervical lymphadenopathy is more likely to be found if infection is acquired orally.
- Some patients have evidence of cardiac and even central nervous system involvement.
- Laboratory abnormalities that may be found include anemia, leukopenia, monocytosis, and eosinophilia.
- The presence of splenomegaly tends to correlate with the finding of leukopenia and with fever of more than 1 week's duration.

Pathologic Features
- Involved tissues show non-necrotizing, sarcoidal granulomas, necrotizing granulomas, sometimes with suppurative necrosis, abscesses, or diffuse mononuclear infiltrates.

Diagnosis
- Blood culture, biopsy of involved lymph node (or other tissue) with microscopic examination and culture, serologic studies, and/or serum agglutination test

Prognosis and Therapy
- Treatment consists of administration of appropriate antibiotics; prolonged use of antibiotics can help prevent relapse.
- Prognosis is good with appropriate therapy.
- Animal testing and treatment could decrease the incidence of disease in humans.

clinical picture varies with the type of mycobacteria and the age and immune status of the affected patients.

TUBERCULOUS LYMPHADENITIS

Clinical Features

Tuberculous lymphadenitis is due to infection by tuberculous mycobacteria, such as *Mycobacterium tuberculosis* or *M. bovis*. Infection by *M. tuberculosis* is a serious global health problem, with approximately one third of the world's population harboring *M. tuberculosis*. Approximately 9×10^6 new cases of tuberculosis and approximately 2×10^6 deaths from tuberculosis occur each year. In Central Africa, for example, tuberculous lymphadenitis is the most common finding among patients who undergo a superficial lymph node biopsy; nearly all of these African patients are human immunodeficiency virus positive (HIV+). *M. tuberculosis* was also the most common specific cause of cervical lymphadenopathy in children in a study from South Africa. Although tuberculosis is much less common among individuals in the United States than in other parts of the world, an increased risk of infection is found in association with poor living conditions. Also, administration of incomplete courses of antibiotics because of poor patient compliance has led to the emergence of increased numbers of drug-resistant strains.

Patients with lymphadenitis due to *M. tuberculosis* are usually adults who present with non-tender lymphadenopathy, constitutional symptoms (fever, fatigue, and weight loss) and have associated pulmonary tuberculosis. When peripheral lymph nodes are involved, they tend to be in the supraclavicular fossa or posterior cervical triangle. Frequently, multiple lymph nodes are involved bilaterally, and there are draining sinuses. The purified protein derivative (PPD) test is usually strongly positive (Table 4.1).

Pathologic Features

Lymph nodes in immunocompetent patients show multiple well-formed granulomas composed of epithelioid histiocytes

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TABLE 4.1
Tuberculous Versus Nontuberculous Lymphadenitis in Immunocompetent Patients

	Tuberculous Mycobacterial Infection	Nontuberculous Mycobacterial Infection
Definition	Lymphadenitis due to tuberculous organisms (*Mycobacterium tuberculosis*, *Mycobacterium bovis*)	Lymphadenitis due to atypical mycobacteria
Age	Any age	Majority <5 yr
Gender	No gender predilection	M = F or M < F (varies by series)
Constitutional	Often present	Infrequent; mild symptoms, if present
Sites	Cervical nodes, mediastinal nodes, lungs	Cervical nodes, unilateral, most often submandibular, jugulodigastric, or preauricular lymph nodes
Chest x-ray	Frequently abnormal	Negative, almost always
Contagious	Yes	No
Purified protein derivative (PPD) test	Usually strongly positive	Negative or weakly positive
Treatment	Antibiotics	Excision or FNA followed by observation

and Langhans-type giant cells; caseation necrosis is present to a variable extent in the centers of the granulomas. Dystrophic calcification may be seen. A Ziehl-Neelsen stain is used to identify these acid-fast bacilli, which are usually few in number in immunocompetent patients. Culture is needed to diagnose cases in which organisms cannot be identified in tissue sections and to distinguish definitively between infections by *M. tuberculosis* and non-tuberculous mycobacteria.

NONTUBERCULOUS MYCOBACTERIAL LYMPHADENITIS

Nontuberculous mycobacterial lymphadenitis is due to infection by the so-called atypical mycobacteria, most often *Mycobacterium avium-intracellulare* and less often *Mycobacterium scrofulaceum*, *Mycobacterium hemophilum, Mycobacterium malmoense*, or *Mycobacterium kansasii*.

Clinical Features

Most immunocompetent individuals who develop nontuberculous mycobacterial lymphadenitis are children, most of whom are younger than 5 years of age. Some studies have described an equal sex ratio; others report a higher proportion of female patients. It is said to be the most common cause of chronic lymphadenitis among previously healthy children, although it should be noted that in some developing countries, tuberculous lymphadenitis is more prevalent than nontuberculous mycobacterial lymphadenitis. The annual incidence is estimated to be 1.2 to 2.1 cases per 100,000 persons. The incidence appears to have increased in recent years.

Children present with firm, nontender cervical lymphadenopathy that is almost always unilateral. Constitutional symptoms are typically absent. Lymph nodes away from the cervical area are rarely affected. The microorganisms are believed to infect the patient via the oropharynx, skin, or conjunctiva, and involved lymph nodes correspond to those with lymphatic drainage from these sites. Over time, necrosis develops within the infected nodes, and they become fluctuant. The overlying skin may become discolored, and sinus tracts may form. The PPD test is usually weakly positive or negative. The chest x-ray is almost always normal.

Patients do not usually respond to antibiotics. Surgical excision of the abnormal lymph nodes is the traditionally accepted optimal therapy, associated with a low rate of recurrence. If incomplete removal of the lymph nodes by incision and drainage or curettage is performed, the infection often persists, resulting in sinus tract formation, and additional surgery may be required. Recently, however, it has been suggested that diagnosis based on fine needle aspiration (FNA) with culture followed by observation only may be considered as an alternative to excision.

Pathologic Features

The children have enlarged, sometimes matted lymph nodes with necrotizing and non-necrotizing granulomas. The necrosis often forms a stellate pattern and may be caseous or suppurative. Necrotic areas may contain neutrophils and nuclear debris focally or diffusely. Langhans-type giant cells can often be found. The diagnosis can be suspected based on clinical features. It can be confirmed and the infection can be treated by complete excision of affected lymph nodes. Special stains for microorganisms and culture will demonstrate the organisms. Ziehl-Neelsen stain typically reveals small numbers of acid-fast bacilli in some cases, particularly at the periphery of the necrotic area, but in other cases, organisms cannot be

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identified without culture. In selected instances, PCR may be used to subclassify the organisms (see Table 4.1).

MYCOBACTERIAL LYMPHADENITIS IN IMMUNOCOMPROMISED PATIENTS

In contrast to atypical mycobacterial infection in immunocompetent patients, in HIV+ patients, atypical mycobacteria, especially *M. avium-intracellulare*, can be associated with disseminated infection. Immunosuppressed patients sometimes have lymphadenopathy with necrotizing or non-necrotizing granulomas; however, sometimes they fail to develop well-formed granulomas. In such cases, involved tissues may contain only loose aggregates and sheets of foamy histiocytes containing numerous microorganisms. Rarely, patients develop so-called mycobacterial pseudotumors, composed of rounded and elongate histiocytes containing numerous acid-fast bacilli. The appearance on routinely stained sections can occasionally suggest a mesenchymal neoplasm (Fig. 4.7). The microorganisms can be seen with the Grocott methenamine silver stain as well as with the Ziehl-Neelsen stain. When the acid-fast bacilli have been phagocytosed and are intracellular they may be periodic acid-Schiff (PAS)-positive. HIV+ patients with subclinical *M. avium-intracellulare* infection who are started on highly active antiretroviral therapy may develop immune reconstitution inflammatory syndrome (IRIS) in the form of symptomatic *M. avium-intracellulare* lymphadenitis. A similar phenomenon can occur with other types of opportunistic infections as well.

Differential Diagnosis

The differential diagnosis of mycobacterial lymphadenitis is broad (Table 4.2). Cases with little or no necrosis resemble sarcoidosis. A Ziehl-Neelsen stain and culture are helpful in establishing a diagnosis; however, the Ziehl-Neelsen stain is often negative in cases of mycobacterial infection unless there is necrosis. A wide variety of microorganisms, including fungi, cat-scratch bacilli, *Brucella* species, spirochetes, and leishmania, can be associated with granulomatous lymphadenitis. Caseation necrosis is more common in mycobacterial infections, although necrosis resembling caseation may be seen in fungal infections and, less often, with other infections. In cases of primary or secondary syphilis, lymph nodes may show non-necrotizing or suppurative granulomas, but they are found in the setting of marked follicular hyperplasia and plasmacytosis. Cat-scratch disease, a common cause of necrotizing granulomatous lymphadenitis in children, is more likely to be associated with exposure to a cat, a cutaneous inoculation site, systemic symptoms, and tender lymphadenopathy compared to non-tuberculous mycobacterial lymphadenitis in immunocompetent children. Uncommon entities that may enter the differential diagnosis include leprosy, particularly the tuberculoid form, and chronic granulomatous disease of childhood. Atypical mycobacterial infection in HIV+ patients in the form of numerous foamy histiocytes can mimic lepromatous leprosy. As intracellular mycobacteria may be PAS+, those cases with foamy histiocytes may mimic Whipple's disease, although the clinical features of Whipple's disease differ from those of mycobacterial infection, and Whipple's bacilli are not expected to be acid-fast (see separate section below). A few cases of Wegener granulomatosis have been described in which there is necrotizing lymphadenitis with granulomatous vasculitis, and such cases could potentially enter the differential diagnosis of mycobacterial lymphadenitis. Results of stains for microorganisms, culture, and clinical information can be helpful in establishing a diagnosis.

Granulomatous inflammation, often accompanied by extensive necrosis, may be found in tissues involved by Hodgkin lymphoma. Identification of Reed-Sternberg cells, which tend to be found in greatest numbers at the periphery of necrotic areas, confirms the diagnosis of Hodgkin lymphoma. The presence of a polymorphous inflammatory cell infiltrate, with eosinophils and plasma cells, is more common in Hodgkin lymphoma than in mycobacterial infection. Non-Hodgkin lymphomas may also occasionally be associated with granulomas, but this is much less common than in Hodgkin lymphoma.

Metastatic carcinoma can occasionally be associated with granulomas in lymph nodes. The granulomatous inflammation may obscure the neoplastic population, and the carcinoma may be overlooked. Granulomas can also be found in lymph nodes draining carcinoma but that are themselves free of metastases.

BACILLE CALMETTE-GUÉRIN LYMPHADENITIS

Bacille Calmette-Guérin (BCG) vaccine has been administered in some developing countries to decrease the risk of developing tuberculosis. Ipsilateral regional lymphadenopathy occurs after BCG vaccination in approximately 1% of individuals who are vaccinated. Other infrequent complications of BCG vaccination include abscesses at the vaccination site and inflammation involving bones, joints, and other sites.

Lymphadenopathy usually develops within 6 months of vaccination. Immunocompetent patients have nontender lymphadenopathy without constitutional symptoms. There are two pathologic forms: suppurative and nonsuppurative. Pathologic examination reveals epithelioid granulomas with or without necrosis. Acid-fast bacilli are present in small numbers. The nonsuppurative form usually resolves spontaneously. The suppurative form is accompanied by erythema and edema of overlying skin and sometimes by ulceration and sinus tract formation. Larger lymph nodes (>5 cm) are also unlikely to resolve without complications. Drainage by needle aspiration can help to prevent sinus tracts and to expedite healing.

Vaccination with BCG is contraindicated in patients who are immunodeficient. When BCG is administered to individuals with an underlying immunodeficiency, such as severe combined

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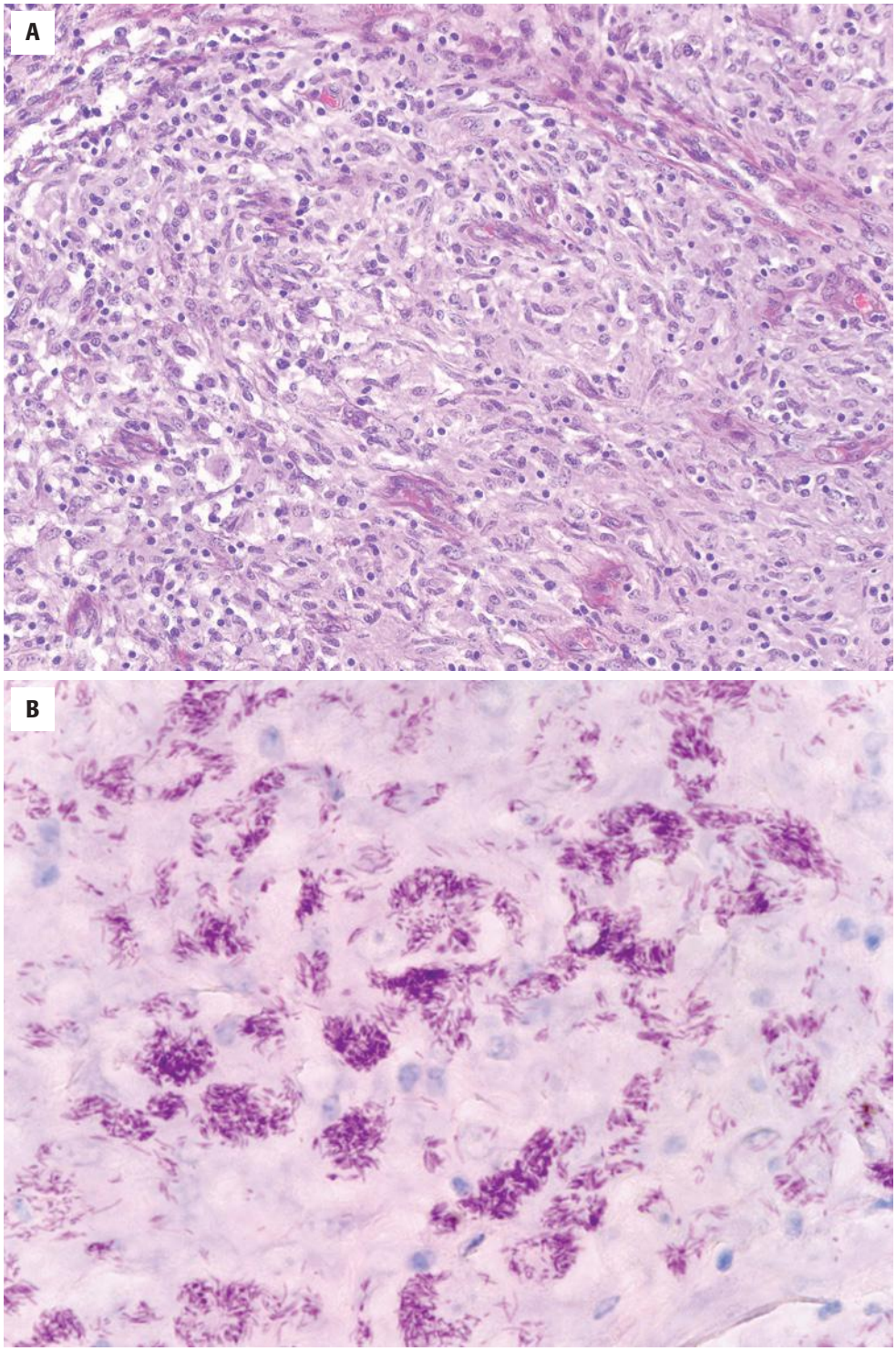


FIG. 4.7

Mycobacterial pseudotumor in a patient with human immunodeficiency virus. A, The lymph node is replaced by histiocytes and spindle cells with finely granular pink cytoplasm. B, A Ziehl-Neelsen stain discloses numerous intracellular acid-fast bacilli.

immunodeficiency, chronic granulomatous disease, or HIV infection, they are at risk of the development of disseminated BCG infection with generalized lymphadenopathy. Depending on the type of immunodeficiency, there may be epithelioid granulomas with few acid-fast bacilli or diffuse infiltrates of histiocytes laden with numerous acid-fast bacilli. This serious complication of the vaccination may be fatal and requires antituberculous therapy as well as treatment of the immunodeficiency.

WHIPPLE DISEASE

Clinical Features

Whipple disease predominantly affects middle-aged to older white men who usually present with diarrhea, weight loss, abdominal pain, and arthritis. Peripheral lymphadenopathy is common; intraabdominal and mediastinal nodes may also

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TABLE 4.2
Causes of Lymphadenopathy and Lymphadenitis by Pattern

Follicular Hyperplasia Prominent	Paracortical Hyperplasia Prominent	Predominantly Sinus Involvement	Granulomatous
Cytomegalovirus (CMV) lymphadenitis	Infectious mononucleosis	Sinus histiocytosis with massive lymphadenopathy	Sarcoidosis
Human immunodeficiency virus (HIV) infection	Herpes simplex lymphadenitis	Lipogranulomas	Berylliosis and reaction to other foreign material
Cat-scratch disease, early stage	Dermatopathic	Reaction to silicone prosthesis, some cases	Cat-scratch disease, later stage
Syphilis	Drug-induced		Tularemia
Toxoplasmosis	Kikuchi's disease		Yersinia
Rheumatoid arthritis	Systemic lupus erythematosus, subset of cases		Lymphogranuloma venereum
Systemic lupus erythematosus, subset of cases			Syphilis
Kimura disease			Brucellosis
IgG4-related disease			Fungal infection
			Tuberculosis
			Non-tuberculous mycobacterial Infection
			Leprosy

BACILLE CALMETTE-GUÉRIN LYMPHADENITIS—FACT SHEET

Definition
- Ipsilateral regional lymphadenopathy occurring after BCG vaccination

Incidence
- Approximately 1% of vaccinated patients

Risk Factors
- Immunodeficiency predisposes to severe BCG infection.

Clinical Features
- Immunocompetent individuals develop lymphadenopathy that is usually nontender within 6 months of vaccination.
- Immunodeficient individuals may develop generalized lymphadenopathy and disseminated BCG infection.

Morphology
- Lymph nodes show granulomas with or without necrosis.
- When present, necrosis is usually suppurative.
- Immunodeficient patients have histiocytic infiltrates with or without granuloma formation.

Prognosis and Therapy
- Nonsuppurative lymphadenitis usually resolves spontaneously.
- Drainage of suppurative lymphadenitis can help expedite healing and prevent development of sinus tracts.
- Immunodeficient individuals require prompt treatment with antibiotics.

be enlarged. Skin may become hyperpigmented. The disease can also affect the heart and lungs, with inflammation or fibrosis of endocardium, myocardium, or pericardium; thickened, distorted cardiac valves; chronic cough; and/or pleuritic pain. Whipple disease can produce a wide variety of manifestations among patients with central nervous system involvement. Ocular involvement, almost always found in patients who also have central nervous system disease, can result in retinitis or uveitis and loss of vision. Although symptoms related to gastrointestinal involvement are generally the most prominent, some patients have presented with lymphadenopathy without diarrhea or malabsorption; in some of these unusual cases, the diagnosis was delayed by years or was made only at autopsy.

Whipple disease usually responds to antibiotics, but the treatment duration must be long to prevent relapse. The antibiotics should cross the blood–brain barrier in order to treat any central nervous system disease and to prevent relapse in the central nervous system, which is associated with a high mortality rate.

Pathologic Features

In lymph nodes, the distinctive finding is the presence of lipogranulomas with clusters of foamy and epithelioid histiocytes and sinuses distended by cystic-appearing, lipid-filled spaces surrounded by foreign body giant cells. The histiocytes have granular, PAS-positive, diastase-resistant cytoplasm.

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WHIPPLE DISEASE—FACT SHEET

Definition
- An infectious disease caused by *Tropheryma whipplei*
- Synonym: intestinal lipodystrophy (older nomenclature)

Incidence
- Rare; estimated annual incidence of less than one per million persons

Gender, Race, and Age Distribution
- Male predominance (male-to-female ratio: 5:1 to 8:1)
- Age ranges from childhood to old age; mean age at diagnosis is approximately 50 years.
- Most patients are white.

Risk Factors
- Possible increased risk for farmers
- Transmission from person to person has not been described.

Clinical Features
- Symptoms are variable.
- Lymphadenopathy (peripheral and/or internal) is found in 50% of cases.
- Weight loss, malabsorption, diarrhea, abdominal pain, arthralgias, anemia, cutaneous hyperpigmentation, and fever occur in 50% or more of patients.
- Cardiac disease (endocarditis, pericarditis, myocardial fibrosis, and/or valvular abnormalities), chronic cough, central nervous system manifestations (cognitive changes, motor and sensory deficits, altered consciousness, and/or hypothalamic dysfunction, and visual changes) are found in 50% or less of patients.

Morphology
- Lymph nodes are often enlarged and may show lipogranulomas, with many histiocytes with PAS-positive cytoplasm, or may show sarcoidal granulomas.

Diagnosis
- Biopsy of affected tissue, especially small intestine for light microscopy, including PAS stain, and for electron microscopy
- PCR on tissue or fluid, such as blood or cerebrospinal fluid

Prognosis and Therapy
- Chronic disease, slowly progressive; untreated, may be fatal
- Treatment consists of long-term administration of antibiotics, preferably those with the ability to cross the blood–brain barrier.

However, in some cases, especially when peripheral lymph nodes have been examined, non-necrotizing granulomas, similar to those seen in sarcoidosis, are identified. In such cases, the PAS stain may be negative or only focally positive, and bacilli may be rare or absent.

Electron microscopy reveals that the PAS-positive histiocytes are filled with intact and degenerated bacilli. The Whipple's bacilli are bacteria with an unusual trilaminar membrane and a cell wall similar to that of gram-positive bacteria.

PCR has been used on fluids, such as cerebrospinal fluid and blood, and on biopsy specimens to detect the presence of Whipple's bacilli. However, *Tropheryma whipplei* DNA has reportedly been found by PCR in healthy individuals, so PCR should not be used in isolation to establish a diagnosis of Whipple disease. PCR is useful in confirming a diagnosis in patients with clinical and/or pathologic features suggestive of Whipple disease. PCR may also play a role in monitoring the efficacy of therapy.

Differential Diagnosis

Based on pathologic features, Whipple disease has a wide differential diagnosis. Clinical features are of great use in narrowing the differential. Lymph nodes in Whipple disease with a lipogranulomatous appearance can resemble lymph nodes in the portal circulation with lipogranulomas related to dietary lipid and/or bile metabolites. Correlation with anatomic site is helpful. Sarcoidosis, Crohn disease, and mycobacterial infections are included in the differential when non-necrotizing granulomas are found. Special stains for acid-fast bacilli and culture are useful in excluding a mycobacterial infection. Intestinal biopsies are helpful in establishing a diagnosis in cases of Crohn disease. PAS staining and electron microscopy may be helpful in differentiating between Whipple disease and sarcoidosis, but in some lymph node biopsy specimens in Whipple disease the findings are indistinguishable from those of sarcoidosis. *M. avium-intracellulare* infection and lepromatous leprosy can be associated with prominent infiltrates of histiocytes with finely granular or vacuolated cytoplasm and can enter the differential diagnosis of Whipple disease. Special stains for microorganisms establish a diagnosis.

If clinical features suggest the possibility of Whipple disease, obtaining additional tissue, especially from the small bowel, should be strongly considered in order to establish a diagnosis.

CHRONIC GRANULOMATOUS DISEASE

Chronic granulomatous disease is a genetically heterogeneous disorder characterized by impaired killing of phagocytosed microorganisms, leading to recurrent infections beginning in early childhood. Phagocytes are able to ingest microbes normally, but microbicidal activity is reduced because of abnormal NADPH (nicotinamide adenine dinucleotide phosphate) oxidase, an enzyme complex found in granulocytes, monocytes, and macrophages that normally generates the superoxide ions and hydrogen peroxide that are toxic to microbes. NADPH oxidase has membrane-bound components (gp91-phox, p22-phox, and rap1a, together comprising cytochrome b_{558}) and cytosolic components (p47-phox, p40-phox, p67-phox, Rac2). Defects in the gp91-phox of cytochrome b_{558} account for the majority of cases; these have

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X-linked inheritance. A defect with autosomal-recessive inheritance accounts for the remaining cases. Among autosomally recessively inherited defects, an abnormal p47-phox is most common; abnormal p67-phox and abnormal p22-phox each account for a small proportion of cases. The severity of the disease is dependent on the underlying genetic defect and on the presence or absence of any residual NADPH oxidase activity; X-linked inheritance with absence of cytochrome b_{558} expression is associated with more severe disease than other defects.

Clinical Features

Lymphadenopathy is very common in chronic granulomatous disease; lymphadenopathy is the most frequent presenting complaint and the most common manifestation of chronic granulomatous disease in some series. Prophylactic use of antibiotics can provide a significant decrease in number of bacterial infections; prophylaxis for fungal infections and interferon-γ are administered to some patients. Bone marrow transplantation and gene therapy have also been proposed as possible modes of therapy. The most common cause of death is infection by *Aspergillus*. Mortality is estimated to be 2% to 5% per year.

Differential Diagnosis

Based on morphology in an involved lymph node (Fig. 4.8), the differential diagnosis includes a variety of other types of necrotizing lymphadenitides, including cat-scratch disease, atypical mycobacteria, brucellosis, and fungal infection. The lack of specificity of the pathologic findings and the rarity of the disease often result in delay, sometimes for years, in establishing a diagnosis. Chronic granulomatous disease is distinctive in that there are recurrent, often severe, pyogenic infections usually beginning in the first year of life and almost always present by the age of 2 years.

Diagnosis can be established using the traditional nitroblue tetrazolium reduction test, with chemiluminescent or fluorescent assays of oxidase activity, or with assays that detect generation of peroxide. When the specific genetic defect is known, a prenatal diagnosis can be made.

CHRONIC GRANULOMATOUS DISEASE—FACT SHEET

Definition
- A disorder characterized by leukocytes with impaired intracellular killing of microorganisms after phagocytosis, leading to recurrent, severe infections by bacteria and less often by fungi

Frequency
- Prevalence: approximately one in 1.2 million individuals
- Incidence: approximately one in 250,000 live births
- Chronic granulomatous disease accounts for <10% of primary immunodeficiencies but is the most common inherited disorder causing phagocytic dysfunction.

Gender, Race, and Age Distribution
- Age at onset is usually <1 year.
- Boys more often affected than girls (male-to-female ratio: 2:1 to 4:1)
- No known racial predisposition

Risk Factors
- Consanguinity, for the autosomal-recessive form

Clinical Features
- Recurrent suppurative infections, with lymphadenitis, pulmonary infection, upper respiratory infection, cutaneous abscesses, gastrointestinal infections, hepatic abscesses, sepsis, and/or osteomyelitis due to bacteria, most often *Staphylococcus aureus*, *Klebsiella*, *Salmonella*, *Burkholderia*, or *Mycobacterium tuberculosis*, or to fungi such as *Aspergillus* or *Candida*
- Noninfectious manifestations: poor wound healing, overly exuberant inflammatory reactions, autoimmune-type phenomena

Morphology
- Microscopic examination reveals necrotizing granulomas with suppurative necrosis and abscesses (see Fig. 4.8).

Diagnosis
- Nitroblue tetrazolium reduction test and others

Prognosis and Therapy
- Treatment with antibiotics for established infections
- Antibiotics often also given prophylactically to prevent infection.
- Prognosis has improved over time.
- Median survival varies in different series, but many patients reach young adulthood or middle age.
- Prognosis is worse with complete absence of cytochrome b_{558}.

FUNGAL LYMPHADENITIS

Fungal lymphadenitis is uncommon. Patients with fungal lymphadenitis are often immunosuppressed because of HIV infection, malignancy (most often, hematolymphoid neoplasia), or iatrogenic immunosuppression; occasionally patients with normal immunologic function are affected. Lymphadenitis may be caused by a variety of fungi, but *Histoplasma capsulatum* and *Cryptococcus neoformans* are among the most common. *Penicillium marneffei* is an uncommon cause of opportunistic infection, but when it does occur, it is often associated with lymphadenopathy. Symptomatic fungal lymphadenitis is uncommonly an isolated finding; patients often have involvement of the lungs, liver, spleen, bone marrow, and/or other sites. Patients with normal immunologic function who develop fungal pulmonary infection may also have hilar lymph node involvement. Lymphadenitis in this setting may be asymptomatic; however, histoplasmosis

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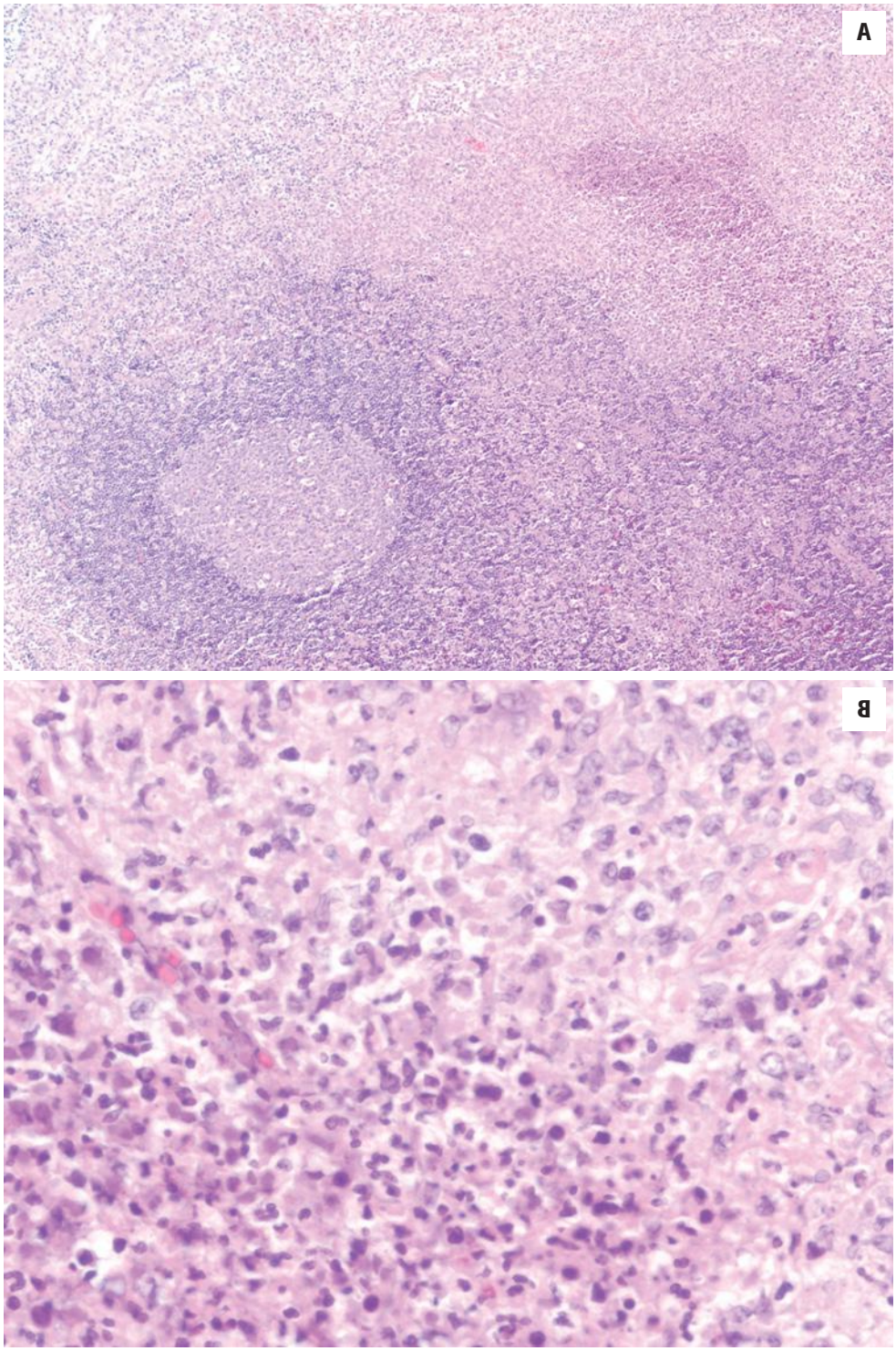


FIG. 4.8

Chronic granulomatous disease. A, This lymph node shows necrotizing granulomatous inflammation adjacent to a reactive follicle. B, Higher power shows palisading histiocytes surrounding suppurative necrosis.

involving mediastinal nodes can be associated with inflammation and severe scarring of the surrounding tissue, producing sclerosing mediastinitis.

Infection is typically associated with granulomatous inflammation, which may be necrotizing. In immunodeficient individuals, granulomas may be poorly formed or absent. Fibrosis and even calcification may develop in old lesions, especially in cases of histoplasmosis. The differential diagnosis is similar to that of *M. tuberculosis* and nontuberculous mycobacteria. During institution of highly active antiretroviral therapy in HIV+ patients harboring fungi, symptomatic fungal lymphadenitis may emerge as an immune reaction to the fungi is mounted (immune reconstitution inflammatory syndrome [IRIS]).

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PROTOZOAL LYMPHADENITIS

TOXOPLASMOSIS

Clinical Features

Although lymphadenitis due to other types of protozoal infections has been reported, infection by *Toxoplasma gondii* is the only one encountered with any frequency in the United States. Toxoplasmosis is a common cause of lymphadenopathy. This type of lymphadenopathy was first described by Piringer-Kuchinka in 1952 and is thus sometimes called Piringer-Kuchinka lymphadenitis. The five modes of presentation of toxoplasmosis are lymphadenopathy, encephalitis, chorioretinitis, disseminated infection, and congenital infection. The most frequent clinical manifestation of *T. gondii* infection is cervical lymphadenopathy. Transmission to humans is thought to be through exposure to cat feces containing oocysts or exposure to uncooked meat. Individuals with toxoplasma lymphadenitis are usually children or young adults with unilateral or bilateral cervical lymphadenopathy. They may have malaise or they may be asymptomatic. An atypical lymphocytosis may be seen. The disease is typically self-limited. Rarely, immunocompetent patients develop serious complications, such as myocarditis or encephalitis, which may be fatal. Immunodeficient patients may develop lymphadenopathy, but they are at high risk of severe manifestations, especially encephalitis. Infection during pregnancy may be associated with fetal morbidity or mortality.

Pathologic Features

Lymph nodes show (1) florid follicular hyperplasia, (2) prominent bands of parasinusoidal and parafollicular monocytoid B cells, and (3) many small, irregular parafollicular clusters of histiocytes, some of which encroach on germinal centers (Fig. 4.9). The microorganisms are identified in lymph nodes only rarely as parasitic cysts, although finding *Toxoplasma* DNA using the PCR reaction may be helpful in establishing a diagnosis. The diagnosis can be confirmed using serologic studies; in 95% of cases with the triad of histologic findings described, results of serology document infection by *T. gondii*.

Differential Diagnosis

The differential diagnosis of toxoplasmic lymphadenitis includes other types of lymphadenitides associated with follicular hyperplasia and/or clusters of histiocytes. Early or partial nodal involvement by cat-scratch disease, sarcoidosis, mycobacteria, or primary or secondary syphilis can result in an appearance that resembles toxoplasmic lymphadenitis, except that in none of these disorders is there a tendency for epithelioid histiocytes to encroach on lymphoid follicles. Significant areas of necrosis and true granuloma formation are unusual in toxoplasmosis and should lead to consideration of other infections. *Leishmania* can produce a lymphadenitis closely resembling that of *Toxoplasma*; it can also be associated with a necrotizing or non-necrotizing granulomatous lymphadenitis. With *Leishmania* infection, however, microorganisms are usually identifiable in epithelioid histiocytes (see the following section).

TOXOPLASMOSIS—FACT SHEET

Definition
- Infectious disease due to *T. gondii*

Incidence and Anatomic Location
- Relatively common
- Toxoplasma lymphadenitis is the most common manifestation of toxoplasmosis.

Gender, Race, or Age Distribution
- Most patients are children and young adults; toxoplasma lymphadenitis is more common among females than males.

Risk Factors
- Immunodeficient patients are at risk of more severe manifestations of toxoplasmosis.

Clinical Findings
- Patients with toxoplasma lymphadenitis typically have unilateral or bilateral cervical lymphadenopathy, especially in the posterior cervical area; they may have malaise or be asymptomatic.
- Other forms of toxoplasmosis include encephalitis, chorioretinitis, disseminated disease, and congenital infection.

Morphology
- Lymph nodes are slightly to moderately enlarged and show florid follicular hyperplasia, monocytoid B-cell hyperplasia, and clusters of epithelioid histiocytes surrounding and encroaching on follicle centers.
- The organisms are not usually identifiable in lymph nodes.

Diagnosis
- Lymph node biopsy specimen shows characteristic changes, and/or serologic studies confirm the diagnosis.

Prognosis and Therapy
- Patients with toxoplasma lymphadenitis typically have a self-limited illness and need no specific therapy.
- Adults with more severe manifestations may respond to drug therapy; congenital infection may cause irreversible damage.

LEISHMANIAL LYMPHADENITIS

Leishmaniasis is a protozoal infection that is transmitted by sandflies (*Phlebotomus* species). *Leishmania* species can infect

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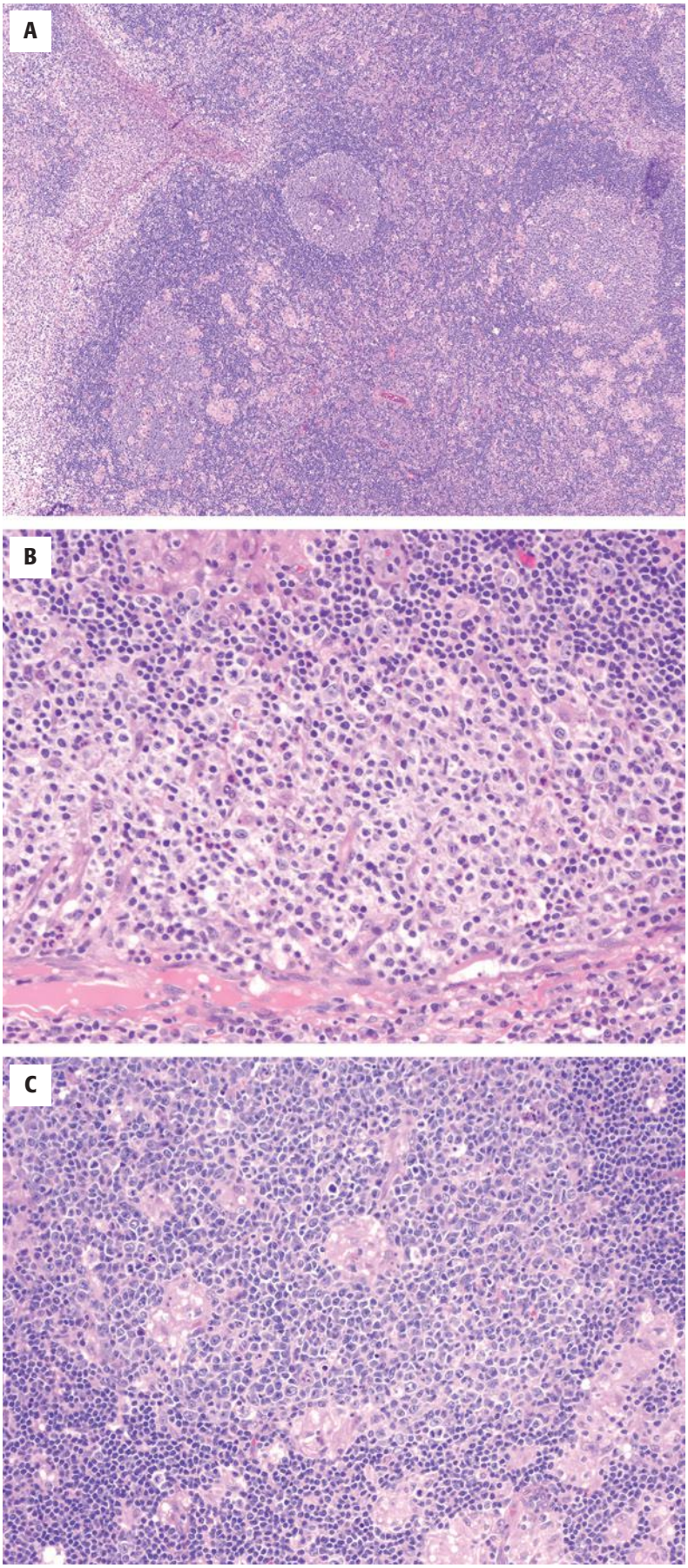


FIG. 4.9

Toxoplasma lymphadenitis. A, Low power shows a lymph node with follicular and monocytoid B-cell hyperplasia. B, A prominent band of monocytoid B cells with abundant clear cytoplasm. C, Small clusters of epithelioid histiocytes surround and invade a reactive follicle.

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lymph nodes in two settings: (1) localized lymphadenopathy, often draining a focus of cutaneous infection, and often due to *L. tropica*, and (2) widespread lymphadenopathy in the setting of visceral leishmaniasis (kala-azar), typically due to *L. donovani*.

The localized lymphadenitis associated with cutaneous leishmaniasis can show follicular and paracortical hyperplasia with necrotizing or non-necrotizing granulomatous inflammation, sometimes with numerous plasma cells. The necrosis may be suppurative or caseating. The granulomas may resemble those seen in tuberculosis or can mimic the granulomas of cat-scratch disease. In visceral leishmaniasis, there is a marked increase in histiocytes throughout the reticuloendothelial system. Organisms reside in histiocytes, but follicular hyperplasia and paracortical hyperplasia may not be prominent, and granulomas are typically absent.

Leishmaniasis may be seen in the Middle East, in parts of Africa, South America, and Asia, and in Central America; information about the patient's origin or travel history gives a clue to the diagnosis. The organisms can be seen on routinely stained sections, on Giemsa stain, and on reticulum stains, although in some cases, they are present in only small numbers. The microorganisms are found mainly within histiocytes, but they are sometimes found in the extracellular space, and their presence in neutrophils has been reported. Individual organisms are 1.5 to 3 μm in diameter, round to oval in shape with a delicate cellular membrane, a nucleus, and a rod-shaped kinetoplast. In some instances the diagnosis can be established or suspected based on a fine needle aspiration biopsy, so that some patients may not require a more invasive procedure to establish the diagnosis.

PNEUMOCYSTIS CARINII LYMPHADENITIS

See Fact Sheet.

PNEUMOCYSTIS CARINII **LYMPHADENITIS—FACT SHEET**

Incidence and Anatomic Location
- Rare
- Less than 1% of all cases of pneumocystosis affect extrapulmonary sites.
- Among extrapulmonary sites, lymph nodes are the site most often affected.

Gender, Race, and Age Distribution
- Patients include HIV+ individuals with male preponderance, children with congenital immunodeficiency, organ transplant recipients, and patients with malignancy.
- No known racial predisposition

Risk Factors
- Treatment with aerosolized pentamidine, which prevents *P. carinii* pneumonia but not extrapulmonary infections

Clinical Features
- *P. carinii* lymphadenitis usually occurs in association with *P. carinii* pneumonia or in the setting of widespread extrapulmonary infection.

Morphology
- Tissue shows frothy material with scattered helmet-shaped organisms that can be detected with a Gomori methenamine silver stain.

Diagnosis
- Can be established with biopsy of affected tissue, although in many cases, the diagnosis is made only at autopsy

Prognosis and Therapy
- Treatment is with appropriate antibiotics.
- Without therapy, mortality is high.

FILARIAL LYMPHADENITIS

See Fact Sheet.

LYMPHOID HYPERPLASIA AND LYMPHADENITIS IN IMMUNOLOGIC DISORDERS

RHEUMATOID ARTHRITIS

Clinical Features

Rheumatoid arthritis is a chronic systemic illness characterized by a destructive arthritis and a variety of extra-articular manifestations, including hematologic, cardiovascular, neurologic and pulmonary abnormalities, as well as laboratory abnormalities that include anemia, polyclonal hypergammaglobulinemia, elevated C-reactive protein, and elevated IL-6. As many as 75% of patients develop lymphadenopathy due to reactive hyperplasia during periods of active disease.

Pathologic Features

Reactive hyperplasia in rheumatoid arthritis typically shows prominent follicular hyperplasia. The follicles are present in the cortex and also frequently extend into the medulla. Infiltration of the capsule and perinodal fat by lymphocytes is common. Plasmacytosis of the interfollicular region and medulla is often conspicuous; Russell bodies may be found. The interfollicular area may also show admixed small lymphocytes, immunoblasts, and neutrophils. Sinuses, in areas, may be compressed by the follicular and interfollicular expansion, but patent and even dilated sinuses can be found. Sinuses show sinus histiocytosis, sometimes including erythrophagocytic histiocytes, neutrophils, and stainable iron

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FILARIAL LYMPHADENITIS—FACT SHEET

Definition
- Lymphadenitis due to infection by a filarial worm, usually *Wuchereria bancrofti* or *Brugia malayi*.

Incidence
- Overall common, but with a restricted geographic distribution; 120 million individuals are affected worldwide.
- *Wuchereria bancrofti* affects patients in equatorial Africa, India, the Mediterranean coast, the Caribbean, coastal areas of South America, and parts of Central America; the infection is referred to as Bancroftian filariasis.
- *Brugia malayi* affects patients in coastal areas of Southeast Asia and India; the infection is referred to as Malayan filariasis.
- Rare in the United States, although zoonotic *Brugia* species may rarely cause human infection outside endemic areas.

Age, Gender, Racial Distribution
- None known

Risk Factors
- Living in or traveling to endemic areas; the vector is the mosquito.

Clinical Features
- Severity of manifestations varies greatly from asymptomatic or mildly symptomatic infections to severe infections with lymphedema, lymphadenopathy, and elephantiasis of extremities due to lymphatic obstruction.

Morphology
- Adult worms are found in lymphatics and in lymph node sinuses. They have a thin, finely striated cuticle and a layer of somatic muscle. Cross section of the midbody in females reveals an intestine and two uteri. *Brugia* species have smaller maximum midbody diameters (females, ≈160 μm; males, ≈90 μm) than *Wuchereria* species (females, 250 μm; males, 150 μm). Dead worms may undergo dystrophic calcification.
- Lymph nodes show changes such as prominent follicular hyperplasia, monocytoid B-cell hyperplasia, and a mixed infiltrate of lymphocytes, plasma cells, eosinophils, and neutrophils.
- Dead worms may elicit a striking infiltrate of eosinophils, sometimes with eosinophilic microabscesses and granuloma formation. Capsular and intranodal fibrosis may be prominent.

Treatment and Prognosis
- Infections by *Wuchereria bancrofti* and *Brugia malayi* require anthelmintic therapy. Prognosis is good with prompt diagnosis and treatment. Zoonotic infections may be cured by excision of the infected lymph node, as infection often consists of the presence of a single adult worm.

RHEUMATOID ARTHRITIS—FACT SHEET

Definition
- Rheumatoid arthritis is a chronic, systemic autoimmune disease causing a destructive arthritis as well as a variety of extra-articular manifestations, including lymphadenopathy.

Incidence
- Common

Gender, Race, or Age Distribution
- Rheumatoid arthritis affects women three times as often as men.
- Nearly all cases appear in adults, from the third to the seventh decades.

Risk Factors
- Genetic predisposition is probable.

Clinical Features
- Lymphadenopathy occurs in approximately 75% of patients and is especially common during periods of active disease.
- Lymphadenopathy may be localized or generalized. When localized, it is most frequent in the axillary area, as these nodes receive lymphatic drainage from inflamed joints of the hand.

Morphology
- Lymph nodes show florid follicular hyperplasia, sinus histiocytosis, and plasmacytosis and may show areas of hyalinization, sinus neutrophils, and deposition of iron.
- Patients with joint replacement may have lymphadenopathy showing a reaction to foreign material if the prosthesis deteriorates.
- Patients receiving gold therapy may develop lymphadenopathy showing a foreign body reaction to gold.

Diagnosis
- Diagnosis is usually established based on clinical and laboratory abnormalities.

Prognosis and Therapy
- Lymphadenopathy itself does not require therapy.
- The goal of therapy is to decrease inflammation and minimize joint damage.
- Rheumatoid arthritis is, however, a chronic, slowly progressive disease that may eventually result in significant morbidity.

(Fig. 4.10). Lymph nodes may have areas of hyaline material, possibly due to long-standing nonspecific inflammation. Amyloid rarely is deposited in lymph nodes. Lymphadenopathy associated with gold therapy has been described; in such cases, gold is present in the form of crystalline material and is associated with a multinucleated giant cell reaction. Rarely, lymph nodal infarction has been attributed to gold therapy. Patients with Silastic joint prostheses may develop silicone lymphadenopathy. Patients with lymphadenopathy associated with juvenile rheumatoid arthritis may have reactive hyperplasia similar to that seen with rheumatoid arthritis.

Differential Diagnosis

The differential diagnosis of rheumatoid arthritis includes follicular lymphoma and other reactive conditions. The extensive follicular proliferation characteristic of rheumatoid arthritis lymph nodes may raise the question of follicular lymphoma. Criteria conventionally used to distinguish

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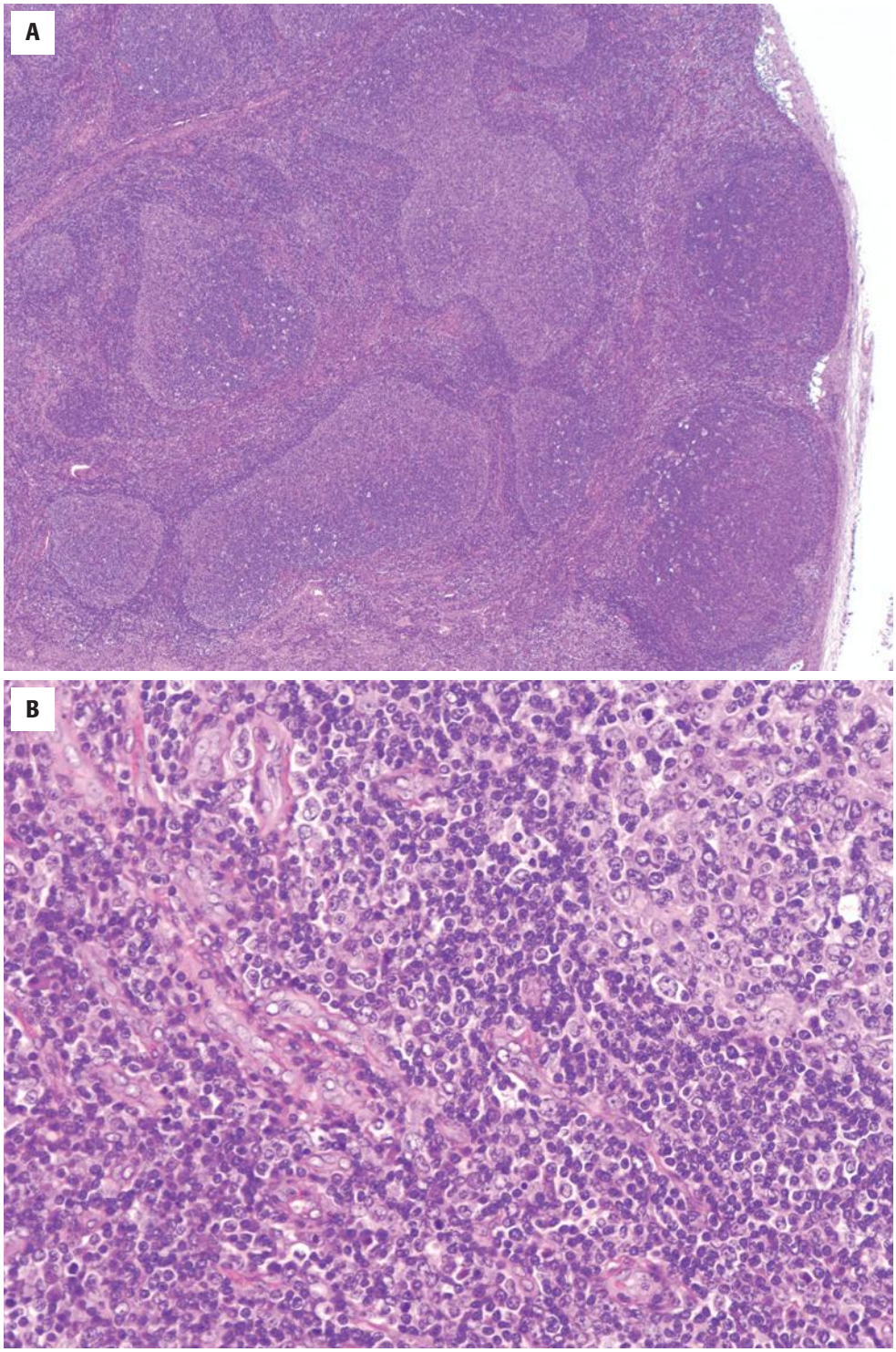


FIG. 4.10

Rheumatoid arthritis–associated lymphadenopathy. A, Low power shows florid follicular hyperplasia, with large, sometimes irregularly shaped follicles. B, The interfollicular area shows numerous plasma cells.

follicular lymphoma and follicular hyperplasia can be used in this setting. In the differential with other causes of reactive hyperplasia, clinical correlation is required to confirm a diagnosis of lymphadenopathy due to rheumatoid arthritis. Other causes of lymphadenopathy that may mimic changes seen in rheumatoid arthritis include syphilis, HIV infection, plasma cell Castleman disease, and nonspecific follicular hyperplasia.

SYSTEMIC LUPUS ERYTHEMATOSUS

Clinical Features

Systemic lupus erythematosus is an autoimmune disorder that predominantly affects adolescents and young adults, with a female preponderance. However, it may occur at

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any age, and males are occasionally affected. It is characterized by a broad spectrum of clinical manifestations, including arthritis or arthralgias, fever, rash, renal disease, anorexia, nausea and vomiting, serositis, and neurologic manifestations. As many as two thirds of patients with active disease develop lymphadenopathy (lupus lymphadenitis), the most common site of which is the cervical area (43% of cases), although generalized lymphadenopathy is also relatively common. Patients with lymphadenopathy tend to be slightly younger (mean, 36 years with adenopathy versus 45 years without adenopathy) and to have more complaints of fatigue, fever, and weight loss; more cutaneous abnormalities; more frequent hepatomegaly and splenomegaly; and higher titers of anti-dsDNA antibodies than those without lymphadenopathy.

Pathologic Features

Lymph nodes in lupus lymphadenitis may be edematous, with areas of hemorrhage. The lymph node architecture may be distorted, but it is not obliterated. The paracortex often contains foci of necrosis, with a central zone of amorphous pink material. In early lesions, there may be little or no cellular reaction around the necrotic areas. In older lesions, large numbers of histiocytes, with admixed small and large lymphoid cells, surround the necrosis. Immunoblasts may form a prominent component of the infiltrate. Granulocytes are sparse or absent. Plasma cells are usually infrequent but are found in large numbers in occasional cases. Hematoxylin bodies, ill-defined violet structures thought to represent degenerated nuclei that have reacted with antinuclear antibodies, are sometimes found in areas of necrosis; they are virtually pathognomonic of lupus (Fig. 4.11). Blood vessels in the necrotic foci may show the Azzopardi phenomenon, in which dark blue nuclear material is deposited on the basement membrane of blood vessels. Periarterial and periarteriolar fibrosis may also be seen. In some cases, lymph nodes lack necrosis and instead show mainly follicular hyperplasia, sometimes with unusual features. Rare patients with systemic lupus erythematosus have lymph nodes with features reminiscent of Castleman disease.

Differential Diagnosis

Because in some cases of lupus lymphadenitis there are numerous large lymphoid cells, a diagnosis of non-Hodgkin lymphoma may be entertained. In favor of lupus lymphadenitis are preservation of nodal architecture, characteristic necrotic areas, admixed histiocytes, and hematoxylin bodies. In difficult cases, immunophenotyping may be helpful in excluding lymphoma. Because of the extensive necrosis often found in lupus lymphadenitis, an infectious process may enter the differential diagnosis. However, neutrophils are sparse to absent in lupus and abundant in many different types of infectious lymphadenitides. The necrotic material in lupus lymphadenitis may resemble caseous necrosis, raising the possibility of tuberculous lymphadenitis, but epithelioid histiocytes, Langhans-type giant cells, and granulomas are not features of lupus lymphadenitis.

SYSTEMIC LUPUS ERYTHEMATOSUS—FACT SHEET

Definition
- A systemic autoimmune disease characterized by a wide variety of clinical findings and laboratory abnormalities, such as autoantibodies, especially antinuclear antibodies

Incidence
- Relatively common

Gender, Race, and Age Distribution
- This disease mainly affects females (male-to-female ratio: ≈1:10) and usually presents during adolescence or young adulthood.
- More frequent among blacks and Asians than among whites

Risk Factors
- Genetic susceptibility is well described.
- Hormonal changes may play a role, and a role for certain environmental factors has been suggested in the development of lupus.

Clinical Features
- Patients have manifestations that vary from case to case.
- Abnormalities include fever, rash, anorexia, renal disease, serositis, neurologic findings, arthralgias, and positive antinuclear antibodies.
- Most patients have lymphadenopathy at some time during the course of their disease.
- Lymphadenopathy is most often cervical, but it can affect any site and can be generalized.

Morphology
- Lymph nodes may show edema, hemorrhage, and paracortical areas of necrosis with amorphous pink debris surrounded by histiocytes and immunoblasts but without granulomas.
- Occasional cases show hematoxylin bodies or the Azzopardi phenomenon, features that are nearly pathognomonic of lupus.
- Some cases show follicular hyperplasia with unusual features that could potentially mimic follicular lymphoma.

Diagnosis
- Although the pathologic findings are distinctive, diagnosis is usually established mainly based on clinical findings and laboratory abnormalities.

Prognosis and Therapy
- As for rheumatoid arthritis, treatment focuses on controlling inflammation and minimizing tissue damage.
- The course is chronic, often remitting and relapsing, and the disease may result in shortening of the normal life span.

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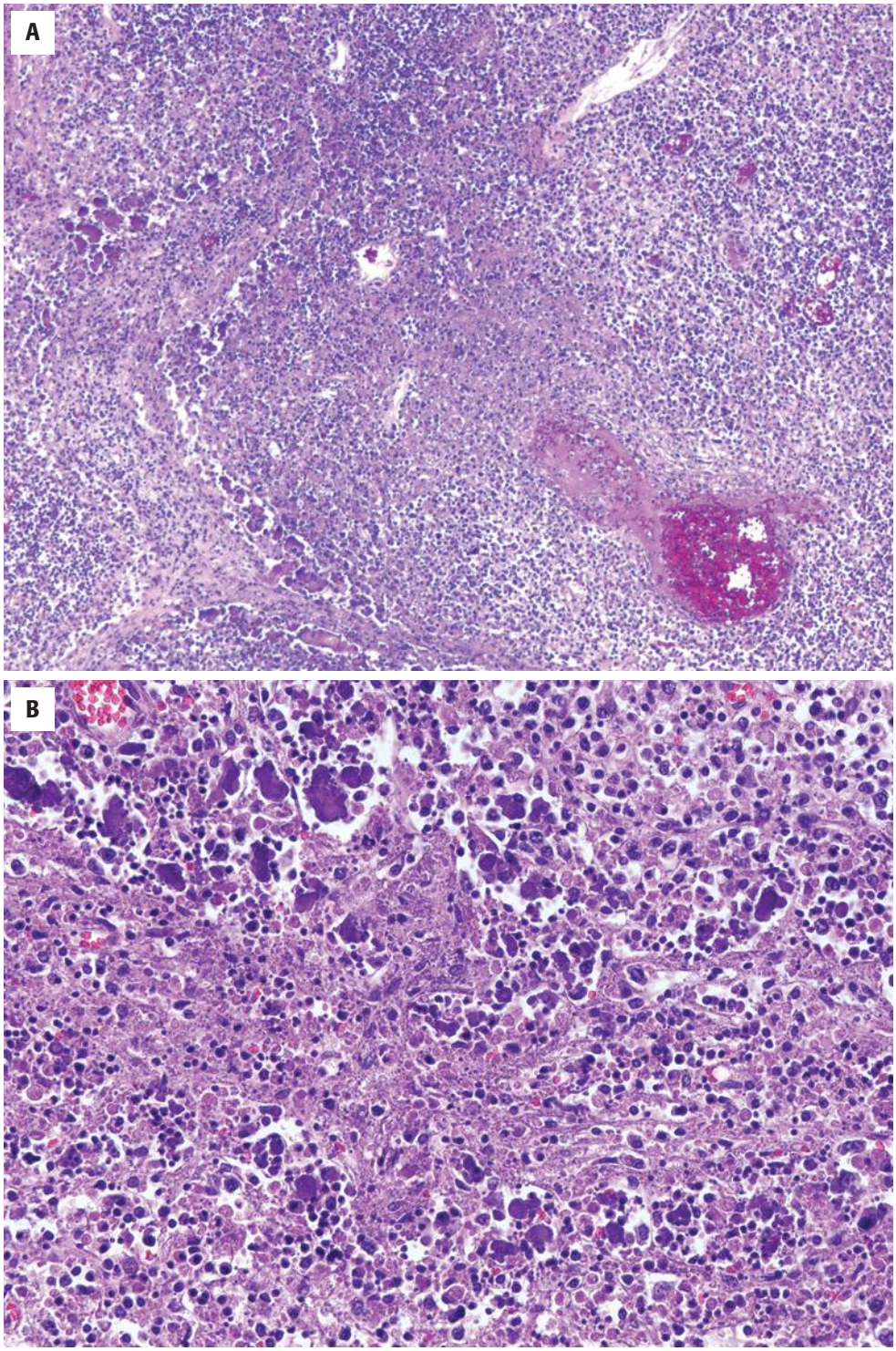


FIG. 4.11
Systemic lupus erythematosus–associated lymphadenitis. The patient died of systemic lupus erythematosus, and the lymph node illustrated was obtained at autopsy. A, Low power shows extensive necrosis as well as vascular thrombosis. B, Higher power shows numerous violet-colored hematoxylin bodies.

KIKUCHI DISEASE

Kikuchi disease is of uncertain cause, but, in many cases, it may represent a reaction to an infectious process and thus could be included in other sections of this chapter. It is included in the section on immunologic disorders because it is an important entity in the differential diagnosis of lupus lymphadenitis.

Clinical Features

Kikuchi disease, also known as histiocytic necrotizing lymphadenitis, Kikuchi lymphadenitis, Kikuchi-Fujimoto disease, and subacute necrotizing lymphadenitis, is of unknown cause. Kikuchi disease seems to be more prevalent among Asians than in Western populations. Most patients are young

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adults (mean age in the third decade), with a female preponderance in nearly all series. The typical presentation is with unilateral, frequently painful, cervical lymphadenopathy. Other lymph nodes are affected much less often. Infrequently, there is generalized lymphadenopathy or involvement of extranodal sites, the most common of which is the skin. Approximately one half of patients have fever at presentation, and some have flulike symptoms. Common peripheral blood abnormalities include anemia and neutropenia; an atypical lymphocytosis is found in 25% of cases. Less than 5% of patients have a leukocytosis. Occasionally, patients have a rash. The disease is self-limited, and most patients recover without therapy. Less than 5% develop recurrent lymphadenopathy. In a few cases, usually in iatrogenically immunosuppressed organ transplant recipients or very young children, Kikuchi disease has resulted in widespread disease with multiorgan involvement and may be fatal.

A variety of viral and other types of infectious causes have been suggested to cause Kikuchi disease. The similarity of some of the clinical and pathologic features of Kikuchi disease and systemic lupus erythematosus has raised the question of Kikuchi disease being a self-limited autoimmune disease. Some patients have developed Kikuchi disease in association with foreign material or other types of noninfectious conditions. It is possible that the histologic and immunohistologic features of Kikuchi disease can be produced by a variety of stimuli in susceptible individuals.

Pathologic Features

Lymph nodes show prominent paracortical hyperplasia with one or more round or irregular, discrete, or confluent eosinophilic areas in the cortex or paracortex containing histiocytes, lymphocytes, immunoblasts, plasmacytoid dendritic cells, and karyorrhectic and eosinophilic granular debris. The necrotic debris is most abundant in the centers of these areas, whereas immunoblasts are most numerous at the periphery. The histiocytes are of a variety of types, including phagocytic, nonphagocytic, and foamy histiocytes. Nonphagocytic histiocytes with eccentric sickle-shaped nuclei have been called *crescentic histiocytes*. Plasmacytoid dendritic cells are medium-sized cells with round nuclei, dispersed chromatin, small nucleoli, and a moderate amount of faintly amphophilic cytoplasm. In Kikuchi lymphadenitis, aggregates of plasmacytoid dendritic cells are frequently seen in non-necrotizing areas; early foci of necrosis appear to begin within clusters of plasmacytoid dendritic cells. The necrosis is apoptotic rather than suppurative. Thrombosed vessels may be seen around necrotic foci. Epithelioid histiocytes, plasma cells, eosinophils, and neutrophils are virtually absent. The characteristic foci may be relatively small and confined to the paracortex or may occupy the majority of the lymph node. The infiltrate may extend beyond the capsule into perinodal soft tissue. Follicular hyperplasia may be seen, but it is not a constant or prominent feature.

KIKUCHI DISEASE—FACT SHEET

Definition
- An illness of uncertain cause characterized by lymphadenopathy with distinctive histologic features

Incidence
- Uncommon

Gender, Race, and Age Distribution
- Most patients are young women, with a higher incidence among Asians.

Risk Factors
- Rarely, severe cases of Kikuchi disease occur in immunocompromised patients.

Clinical Features
- Patients usually present with unilateral tender cervical lymphadenopathy.
- Other common findings include fever, rash, anemia, and neutropenia.
- Some patients have an atypical lymphocytosis.

Morphology
- Lymph nodes show small or large foci of necrosis containing amorphous eosinophilic and apoptotic debris.
- Necrotic areas are surrounded by numerous histiocytes and by variable numbers of immunoblasts.
- Granulocytes, plasma cells, and granulomas are absent.

Immunophenotype
- Lymphoid cells are T cells, often with numerous cytotoxic (CD8+) cells.
- Histiocytes may express myeloperoxidase.
- B cells are rare to absent in and around the necrotic foci.

Diagnosis
- Lymph node biopsy

Prognosis and Therapy
- Nearly all patients have a self-limited illness requiring no specific therapy.
- A few patients develop recurrent lymphadenopathy.
- Rare patients, usually with an underlying immunodeficiency, have a severe illness with a poor outcome.
- A few patients thought to have Kikuchi disease later develop systemic lupus erythematosus; this may be coincidental, or the patient may have had lupus mimicking Kikuchi disease from the outset.

Kikuchi disease can been subclassified into three histologic subtypes; these may represent different stages in the evolution of the disease. In the proliferative type, microscopic examination reveals the mixture of cells described, with apoptosis but without coagulative necrosis (Fig. 4.12). In the necrotizing type, lymph nodes show large areas of necrosis in addition to the changes characteristic of the proliferative type. In the xanthomatous type, there is a predominance of foamy histiocytes, and necrosis can be present or absent.

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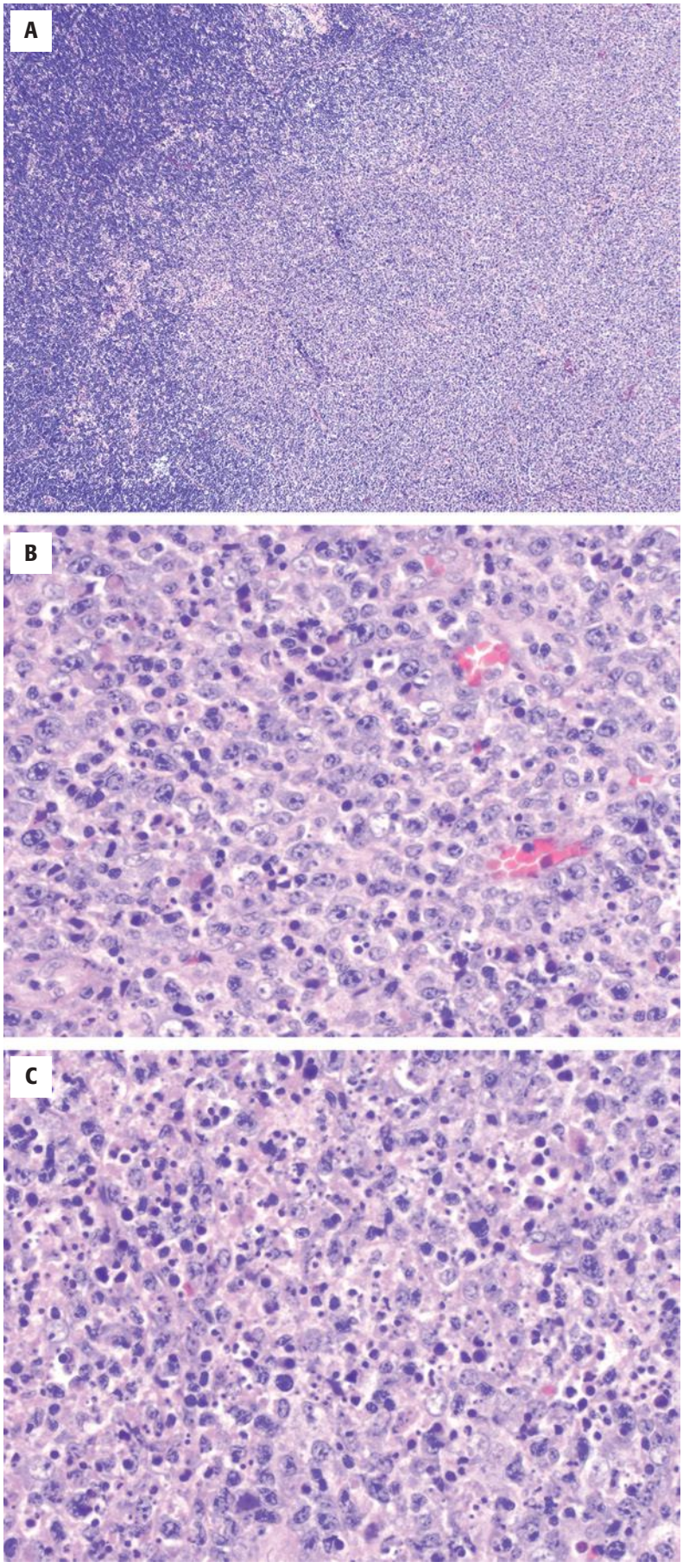


FIG. 4.12

Kikuchi disease. A, Low power shows a rounded, slightly pale area within a hyperplastic paracortex. B, High power in areas shows numerous immunoblasts, admixed with variable numbers of histiocytes and some apoptotic debris. C, In other areas, immunoblasts are less numerous, and apoptotic and eosinophilic cellular debris and histiocytes predominate.

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Immunohistochemical analysis confirms the presence of a mixture of lymphoid cells, histiocytes, and plasmacytoid dendritic cells. The lymphoid cells, including the immunoblasts, express pan–T-cell antigens. There is a varying admixture of CD4+ and CD8+ T cells, but CD8+ T cells are often abundant. CD8+ T cells coexpress cytotoxic granule proteins, such as TIA-1 and perforin. CD56+ natural killer cells are infrequent. The histiocytes are lysozyme+, CD68+, and, surprisingly, they are reported to coexpress myeloperoxidase, an unusual feature also reported in lupus lymphadenitis. The plasmacytoid dendritic cells express CD2, CD4, CD43, CD68, and CD123 but not CD3. Although B cells are found if reactive follicles are present, B cells are scarce in the areas of the node with histiocytes and cellular debris.

Differential Diagnosis

The most important entity in the differential diagnosis is non-Hodgkin lymphoma. The distinction may be difficult when immunoblasts are abundant. Familiarity with the spectrum of changes found in Kikuchi disease, and identification of areas with the characteristic polymorphous infiltrate, are essential to rendering the correct diagnosis. In addition, Kikuchi disease more often shows partial nodal involvement than lymphoma. The vast majority of lymphomas in Western countries are B-cell lymphomas. Only a minority of lymphomas in the United States are T-cell lymphomas, but most of those arising in lymph nodes are CD4+. The predominance of CD8+ T cells in Kikuchi disease is an important clue in avoiding a misdiagnosis of lymphoma.

Because necrosis is such a prominent component of Kikuchi disease, infectious lymphadenitis is often a consideration. However, neutrophils, suppurative necrosis, granulomas, and viral inclusions, features characteristic of various types of infectious lymphadenitides, are not found in Kikuchi disease. When crescentic histiocytes are prominent, metastatic carcinoma of the signet ring cell type can be considered in the differential diagnosis. However, histiocytes in Kikuchi disease lack nuclear atypicality, may contain cellular debris, but do not contain mucin and express histiocyte-related antigens rather than keratins.

Lymphadenitis in patients with systemic lupus erythematosus can closely resemble, or even be indistinguishable from, the lymphadenitis of Kikuchi disease. Finding hematoxylin bodies, plasma cells, or deposition of nuclear material on blood vessels (the Azzopardi phenomenon) supports a diagnosis of lupus lymphadenitis over Kikuchi disease, but these features may not be found in every case of lupus lymphadenitis. Clinical features are sometimes helpful in making a definitive distinction. Because of the difficulty in definitively excluding lupus on microscopic examination and because a minority of patients diagnosed with Kikuchi disease have developed lupus later, clinicians may consider performing a workup for autoimmune disease in cases showing histologic features of Kikuchi disease.

KIMURA DISEASE

Clinical Features

Kimura disease is a chronic inflammatory disorder that mainly affects the area of the head and neck; it is associated with one or more relatively large, painless, slowly enlarging lesions (typically between 2 and 5 cm, but with a range of 1 to 20 cm and an average of approximately 3 cm) in subcutaneous tissue and deep soft tissue, and lymphadenopathy. Lymphadenopathy usually affects the cervical area; it may be bilateral and is usually painless. In 40% of cases, there is involvement of major salivary glands. Kimura disease affecting the lacrimal gland has been described. Rarely, there are soft-tissue lesions and lymphadenopathy away from the head and neck, in sites that include the groin, axilla, forearm, and popliteal area. Kimura disease affects patients from childhood to middle age; most patients are young adults, with a significant male preponderance (6:1 in a recent series). The disease appears to be more common among Asians but may also be seen in whites and blacks. Patients nearly always have peripheral eosinophilia and elevated serum IgE levels. Some patients also have asthma, proteinuria, or nephrotic syndrome. Lesions may persist or recur over a period of months or years. Findings on physical examination often suggest a diagnosis of a salivary gland tumor or lymphoma, so that a neoplastic process is often in the clinical differential diagnosis. Kimura disease is of uncertain etiology but may represent an aberrant immune reaction to an unknown stimulus.

Pathologic Features

Lesions in salivary glands and subcutaneous tissue are poorly circumscribed and consist of a dense infiltrate of lymphocytes, eosinophils, mast cells, and plasma cells with many small blood vessels. Numerous reactive follicles are present; their germinal centers may be pierced by blood vessels or may be invaded and disrupted by eosinophils. In about one half of cases, there is deposition of homogeneous eosinophilic material in the interstitium of the germinal centers. Polykaryocytes may be present, most often in the follicles. Fibrosis is very common, but its extent is variable. Collagen deposition may be perivascular or, in salivary glands, periductal, but over time, it may progress to diffuse, extensive hyalinization of the involved tissues with only scattered lymphocytes, plasma cells, eosinophils, and mast cells. Involved lymph nodes show follicular hyperplasia with increased numbers of eosinophils in the paracortex, sinuses, and perinodal soft tissue and within follicles, sometimes with formation of eosinophilic microabscesses. Paracortical postcapillary venules are increased (Fig. 4.13). Fibrosis tends to be less severe in lymph nodes than in subcutis or salivary glands.

Kimura disease has the distinctive finding of IgE-positive dendritic networks in germinal centers. The prominence

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KIMURA DISEASE—FACT SHEET

Definition
- A chronic inflammatory disorder with distinctive histologic features mainly affecting the head and neck that appears to represent an aberrant immunologic reaction

Incidence
- Uncommon

Gender, Race, and Age Distribution
- Most patients are young adult males; Asians are more commonly affected than whites or blacks.

Clinical Features
- Patients typically have cervical lymphadenopathy and/or tumor-like lesions of salivary glands or soft tissue.
- Patients also have peripheral blood eosinophilia, elevated serum IgE, and occasionally asthma, suggesting an allergic process.

Morphology
- Lymph nodes and other involved sites show an infiltrate of lymphocytes, eosinophils, plasma cells, mast cells, reactive follicles, and fibrosis.
- There may be eosinophilic abscesses and polykaryocytes, especially in follicles.

Immunophenotype
- IgE-positive follicular dendritic networks in follicles

Diagnosis
- Biopsy of involved tissue

Prognosis and Therapy
- The lymphadenopathy and other mass lesions do not require specific therapy, although they can be removed if they are large and persistent.

of eosinophils, the presence of mast cells, and the characteristic IgE deposition in germinal centers reinforce the idea that Kimura's disease may be a manifestation of an abnormal immunologic response. An increased number of IgG4+ plasma cells has been described in a few cases of Kimura disease (see IgG4-Related Lymphadenopathy, below), raising the question of a relation between these two disorders.

Differential Diagnosis

The differential diagnosis of Kimura disease includes a variety of neoplastic and reactive conditions, such as Hodgkin lymphoma, angioimmunoblastic T-cell lymphoma, Castleman disease, parasitic infection, nonspecific florid follicular hyperplasia, and angiolymphoid hyperplasia with eosinophilia. In many early reports, Kimura disease and angiolymphoid hyperplasia with eosinophilia were grouped together as one disease. Both angiolymphoid hyperplasia with eosinophilia and Kimura disease tend to produce lesions with prominent blood vessels and eosinophils in the region of the head and neck. However, angiolymphoid hyperplasia with eosinophilia does not show any special predilection for Asians or for males and is associated with smaller lesions (average, 1 cm and typically less than 2 cm) that are better circumscribed and more superficially located (i.e., in the dermis and subcutaneous tissue), especially in the area around the ear. Lymphoid follicles and fibrosis are not always seen in angiolymphoid hyperplasia with eosinophilia. Angiolymphoid hyperplasia with eosinophilia may be associated with regional lymphadenopathy, but salivary gland involvement is distinctly unusual. Peripheral blood eosinophilia, elevated IgE, asthma, and proteinuria are unusual. A characteristic feature of angiolymphoid hyperplasia with eosinophilia is prominent epithelioid, or histiocytoid, endothelial cells, which are not seen in Kimura disease, whereas eosinophils are not always abundant and eosinophilic abscesses are unusual in angiolymphoid hyperplasia with eosinophilia. Angiolymphoid hyperplasia with eosinophilia may represent a benign vascular tumor rather than a type of lymphoid hyperplasia, and for this reason, it has also been called epithelioid hemangioma and cutaneous histiocytoid angioma.

DRUG-RELATED LYMPHADENOPATHY

Infrequently patients receiving medication, usually anticonvulsant therapy, develop a severe hypersensitivity reaction with manifestations that commonly include a skin rash, fever, lymphadenopathy, elevated liver function tests, leukocytosis, peripheral eosinophilia, and elevated C-reactive protein and lactate dehydrogenase. The rash is usually maculopapular or erythrodermic, but a minority of patients have a bullous eruption (toxic epidermal necrolysis or Stevens-Johnson syndrome). The lymphadenopathy is more often multifocal than unifocal (Fig. 4.14). Generally the triad of fever, rash, and multiorgan involvement is suggested to establish a diagnosis of drug-induced hypersensitivity syndrome (DISH), also called drug rash with eosinophilia and systemic symptoms (DRESS).

IgG4-RELATED LYMPHADENOPATHY

The least common of all the IgG subclasses is IgG4. IgG4-related disease, also known as IgG4-related autoimmune disease and IgG4-related sclerosing disease, is a systemic disease characterized by the presence of tumor-like sclerosing lesions in one or more extranodal sites, typically without constitutional symptoms, often with elevated serum IgG4 level, and a good response to steroid or rituximab therapy. Among the more commonly involved extranodal sites are the pancreas (IgG4-related pancreatitis), biliary tract (IgG4-related sclerosing cholangitis), submandibular gland (IgG4-related

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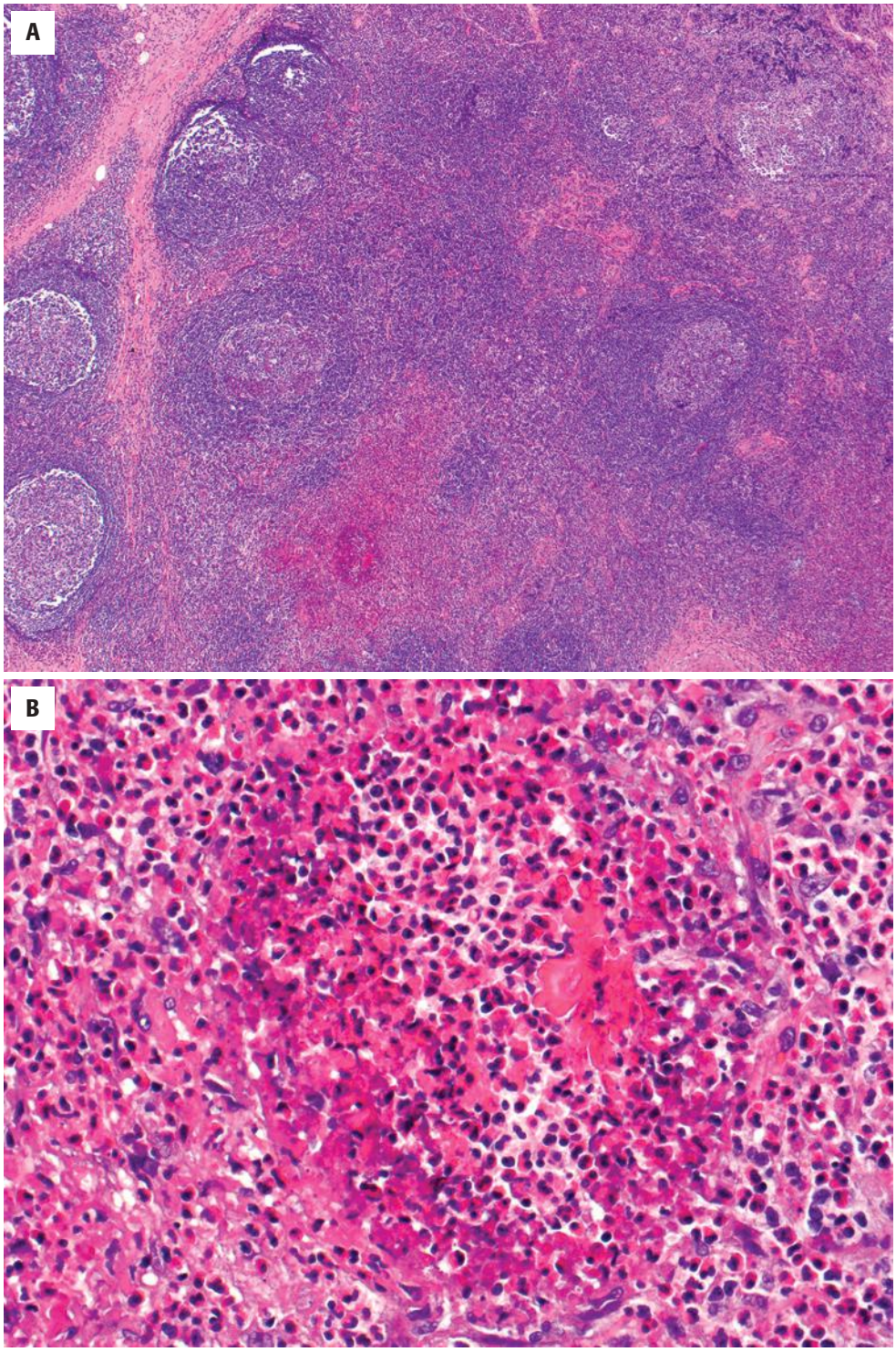


FIG. 4.13

Kimura disease. A, Low power shows a nodule of lymphoid tissue with prominent follicular hyperplasia and focal fibrosis. B, Higher power shows an eosinophilic microabscess. IgE+ dendritic meshworks and IgE+ plasma cells were also present (not illustrated).

sialadenitis/Küttner tumor), and lacrimal gland (sclerosing dacryoadenitis). On microscopic examination the sclerosing lesions typically show variably prominent lymphoplasmacytic infiltrates, sclerosis, and obliterative phlebitis, with increased numbers and increased proportion of IgG4+ plasma cells. In addition to the elevated serum IgG4, common laboratory abnormalities include polyclonal hypergammaglobulinemia, elevated ESR and/or C-reactive protein, rheumatoid factor, antinuclear antibodies, and anemia. Patients with IgG4-related disease often have concurrent lymphadenopathy, which may be localized to the region of the extranodal sclerosing lesion(s) or systemic. Five different patterns of IgG4-related lymphadenopathy are described. In general, lymphadenopathy with similar features is occasionally identified in individuals without an established diagnosis of IgG4-related disease. Only rarely do such patients develop

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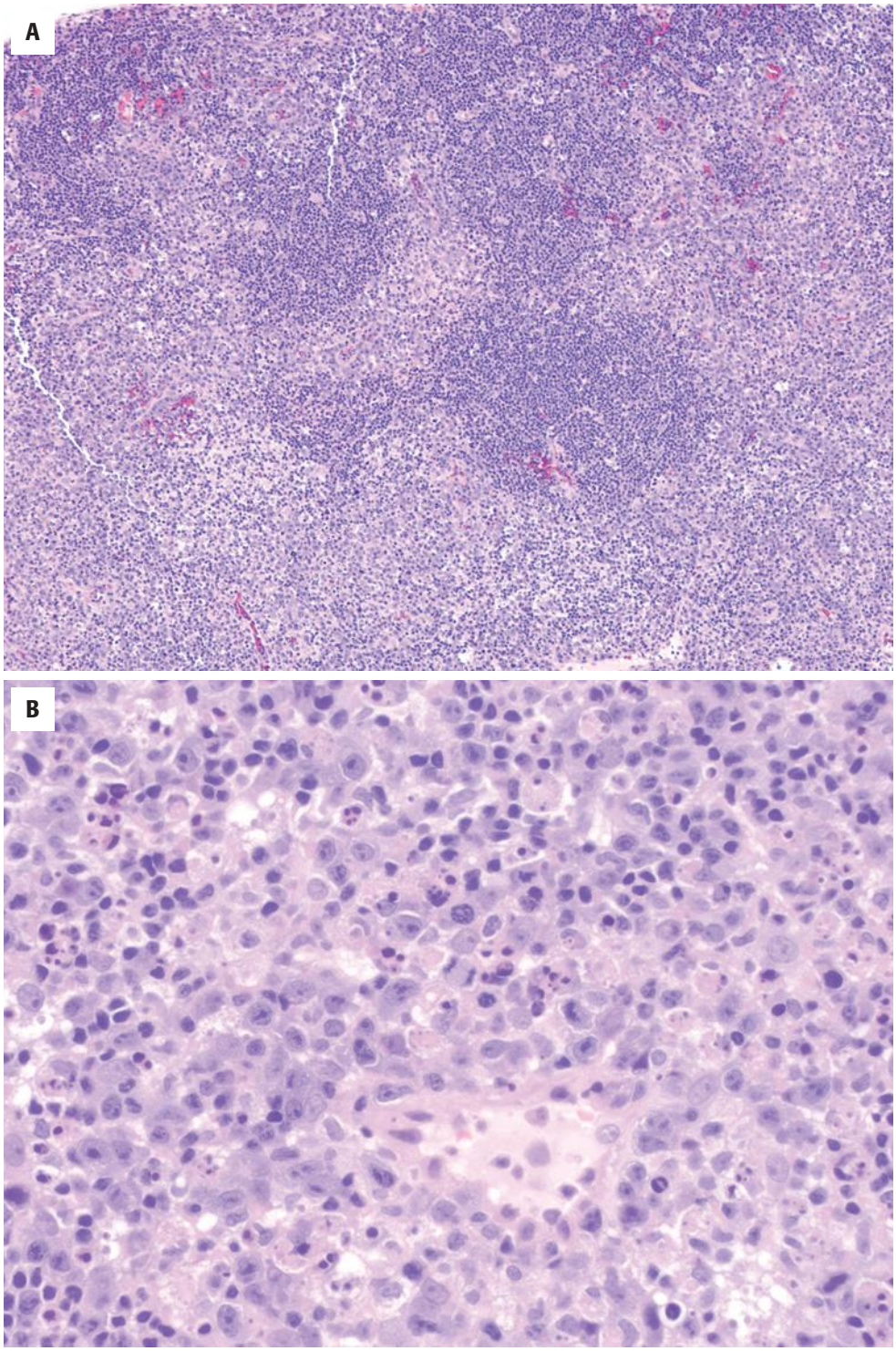


FIG. 4.14

Phenytoin-associated lymphadenopathy. A, The lymph node is slightly enlarged. It shows scattered small, dark primary follicles and an expanded paracortex. B, High-power examination of the paracortex shows numerous immunoblasts, scattered lymphocytes, and apoptotic debris.

extranodal sclerosing lesions. The lymphadenopathy thus has features that are distinctive but not entirely specific (Fig. 4.15); evaluation for other features that are found in IgG4-related disease is required to establish a diagnosis. Thus, when encountering an enlarged lymph node with histologic features of IgG4-related lymphadenopathy, in the absence of other evidence of IgG4-related disease, making a diagnosis of reactive lymphoid hyperplasia with increased IgG4+ plasma cells, rather than IgG4-related lymphadenopathy, is recommended. Clinical correlation and follow-up are suggested. Lymphadenopathy with pattern V, or IgG4-fibrosing lymphadenopathy, however, is reported to be strongly associated with IgG4-related disease, including extranodal involvement and high serum IgG4. Thus, in cases with these histologic features, evaluation for evidence of IgG4-related disease is important. EBV+ cells, usually in modest numbers, are commonly found in lymph nodes with features of IgG4-related

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DRUG-RELATED LYMPHADENOPATHY—FACT SHEET

Definition
- Lymphadenopathy occurring as a reaction, typically a hypersensitivity reaction, to medication
- Antiseizure medication, especially phenytoin and carbamazepine, is most often associated with this type of lymphadenopathy.

Incidence
- One in 1000 to one in 10,000 exposures to anticonvulsant therapy; more frequent in immunocompetent patients

Gender, Race, or Age Distribution
- Patients can be young or old, male or female.

Risk Factors
- Possible link to a defect in the detoxifying enzyme epoxide hydrolase; can be familial; dark skin, vitamin D deficiency, winter months may increase risk.

Clinical Features
- The classic presentation is with a hypersensitivity reaction, with fever, malaise, rash, and lymphadenopathy 2 to 8 weeks after starting therapy, but lymphadenopathy has been reported after years on phenytoin.
- Involved nodes are most often cervical, but other nodes may be involved and lymphadenopathy may be generalized.

Pathologic Features
- Often not biopsied, but the typical picture is a paracortical immunoblastic proliferation, with or without follicular hyperplasia, sometimes with eosinophils.
- The immunoblastic reaction can be so striking in some cases as to suggest lymphoma; such cases have been called pseudolymphoma (Fig. 4.14).
- Rare patients have had lymphadenopathy with an immunoblastic reaction to drug and later developed lymphoma, but the medications have not been proven to cause lymphoma.
- Some individuals have had nonspecific reactive hyperplasia, necrotizing lymphadenitis or, dermatopathic lymphadenopathy, but these may be coincidental rather than caused by the medication.
- Rare cases contain monoclonal lymphoid cells. Clinical history may help in arriving at the correct diagnosis.

Prognosis and Therapy
- Treatment consists of immediate discontinuation of the drug, with the addition of steroids or other anti-inflammatory agents, if needed.
- Many patients do well if treated promptly; the drug-induced hypersensitivity reaction is occasionally fatal.

lymphadenopathy; the significance of EBV in this setting is uncertain.

A variety of other diseases, including sinus histiocytosis with massive lymphadenopathy, Castleman disease, rheumatoid arthritis, Kimura disease, and polyangiitis with granulomatosis, can have lymphadenopathy or other tissues with an inflammatory cell infiltrate that includes many IgG4+ plasma cells. Thus careful clinical correlation and evaluation of other histologic features are required to establish a diagnosis.

IgG4-RELATED LYMPHADENOPATHY—FACT SHEET

Definition
- Lymphadenopathy occurring in the setting of IgG4-related sclerosing disease

Incidence
- Unknown

Gender, Race, or Age Distribution
- Males more often than females; most patients are middle-aged to older.

Risk Factors
- None known

Clinical Features
- Lymphadenopathy in the setting of IgG4-related disease; constitutional symptoms are very unusual

Pathologic Features
- Five main histologic patterns are described, each showing increased numbers of IgG4+ plasma cells (>100 IgG4+ plasma cells/hpf) and increased proportion of IgG4+ plasma cells compared to IgG+ plasma cells (>40% suggested as cut-off, usually higher). Encountering a mixture of patterns in a single lymph node is common (Fig. 4.15):
  - Castleman disease–like: The follicles show changes similar to those of hyaline-vascular Castleman disease, whereas the interfollicular area contains increased numbers of plasma cells.
  - Follicular hyperplasia: The lymphoid follicles are hyperplastic, with increased numbers of intrafollicular plasma cells.
  - Interfollicular expansion: In this pattern, a few follicles are widely separated by an expanded interfollicular area occupied by plasma cells, including some immature forms, occasional immunoblasts, small numbers of lymphoid cells with clear cytoplasm, and arborizing high endothelial venules. The differential diagnosis could include angioimmunoblastic T-cell lymphoma, but the absence of atypical CD10+ T cells and lack of expanded follicular dendritic meshworks tend to exclude this possibility.
  - Progressive transformation of germinal center (PTGC)-like: Lymph nodes show PTGC, occasionally surrounded by clusters of epithelioid histiocytes, often with increased plasma cells, in typical follicles or in PTGCs. The interfollicular area contains small lymphocytes with or without admixed eosinophils, plasma cells, and immunoblasts.
  - Inflammatory pseudotumor-like: In a background of reactive changes, there is partial replacement of the lymph node by sclerosis, sometimes with a storiform pattern. IgG4+ plasma cells are scattered within areas of sclerosis. Eosinophils are usually present. This pattern appears more likely to be associated with IgG4-related disease.
- Features uncommon in IgG4-related lymphadenopathy: Granulomas, neutrophils, and necrosis.
- Lymphadenopathy with features of IgG4-related lymphadenopathy can occur in patients with no other evidence of IgG4-related disease at presentation or follow-up. Such lymph nodes should receive a diagnosis of reactive lymphoid hyperplasia with increased IgG4+ plasma cells, not IgG4-related lymphadenopathy, and suggest clinical correlation.

Prognosis and Therapy
- Treatment with corticosteroids and/or rituximab is often beneficial; prognosis is good.

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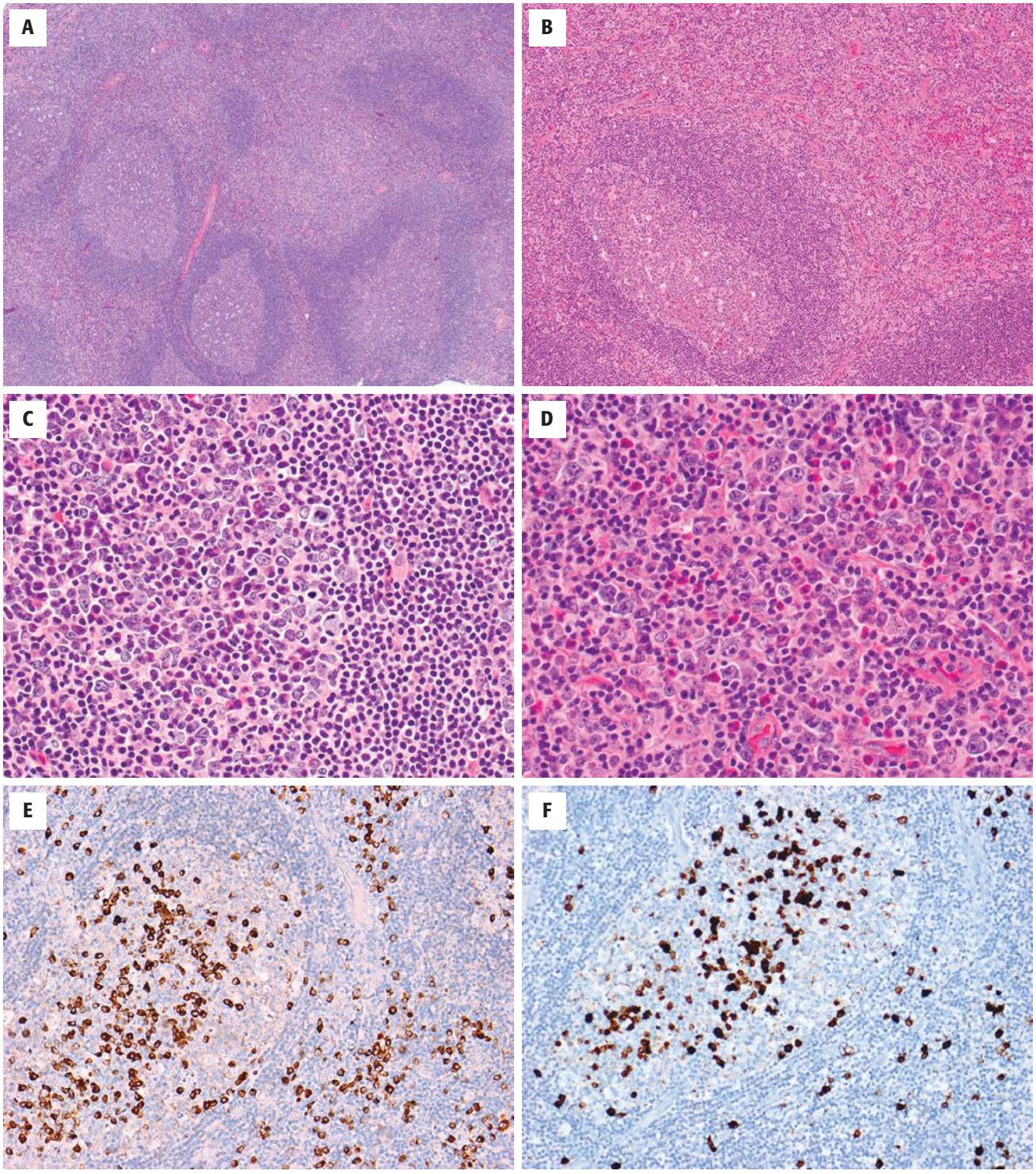


FIG. 4.15

IgG4-related lymphadenopathy. A, Low power shows follicular hyperplasia as well as focal expansion of the interfollicular area. B, Medium power shows one follicle with an active follicle center and a discrete mantle. Cells in the interfollicular area are polymorphous. C, High power of a follicle shows many plasma cells admixed with follicle center cells. D, The interfollicular area contains small lymphocytes, plasma cells, eosinophils, and immunoblasts. With antibody to IgG (E) and IgG4 (F), most IgG+ plasma cells are positive for IgG4; this is most pronounced within follicles. This case shows a mixture of features of patterns 2 and 3 (see Fact Sheet).

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LYMPHADENOPATHY RELATED TO EXOGENOUS, EXCESS ENDOGENOUS, OR PIGMENTED MATERIAL

DERMATOPATHIC LYMPHADENOPATHY

Dermatopathic lymphadenopathy is found in patients with a variety of chronic dermatoses or with mycosis fungoides. The affected lymph nodes are those draining the abnormal skin. In some cases, patients do not have appreciable skin disease. Lymph nodes show marked expansion of the paracortex, with formation of large, pale nodules containing lymphocytes, occasional immunoblasts, and numerous histiocytes with abundant pale cytoplasm including interdigitating dendritic cells, Langerhans cells, and phagocytic histiocytes containing melanin, lipid, and, occasionally, iron (Fig. 4.16). Small numbers of lymphocytes with irregular nuclei,

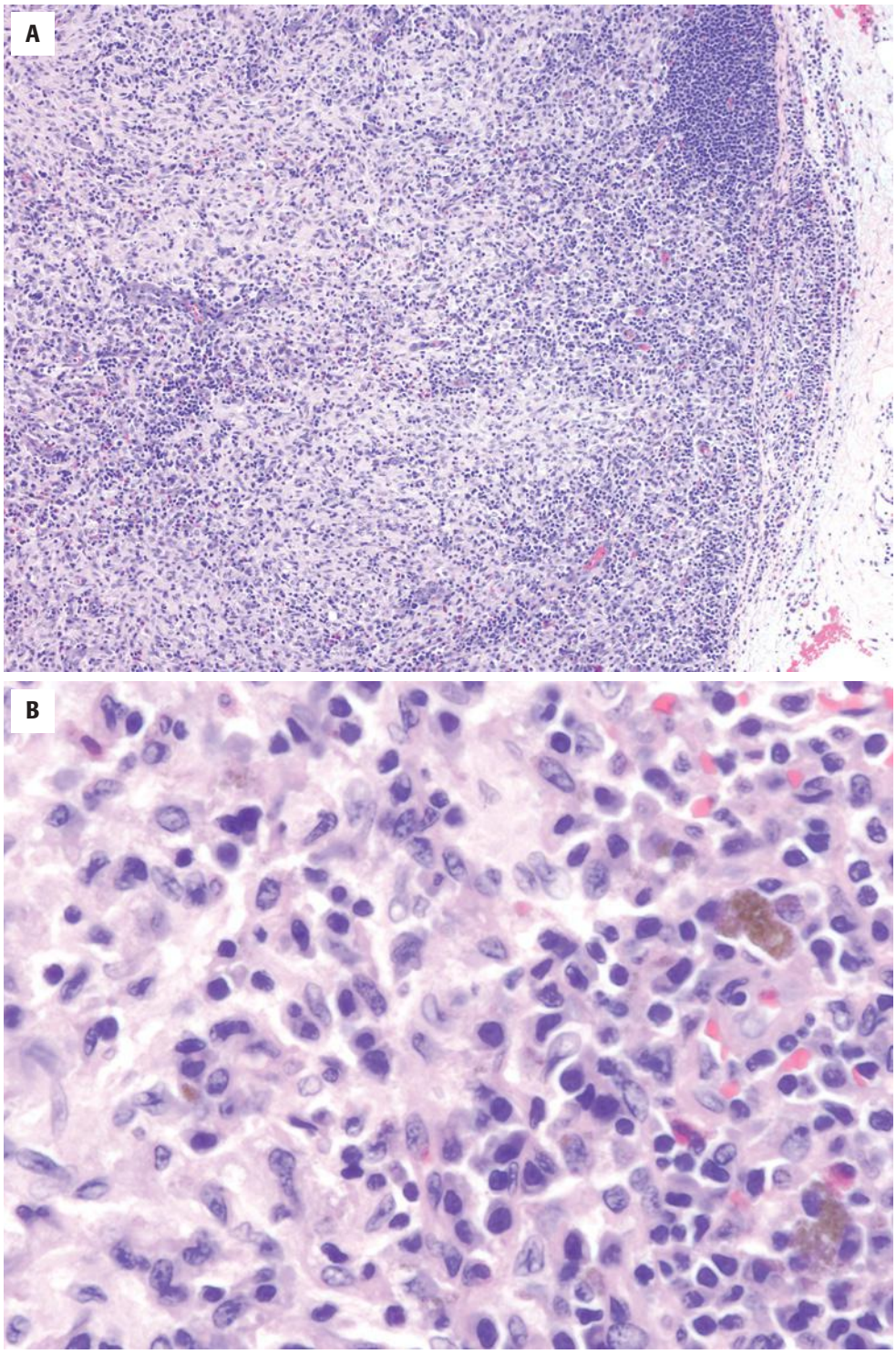


FIG. 4.16

Dermatopathic lymphadenopathy. A, Low power shows vaguely nodular pale areas in the paracortex. A small, dark primary follicle is present beneath the nodal capsule. B, The pale areas correspond to numerous antigen-presenting cells, including Langerhans cells with pale, folded, and grooved nuclei. Histiocytes containing pigment are also present.

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reminiscent of the cerebriform cells that are characteristic of mycosis fungoides, may be found, even in patients with no evidence of lymphoma. Follicular hyperplasia is usually inconspicuous. The paracortical T cells are predominantly T helper cells, with a CD4-to-CD8 ratio that is, on average, higher than that of nonspecific lymphoid hyperplasia. The interdigitating dendritic cells and Langerhans cells are S100 positive. Findings in patients with and without mycosis fungoides are similar. The majority of cases of dermatopathic lymphadenopathy in patients with mycosis fungoides show clonal T-cell receptor gene rearrangement, suggesting that nodal involvement by mycosis fungoides is frequent and may be subtle and difficult to appreciate on routine sections.

MISCELLANEOUS LYMPHADENOPATHIES WITH HISTIOCYTIC INFILTRATION

Hereditary storage diseases, such as Niemann-Pick disease and Gaucher disease, can be associated with nodal infiltration by histiocytes distended by storage products. The appearance of the histiocytes is similar to their appearance in other tissues. Lymphangiography, previously used for the staging of Hodgkin lymphoma, produces lipogranulomas, with sinusoidal distention by foamy histiocytes, multinucleated giant cell, and large and small vacuoles of contrast material. Lipogranulomas are commonly found in lymph nodes related to the portal circulation and are probably the result of deposition of lipids related to the diet and bile metabolites. Hyperlipidemia is a rare cause of lymphadenopathy. Severe hyperlipidemia has been associated with droplets of lipid in nodal sinuses and large numbers of lipid-laden macrophages.

Prosthetic joint replacements may deteriorate over time; occasionally, this process is associated with a reaction to foreign material in regional lymph nodes. The histiocytic reaction may be confined to distended sinuses or may extend to involve the paracortex. Prosthetic devices containing metal, polyethylene, and cement (polymethylmethacrylate) are associated with sheets of histiocytes with granular eosinophilic cytoplasm; metal and refractile foreign materials are seen. Reaction to Silastic joint prostheses (silicone lymphadenopathy) consists of granulomas with multinucleated giant cells containing pale yellow, refractile particles of silicone.

Mammary prostheses (bag-gel prostheses or silicone injection) may also result in adenopathy. Silicone lymphadenopathy in this setting tends to show diffuse infiltrates of histiocytes with marked cytoplasmic vacuolization and extracellular deposits of pale foreign material forming prominent cystic spaces (Fig. 4.17).

Patients with respiratory exposure to silica may develop lymphadenopathy, often in association with lung disease, although, on occasion, lymphadenopathy may be present in isolation. Microscopic examination shows sclerotic nodules associated with a histiocytic and fibroblastic reaction and birefringent crystals on examination with polarized light. These lymph nodes often also show anthracosis (Fig. 4.18).

Anthracosis is a common finding in mediastinal lymph nodes. Involved nodes typically show black discoloration on gross examination and numerous histiocytes with carbon particles on microscopic examination. Lymph nodes draining skin with tattoos may contain histiocytes with pigment that is usually black; however, other colors can be seen. Infrequently, this phenomenon produces clinically significant lymphadenopathy (Fig. 4.19).

SARCOIDOSIS

Clinical Features

Sarcoidosis is an uncommon granulomatous disorder of unknown cause; blacks are more often affected than whites, and women are more often affected than men. Involvement of a wide variety of tissues can occur, but the lymph nodes and lung are the most common. Mediastinal and pulmonary hilar nodes are the most frequently involved, but any lymph node, as well as a wide variety of extranodal sites, may show changes of sarcoidosis. The clinical course is highly variable; some patients are asymptomatic and some develop significant complications, the most common of which is progressive pulmonary fibrosis.

Pathologic Features

Lymph nodes show multiple compact granulomas composed of epithelioid histiocytes and multinucleated giant cells with a narrow cuff of small lymphocytes. The granulomas are often confluent, replacing nearly the entire node; when the node is partially involved, the granulomas are usually in the paracortex. A small amount of fibrin and cellular debris may be seen in the centers of some granulomas, but necrosis is not prominent, and caseation is absent. Over time, the granulomas may become hyalinized.

Differential Diagnosis

Sarcoidosis is a diagnosis of exclusion; its histologic differential is broad and includes tuberculosis, fungal infection, tuberculoid leprosy, brucellosis, Whipple disease, syphilis, Crohn disease, and reaction to foreign material such as beryllium. Hodgkin lymphoma and certain types of non-Hodgkin lymphomas may be associated with a granulomatous reaction resembling sarcoidosis. Examination of the areas between granulomas in these cases reveals an atypical lymphoid infiltrate. Patients with Hodgkin lymphoma may have sarcoidal granulomas in multiple tissues, including liver, spleen, and lymph nodes, in the absence of involvement by Hodgkin lymphoma. Sarcoidal granulomas may

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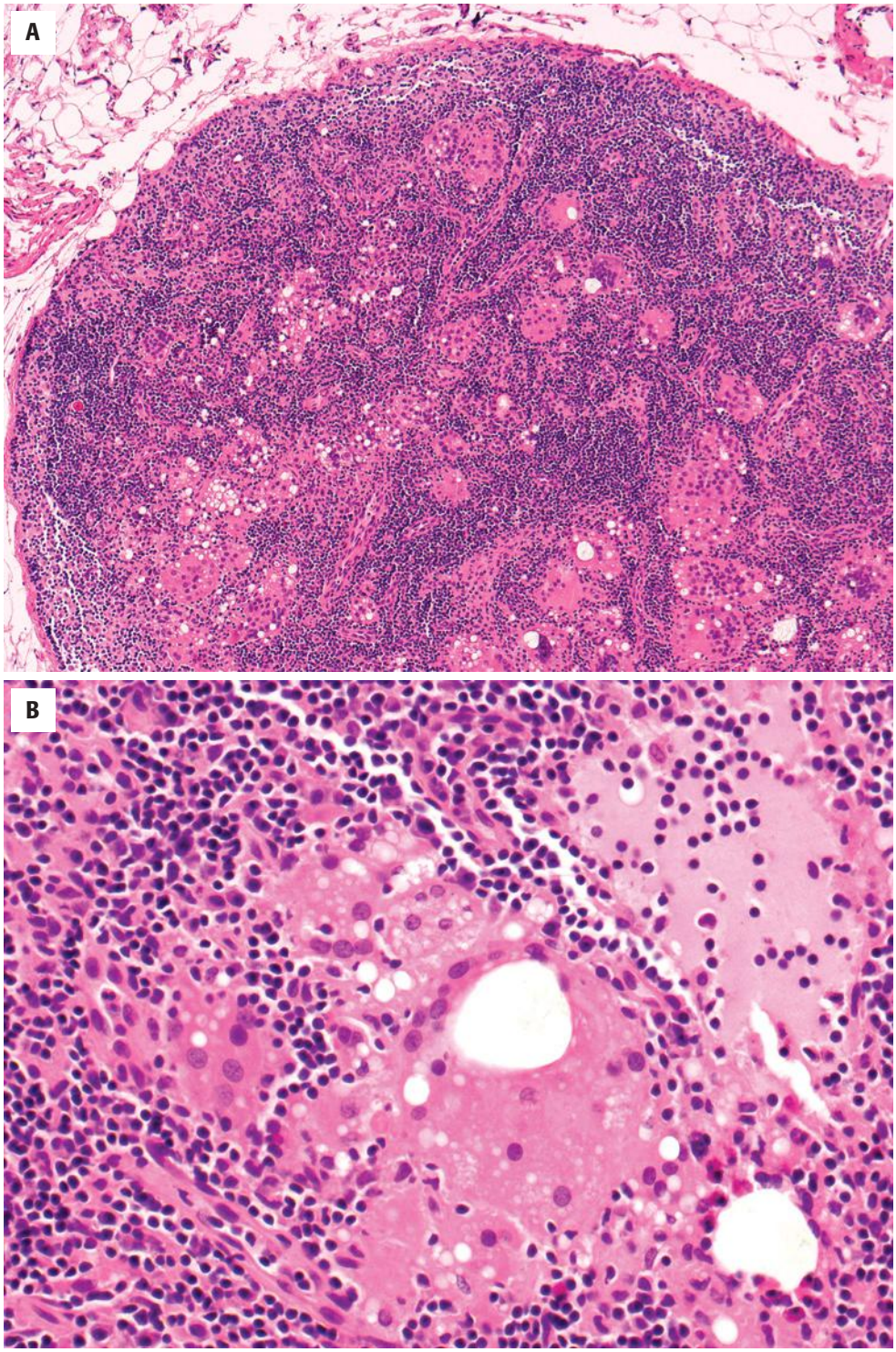


FIG. 4.17

Silicone lymphadenopathy, related to a breast implant. A, Low power shows increased numbers of histiocytes, with multinucleated histiocytes and many histiocytes with prominently vacuolated cytoplasm. B, Higher power shows an aggregate of vacuolated histiocytes; some vacuoles contain pale material consistent with silicone.

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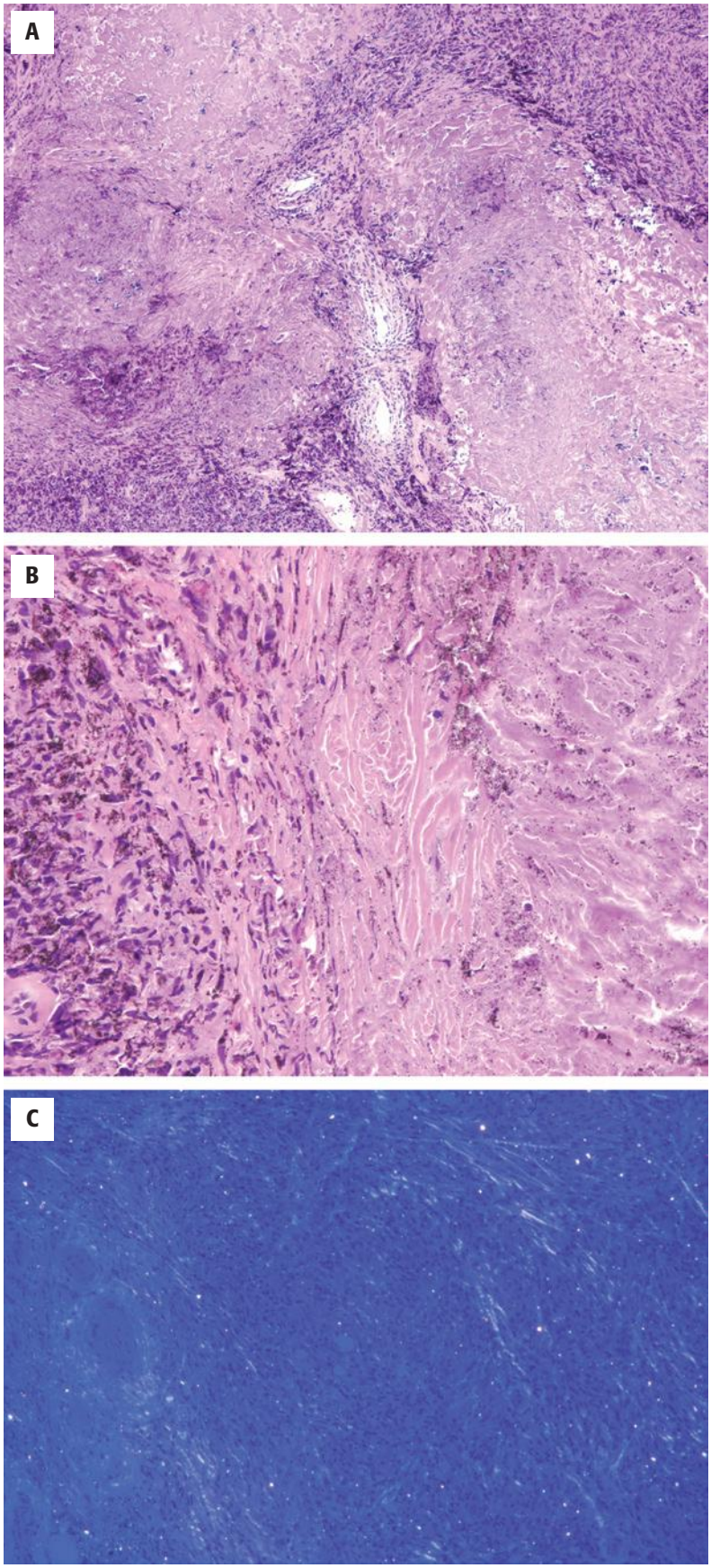


FIG. 4.18

Lymph nodes with sclerotic nodules related to silica. A, Nodular sclerotic areas occupy much of the lymph node. The lymph node also shows anthracosis. B, Higher power shows hyalinized lymphoid tissue. C, Birefringent particles of silica are seen with polarized light.

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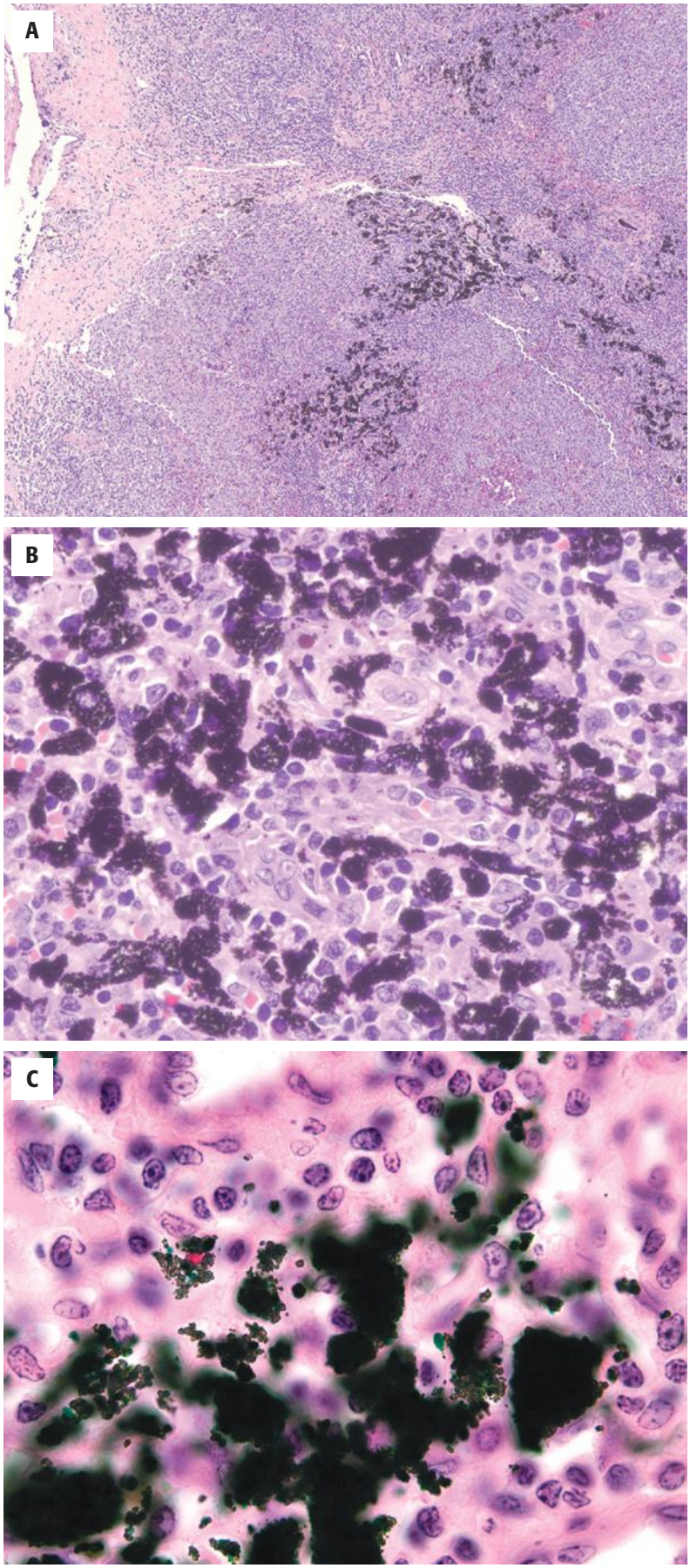


FIG. 4.19

Lymph node with tattoo pigment. A, This axillary lymph node contains numerous histiocytes with black pigment. The patient had multiple tattoos. B, Higher power shows histiocytes with black particulate material. C, In another example, the pigment has a distinct green color.

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be found in lymph nodes draining tissues involved by carcinoma.

CASTLEMAN DISEASE

Castleman disease has been subclassified based on pathologic features into hyaline-vascular and plasma cell variants. Occasional cases with features intermediate between the two types (transitional or mixed type) may be seen. An additional subtype of Castleman disease has been described more recently: HHV8-associated multicentric Castleman disease. Castleman disease occurs in two clinical forms: localized and multicentric.

LOCALIZED CASTLEMAN DISEASE

Nearly all cases of hyaline-vascular Castleman disease, and a minority of plasma cell Castleman disease, are localized—that is, the disorder affects a single anatomic site or a single group of lymph nodes. Approximately 80% of localized Castleman disease is of the hyaline-vascular type, whereas approximately 20% is of the plasma cell type, with occasional cases of mixed type. Excision of the lesion is almost always curative.

MULTICENTRIC CASTLEMAN DISEASE

Multicentric Castleman disease (MCD) is not one disease, but rather a group of incompletely understood lymphoproliferative disorders with systemic manifestations believed to be related to proinflammatory cytokinemia, with a relatively poor prognosis. Patients have lesions of Castleman disease in two or more separate anatomic sites. Most patients are middle-aged to older adults. Patients present with peripheral and/or internal lymphadenopathy and often with hepatomegaly and splenomegaly. Patients usually have systemic symptoms such as fatigue, fever, weight loss, and/or night sweats; such symptoms tend to be more severe than those in localized plasma cell Castleman disease. Laboratory abnormalities usually include anemia, elevated sedimentation rate, and others. Manifestations may be related to elevated levels of human or viral IL-6, likely produced in the affected tissues. The levels of IL-6 appear to correlate with the systemic inflammatory manifestations. Elevated IL-6 results in production of acute-phase reactants in liver, with constitutional symptoms, as well as stimulation of B cells and plasma cells, and increased vascular endothelial growth factor (VEGF), which promotes angiogenesis. Cases of MCD may have the histologic features of plasma cell Castleman disease or of mixed-type Castleman disease, and infrequently of the hyaline-vascular type. A subset of cases is related to human herpesvirus 8 infection (see separate section below). A subset of patients has a monotypic plasma cell proliferation (almost always lambda+) that may be associated with POEMS (Peripheral neuropathy, Organomegaly, Endocrinopathy, Monoclonal plasma cell proliferation, and Skin changes) syndrome (see below). Other cases are idiopathic. Patients with multicentric Castleman disease may have systemic disease with manifestations in multiple organ systems; rarely, these take the form of TAFRO (Thrombocytopema, Anasarca, myeloFibrosis, Renal dysfunction and Organomegaly) syndrome or POEMS syndrome (see below, and also Table 4.3).

Patients with idiopathic multicentric Castleman disease present with clinical and laboratory manifestations that often

TABLE 4.3

Multisystem Disorders With Lymphadenopathy With Features of Castleman Disease

TAFRO

Immunologic disorder; pathogenesis due to increased IL-6 and VEGF

T: Thrombocytopenia (and/or anemia)
A: Anasarca (pleuritis/peritonitis), pleural effusion, ascites
F: Myelofibrosis
R: Renal dysfunction
O: Organomegaly (lymphadenopathy, hepatomegaly, splenomegaly)
(Alternatively: T: thrombocytopenia, A: anasarca, F: fever, R: reticulin fibrosis, O: organomegaly)
Affected patients: young to older adults; men and women affected
Variety of autoantibodies including rheumatoid factor and ANA (antinuclear antibody)
Elevated alkaline phosphatase
Elevated IL-6 and VEGF (vascular endothelial growth factor) in serum and effusions
Lack of marked hypergammaglobulinemia
Lymphadenopathy, usually <1.5 cm, typically with features of mixed type MCD (multicentric Castleman disease), less often HVCD. Follicles are of hyaline-vascular type. Interfollicular area shows mature polyclonal plasma cells (although usually not as abundant as in PCCD), and increased blood vessels of high endothelial type. Immunoblasts, immature plasma cells, and plasmacytoid dendritic cells are scarce.
May respond to high-dose steroids or to monoclonal antibodies to IL-6 or IL-6R. Some have a prolonged, indolent course; some do not achieve complete remission. Some patients have a rapid course. Some die of progressive disease and infection.
Not related to HIV (human immunodeficiency virus), EBV (Epstein-Barr virus), or HHV8 (human herpesvirus 8)

POEMS syndrome

P: Polyneuropathy
O: Organomegaly (lymphadenopathy, splenomegaly, and/or hepatomegaly)
E: Endocrinopathy (affecting thyroid, adrenal, pituitary, gonadal, or other function)
M: Monoclonal plasma cell proliferation (almost always λ+)
S: Skin changes (hyperpigmentation, hypertrichosis, hemangiomas, others)
Other findings:
Osteosclerotic bone lesions (often osteosclerotic plasma cell myeloma)
VEGF elevation
Fluid overload (edema, effusions)
Polycythemia

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include multicentric lymphadenopathy, elevated C-reactive protein, decreased albumin, elevated IL-6, hypergammaglobulinemia, increased ESR, positive antinuclear antibody (ANA), hepatomegaly, splenomegaly, fever, edema/ascites/anasarca, night sweats, weight loss, increased soluble IL-2R, increased levels of VEGF, and possibly increased risk of malignancy. Symptoms tend to wax and wane and to correlate with IL-6 levels. IL-6 induces plasmacytosis, hyperglobulinemia, increased VEGF leading to hypervascularity, thrombocytosis, and possibly autoimmune phenomenon through expansion of autoantibody-inducing CD5+ B cells. A recent study proposed diagnostic criteria for the diagnosis of idiopathic MCD. Criteria included a combination of major and minor clinical and pathological criteria. To establish a diagnosis, both major criteria: (1) lymphadenopathy ≥ 1 cm in at least two sites and (2) histology showing hyaline-vascular-type follicles (with regressed germinal centers, prominent follicular dendritic cells and expanded mantles) and prominent polytypic plasmacytosis (with or without interfollicular vascularity and hyperplastic germinal centers) should be fulfilled. In addition, at least 2 of 11 minor criteria (including elevated CRP or ESR, anemia, thrombocytopenia or thrombocytosis, constitutional symptoms, evidence of fluid overload and others) must be fulfilled, and other diseases (including infection by HHV8, EBV and others; autoimmune disease; and lymphoid and plasma cell neoplasms) must be excluded. Possible causes that are proposed include an unusual form of autoimmune disease, germline mutations in genes regulating the immune system, a paraneoplastic process related to an unrecognized malignancy, and an effect of a virus other than HHV8. Treatment has included steroids, immunomodulatory agents, anti-human IL-6 (siltuximab) and anti-IL-6 receptor (tocilizumab), rituximab, and chemotherapy. Although some patients respond, a subset of patients succumbs to progressive disease, organ failure, sepsis, or malignancy.

In POEMS, the neoplastic plasma cells may be the source of the increased cytokines. In HHV8+ MCD the HHV8-infected cells are the source of the increased cytokines.

HYALINE-VASCULAR VARIANT

Clinical Features

Hyaline-vascular Castleman disease (HVCD) can affect patients of any age, but most are young adults. Females may be affected slightly more often than males. Hyaline-vascular Castleman disease is often an incidental finding on radiographic examination, but patients may also present with symptoms related to compression of adjacent structures or rarely with pain or a palpable mass. Constitutional symptoms and hematologic abnormalities are very unusual. Patients have lymphadenopathy in a single site in nearly all cases; in rare cases, the lesion involves an extranodal site. There may be a main mass lesion with enlargement of adjacent lymph nodes, sometimes also showing changes of Castleman disease. Rarely, more than one site is involved. The mediastinum is most commonly affected, followed by lymph nodes in the abdomen, neck, and other sites. HVCD almost never recurs after excision. The cause is uncertain but is most likely related to abnormal follicular dendritic cells. An uncommon but serious complication of Castleman disease (typically unicentric HVCD) is a paraneoplastic pemphigus. It is associated with a poor prognosis.

Pathologic Features

Involved lymph nodes take the form of rounded masses 1.5 to 16 cm in diameter (median, about 6 cm). HVCD is characterized by lymphoid follicles with small, hyalinized germinal centers and broad mantle zones. Each follicle may contain one or more germinal center; some follicles contain no recognizable germinal center. The germinal centers contain an increased proportion of follicular dendritic cells and endothelial cells. Mantle zone lymphocytes are arranged in concentric rings ("onion skin" pattern) around the germinal center. Large, hyperchromatic, occasionally bizarre, dystrophic cells with scant cytoplasm, consistent with abnormal follicular dendritic cells, are sometimes found in the follicles, usually in the atrophic follicle centers, and less often in the mantles. Follicles are often radially penetrated by a blood vessel ("lollipop follicle"). The interfollicular region shows increased numbers of high endothelial venules and of small vessels with flat endothelium and hyalinized walls (Fig. 4.20). Lymphocytes are fewer in number than in a normal lymph node, and there may be an admixture of scattered plasma cells, few immunoblasts, few eosinophils, and clusters of plasmacytoid dendritic cells. Sheets of plasma cells are absent. Epithelioid histiocytes and granulomas are inconspicuous or absent. In some cases, patent sinuses may be found in residual normal lymphoid tissue at the periphery of the lesion, but, in general, they are absent within the lesion. Fibrosis is common peripherally and also in bands running through the lesion. There may be prominent perivascular fibrosis. Rarely, dystrophic calcification occurs in fibrotic areas. Rarely, HVCD is complicated by the development of a follicular dendritic cell tumor (Fig. 4.21) or a vascular neoplasm that appears to be an outgrowth of the abnormal follicular dendritic cells or of the floridly proliferating blood vessels, respectively.

Follicles contain decreased numbers of B cells and relatively increased numbers of follicular dendritic cells (CD21+, CD23+). B cells are polytypic. In some cases (mainly in HVCD, but occasionally in plasma cell Castleman disease), a subset of polytypic B cells in the follicle mantles co-expresses CD5; the co-expression of CD5 in this setting is not indicative of a diagnosis of B-cell lymphoma. T cells (CD3+) are present in smaller than normal numbers in follicles and interfollicular area. Many interfollicular blood vessels are factor VIII positive, CD34+, HECA-452

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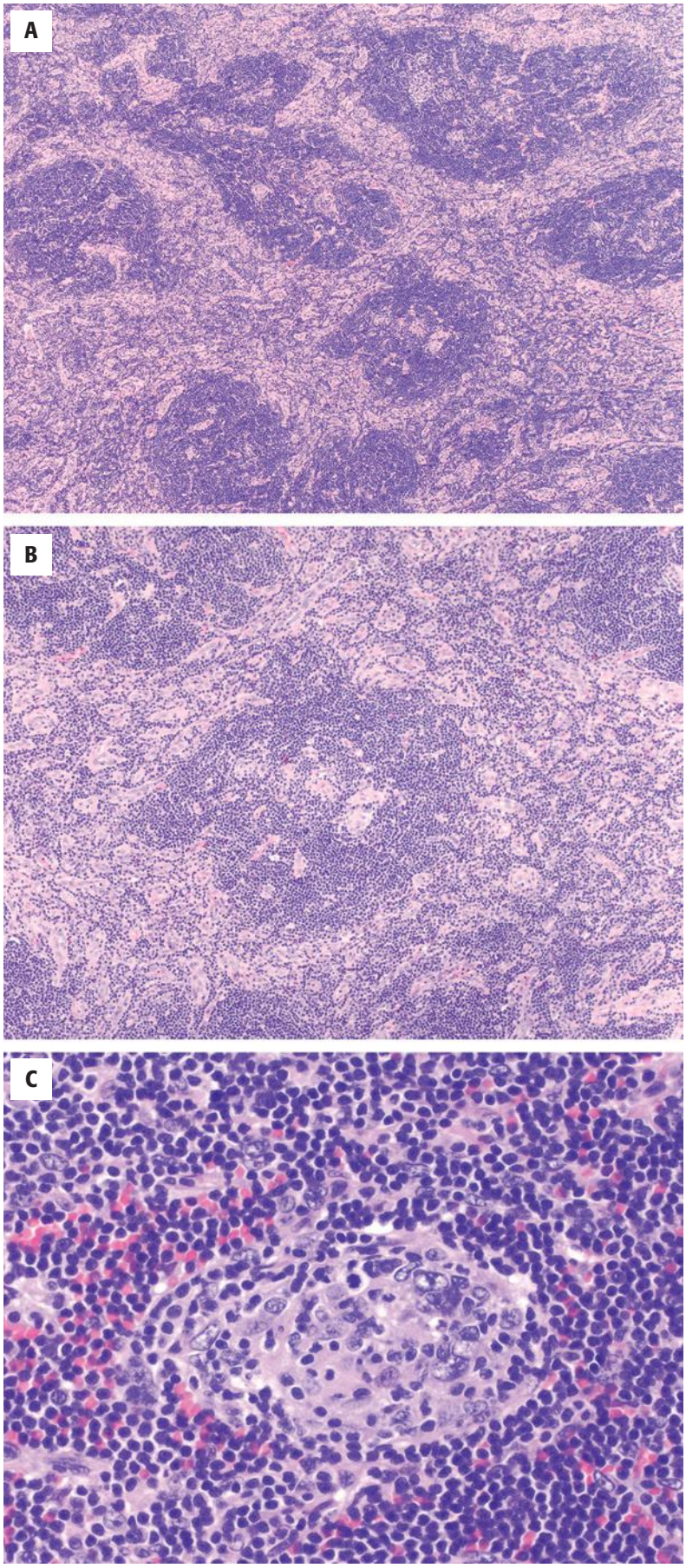


FIG. 4.20

Hyaline-vascular Castleman disease. A, Low power shows a lymph node replaced by follicles with broad mantles, inconspicuous follicle centers, and a hypervascular interfollicular area. Patent sinuses are absent. B, Higher power shows a follicle consisting mainly of small lymphocytes, with a suggestion of tiny sclerotic follicle centers, surrounded by numerous blood vessels. C, This follicle center contains few follicle-center B cells. Scattered large, oval, slightly atypical follicular dendritic cells are present.

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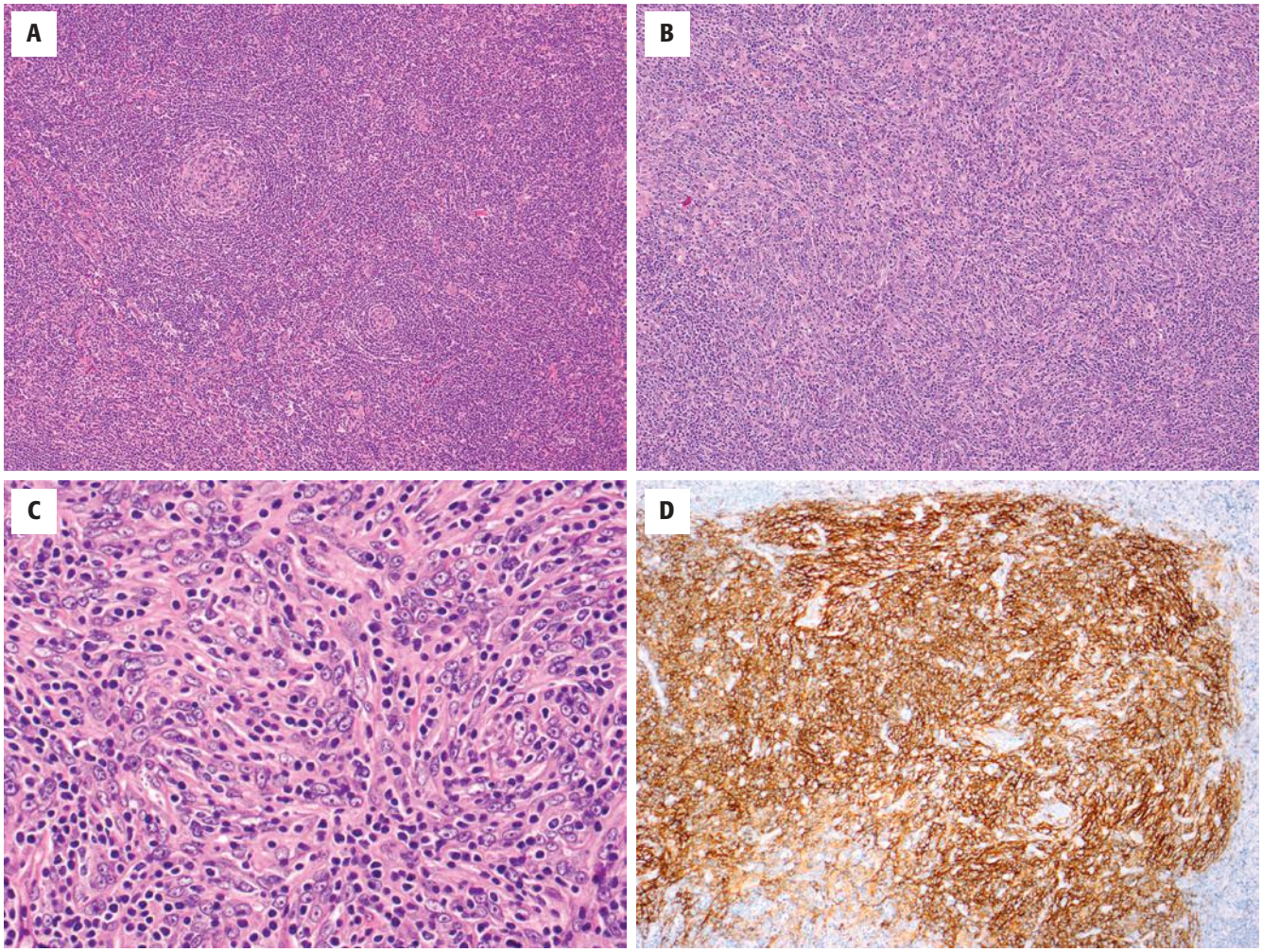


FIG. 4.21

Follicular dendritic cell sarcoma, probably arising in association with hyaline-vascular Castleman disease. A, The enlarged lymph node shows areas with changes consistent with hyaline-vascular Castleman disease; two hyaline-vascular follicles are seen here. B, In other areas there is a prominent, disorderly proliferation of spindle cells with many admixed small lymphocytes. C, High power shows spindle cells with oval to slightly elongate nuclei, vesicular chromatin, small nucleoli, and indistinct cytoplasmic borders. D, Spindle cells are intensely positive for CD21, consistent with follicular dendritic cells.

positive, MECA-79 positive, consistent with high endothelial venules. The interfollicular area also contains CD68+, CD123+ plasmacytoid dendritic cells and S-100-positive interdigitating dendritic cells. Follicular dendritic cells have aberrant expression of adhesion molecules and may show cytogenetic abnormalities. Increased numbers of TdT+ T cells may be found, but in the absence of destructive growth, cytologic atypia, and clonally rearranged T-cell receptor genes, they do not indicate a diagnosis of T-lymphoblastic lymphoma. Human androgen receptor (HUMARA) assay in female patients shows a monoclonal pattern in the majority of cases, thought to be originating from follicular dendritic cells or stromal cells. HVCD is thus best considered a benign neoplasm, most likely of follicular dendritic cell origin, with abundant reactive lymphoid cells.

PLASMA CELL VARIANT

Clinical Features

When plasma cell Castleman disease (PCCD) is localized, it affects a single anatomic site, usually mediastinal or intraabdominal lymph nodes. Patients of any age can be affected, but most are young adults. They may be asymptomatic, but systemic symptoms, including fever, weight loss, and fatigue, are common, and many have anemia, elevated erythrocyte sedimentation rate, and hypergammaglobulinemia. Following excision of the mass, the symptoms and laboratory abnormalities disappear, and patients do well. Clinical features of multicentric PCCD are described above, under Multicentric Castleman Disease.

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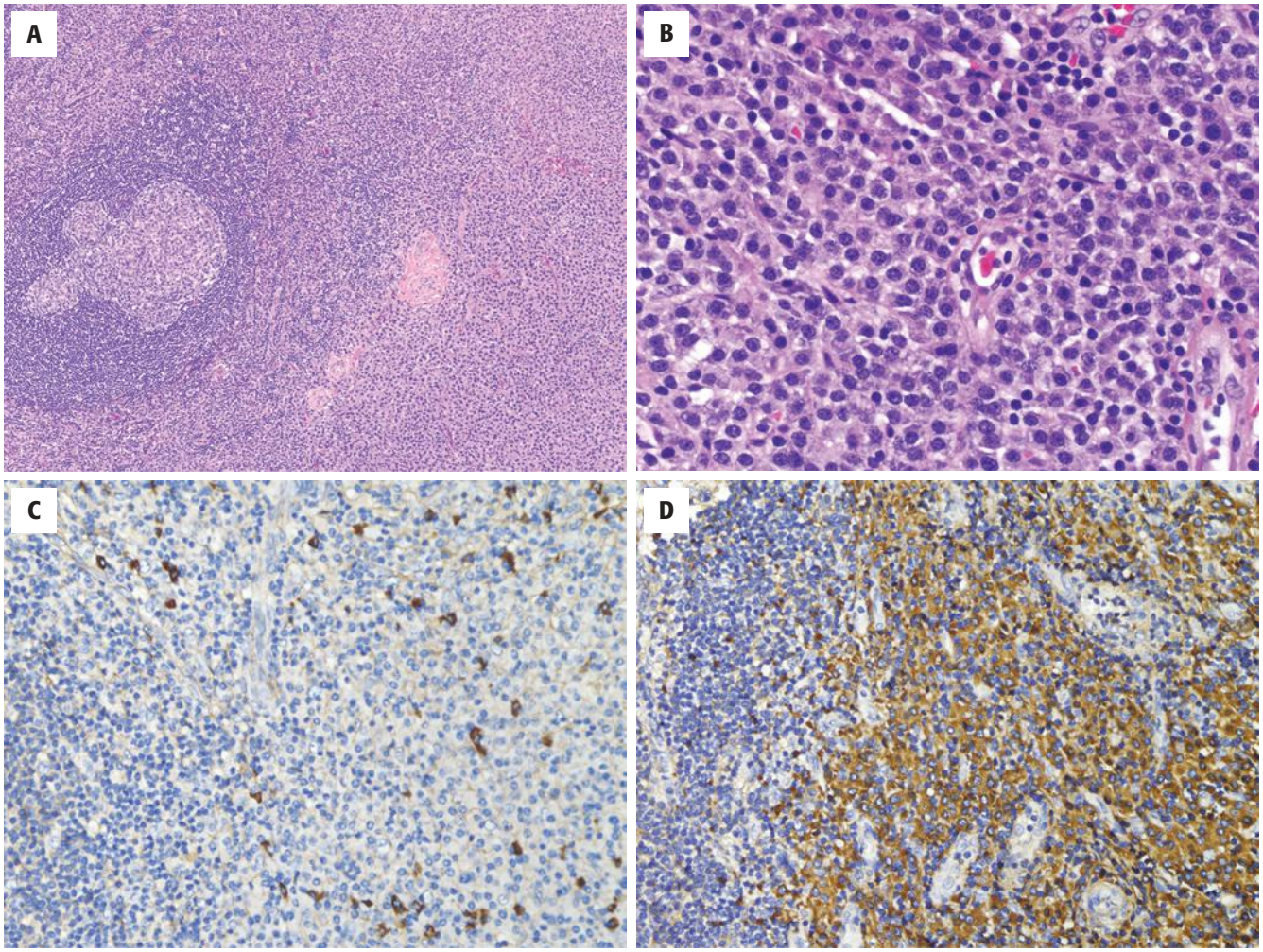


FIG. 4.22

Plasma cell Castleman disease. A, The lymph node shows a reactive follicle and sheets of interfollicular plasma cells. B, Higher power of the interfollicular area shows a monotonous population of plasma cells. C and D, In this case, plasma cells express monotypic immunoglobulin (Ig), IgGλ type (*C,* κ; *D,* λ).

Pathologic Features

Pathologic changes are similar in localized and multicentric PCCD. The mass lesion is composed of one enlarged lymph node or an aggregate of enlarged nodes, measuring about 3 to 11 cm, with individual nodes measuring 2.5 to 7 cm. There are sheets of mature plasma cells in the interfollicular area, sometimes with Russell bodies; occasional immunoblasts may be seen. The follicles may be hyperplastic follicles of the usual type or hyaline-vascular follicles or a mixture of the two types of follicles. Blood vessels are less conspicuous than in HVCD. Sinuses are usually obliterated but may be patent focally. In some cases (which may represent an early stage of disease), immunoblasts and high endothelial venules, in addition to plasma cells, are present in large numbers in the interfollicular region. Plasma cells in a subset of cases express monotypic immunoglobulin, which is nearly always IgGλ or IgAλ (Fig. 4.22). In the remainder of cases, plasma cells express polytypic Ig. Some patients with monotypic plasma cells have POEMS syndrome (see below and also Table 4.3).

MIXED PATTERN CASTLEMAN DISEASE

Criteria for this type of Castleman disease are somewhat ill defined. Typically, there are some hyaline-vascular follicles and many interfollicular plasma cells, although they may not form sheets as in PCCD. There is often prominent interfollicular vascularity.

POEMS SYNDROME

POEMS syndrome is a rare systemic disease with paraneoplastic manifestations. All patients have peripheral neuropathy

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and a monoclonal plasma cell neoplasm, with a variable number of other manifestations of POEMS syndrome and commonly sclerotic bone lesions (osteosclerotic myeloma). The peak onset is in the fifth to sixth decade of life; these patients are younger overall than in other cases of myeloma. Patients with POEMS syndrome have a chronic, sometimes slowly progressive course, with a median survival of about 14 years.

Plasmacytoma and plasma cell myeloma in the setting of POEMS syndrome is typically characterized by less than 5% plasma cells, bony sclerosis, and lambda+ plasma cells. Anemia, hypercalcemia, and renal insufficiency are uncommon compared to myeloma occurring without associated POEMS syndrome. The plasma cells almost always express lambda light chain (usually IgGλ or IgAλ). The light chain expressed is typically restricted to the Vλ1 subfamily with a pattern of somatic hypermutation suggesting antigen-driven selection. The plasma cells express vascular endothelial growth factor (VEGF). Serum VEGF levels may be elevated. The increased levels of VEGF and the specific λ light chain usage may contribute to the pathogenesis of POEMS. VEGF may promote organomegaly, edema, skin hemangiomas, and bony sclerosis due to osteoblastic activation. Elevated levels of VEGF could increase vascular permeability, leading to endoneural edema and then to nerve damage.

The neuropathy is typically demyelinating. The organomegaly consists of hepatosplenomegaly or lymphadenopathy. Lymphadenopathy in POEMS syndrome often has the appearance of Castleman disease or, if not diagnostic, has at least some histologic features of Castleman disease. Not all patients with POEMS syndrome have documented Castleman disease. Other manifestations include papilledema and evidence of extravascular volume overload. The endocrinopathy in POEMS syndrome commonly consists of gonadal or thyroid dysfunction, abnormal glucose metabolism, or adrenal insufficiency. Although in most cases there is no evidence of a viral infection, POEMS syndrome has been reported rarely in association with HHV8+ Castleman disease.

HHV8-ASSOCIATED MULTICENTRIC CASTLEMAN DISEASE

Some years ago, the finding of Kaposi sarcoma–associated herpesvirus (KSHV)-like DNA sequences was reported in 100% of cases classified as multicentric Castleman disease in HIV+ patients and in some cases of multicentric Castleman disease in HIV-negative patients. Subsequently, cases of multicentric Castleman disease in HIV+ patients were described in which immunoblasts or plasmablasts in mantle zones were positive for KSHV (also known as human herpesvirus 8, HHV8) and expressed monotypic IgMλ. Interfollicular plasma cells were polytypic. This entity has been named HHV8-associated multicentric Castleman disease (Fig. 4.23). Additional studies showed that the plasmablasts could also proliferate to form solid aggregates (microlymphoma) or to form sheets of plasmablasts (referred to as frank lymphoma, HHV8+ plasmablastic Castleman disease, or large B-cell lymphoma arising in HHV8+ multicentric Castleman disease). The HHV8+ plasmablasts are CD20+ or -, CD138-, monotypic IgMλ+ (as noted above) and typically negative for Epstein-Barr virus (EBV), although in rare cases the HHV8+ cells are co-infected by EBV. Surprisingly, the monotypic plasmablasts in HHV8-associated Castleman disease and in the microlymphomas are usually polyclonal by molecular techniques, although the frank lymphomas are usually clonal. The HHV8+ plasmablastic proliferations arising on the background of HHV8-associated Castleman disease must be distinguished from plasmablastic lymphoma of the oral cavity type, which is usually positive for EBV but is, by definition, negative for HHV8.

Differential Diagnosis

Castleman disease is a diagnosis of exclusion. A wide variety of specific diseases may be associated with morphologic features resembling those of Castleman disease, and these should be ruled out before making a diagnosis of Castleman disease.

HYALINE-VASCULAR CASTLEMAN DISEASE

Mantle Cell Lymphoma

Because hyaline-vascular Castleman disease is characterized by prominent mantle zones, mantle cell lymphoma with a mantle zone pattern can be considered in the differential of hyaline-vascular Castleman disease. Residual follicle centers in mantle cell lymphoma do not show hyaline-vascular features, and the interfollicular region lacks the distinctive appearance seen in hyaline-vascular Castleman disease. Immunophenotyping is definitive in difficult cases.

Follicular Lymphoma

Some cases of follicular lymphoma have sclerotic neoplastic follicles. When there is early nodal involvement in such cases, that is, the architecture is not effaced, the appearance can resemble hyaline-vascular Castleman disease. In follicular lymphoma, mantle zones should not be prominent, and the interfollicular area should not be hypervascular. Immunophenotyping is definitive in difficult cases.

Thymoma

In the first reports of Castleman disease, the resemblance of hyaline-vascular follicles to Hassall's corpuscles was noted. However, hyaline-vascular Castleman disease and thymoma do not otherwise resemble each other; Castleman disease arises in lymph nodes rather than the thymus, and patients with thymomas are usually older than those with hyaline-vascular Castleman disease.

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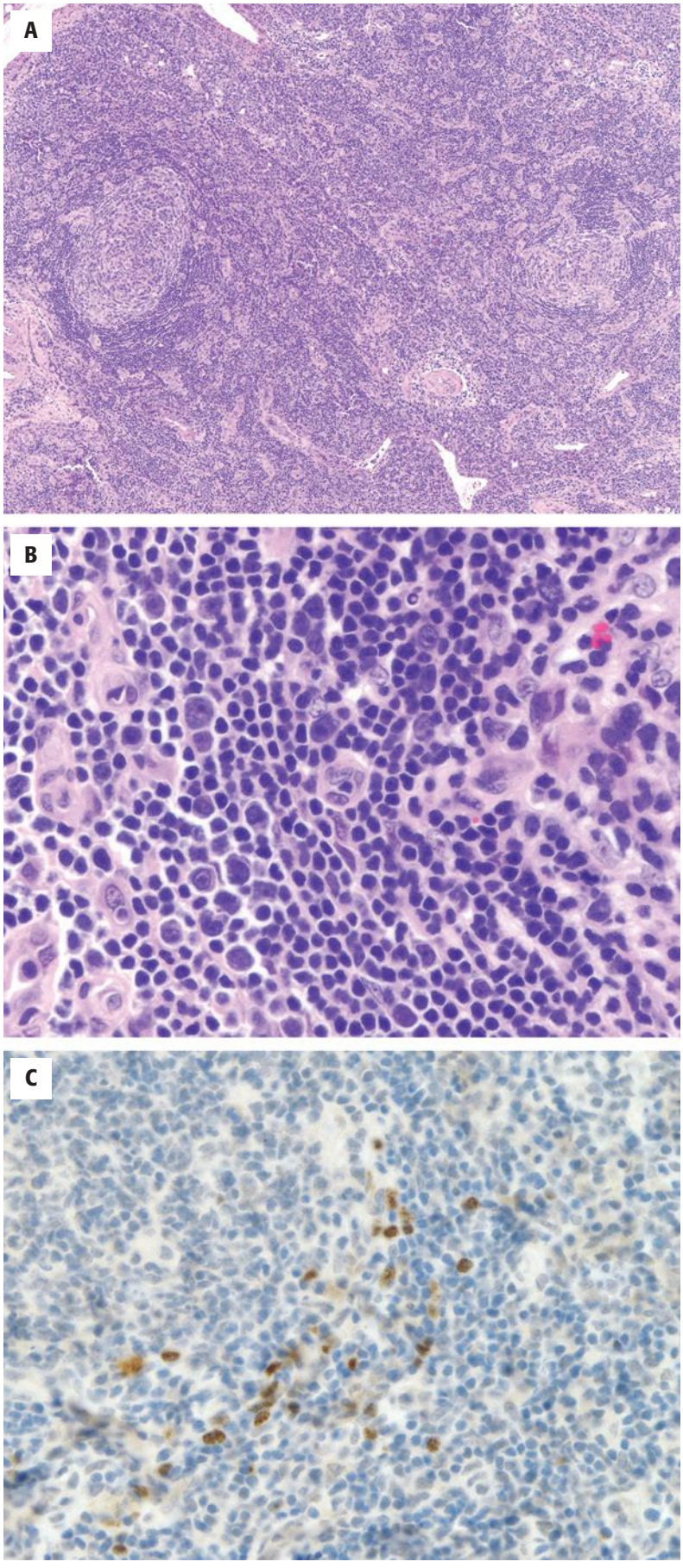


FIG. 4.23

HHV8-associated multicentric Castleman disease in a human immunodeficiency virus–positive patient. A, Low power shows a lymph node with a few hyaline-vascular follicles and an interfollicular area with increased numbers of blood vessels. B, High power of a portion of a follicle shows a mantle zone composed of small lymphocytes with scattered large lymphoid cells. A small area of follicle center is seen within the mantle, and a few blood vessels are seen in the interfollicular area on the opposite side of the mantle. C, The large lymphoid cells in the mantle (plasmablasts) are HHV-8 positive.

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PLASMA CELL CASTLEMAN DISEASE

Autoimmune Disease

Lymphadenopathy in patients with rheumatoid arthritis shows features that overlap those of plasma cell Castleman disease. Rare patients with systemic lupus erythematosus show lymphadenopathy with features of Castleman disease. Clinical correlation is helpful in establishing a diagnosis.

Plasmacytoma

Nodal plasmacytomas are rare, and a proliferation of lymphoid follicles is not a feature. Polytypic plasma cells exclude plasmacytoma but are often seen in plasma cell Castleman disease. Finding monotypic κ-positive plasma cells is unusual in plasma cell Castleman disease. However, among cases with monotypic λ+ plasma cells, the distinction is to some extent a matter of terminology.

Lymphoplasmacytic Lymphoma

Lymphoplasmacytic lymphoma often partially involves nodes and may produce an interfollicular infiltrate of lymphocytes and plasma cells with sparing of follicles that resembles plasma cell Castleman disease. Demonstration of an interfollicular population of B lymphocytes is evidence against plasma cell Castleman disease. Unlike plasma cell Castleman disease, lymphoplasmacytic lymphoma usually expresses monotypic IgM and does not preferentially express λ light chain.

HYALINE-VASCULAR AND PLASMA CELL CASTLEMAN DISEASE

Hodgkin Lymphoma

Occasional cases of Hodgkin lymphoma are associated with focal changes reminiscent of hyaline-vascular Castleman disease or plasma cell Castleman disease. The Hodgkin lymphoma may be in the interfollicular area of the tissue with the Castleman disease–like changes or may occupy an area adjacent to the Castleman disease–like area.

HIV-Associated Lymphadenopathy

In later stages of HIV infection, follicles may become small and burned out, and interfollicular plasma cells may be abundant, potentially mimicking Castleman disease. Such lymph nodes should not receive a diagnosis of Castleman disease unless there are also HHV8+ cells in follicle mantles, in which case a diagnosis of HHV8+ Castleman disease can be made.

Miscellaneous

One or more features of Castleman disease can be found in lymph nodes showing reactive hyperplasia that is otherwise nonspecific. Such cases should not be interpreted as Castleman disease.


The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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5

Bone Marrow Failure Syndromes

James R. Cook, MD, PhD

INTRODUCTION

Bone marrow failure syndromes, defined as peripheral cytopenias due to decreased hematopoiesis in the bone marrow, may be divided into acquired and inherited conditions and further divided into disorders that alter only one hematopoietic lineage (erythroids, myeloids, or platelets) and those that involve all three lineages. This chapter contains detailed discussion of the two major entities leading to acquired pancytopenia (aplastic anemia and paroxysmal nocturnal hemoglobinuria) as well as Fanconi anemia (FA) and dyskeratosis congenita (DKC), two of the most thoroughly characterized congenital bone marrow failure syndromes. Other acquired and congenital disorders associated with peripheral cytopenias caused by bone marrow failure are also discussed briefly. Each of these disorders is rare and diagnostically challenging. In addition, because the characteristic morphologic findings are generally nonspecific, these entities are often best defined as clinicopathologic syndromes. Establishing the most precise diagnosis requires the correlation of the clinical and morphologic findings as well as special ancillary testing. A close working relationship between the clinicians treating the patient and the pathologist interpreting the morphologic findings is essential for optimal patient care.

ACQUIRED APLASTIC ANEMIA

Aplastic anemia (AA) is broadly defined as peripheral blood pancytopenia secondary to bone marrow hypocellularity (<30% cellular). Acquired AA can be either primary (idiopathic) or secondary.

Clinical Features

In the United States, acquired AA is a rare disorder, with an approximate incidence of two cases per 1 million population per year. Worldwide, there is substantial geographic variability, with the incidence of AA being two to three times higher in Asia than in the United States or Western Europe. Although acquired AA can occur at any age, there are two characteristic peaks in the age of onset: one from 15 to 25 years and a second peak after 60 years of age. Overall, the incidence in males and females is approximately equal. AA can occur secondary to a wide variety of toxic agents, including numerous drugs, viruses, environmental chemicals, and other agents (Box 5.1). In approximately 70% to 80% of cases, AA is idiopathic; therefore, the remainder of this section discusses only idiopathic AA. The pathophysiology of idiopathic AA is complex, but, in general, idiopathic AA appears to be an autoimmune process, with both T-cell– and B-cell–mediated mechanisms implicated in the disease. The presenting clinical features are nonspecific but relate directly to the presence of peripheral blood cytopenia. Patients may exhibit bruising or mucocutaneous bleeding due to thrombocytopenia, fatigue due to anemia, or infections secondary to neutropenia.

Pathologic Features

Peripheral Blood

The peripheral blood usually displays pancytopenia (Fig. 5.1). By definition, at least two of the following criteria are present: hemoglobin less than 10 g/dL, platelet count less than 50×10^9/L, absolute neutrophil count less than 1.5×10^9/L. The residual neutrophils and platelets are morphologically unremarkable, and immature myeloid cells are not detected. The red blood cells are often macrocytic but are normocytic in some cases. There is reticulocytopenia. In occasional cases, there is initially unilineage cytopenia (often thrombocytopenia) with a progression to pancytopenia over time. The degree of the peripheral blood cytopenia has been used to separate patients into prognostically distinct groups. Cases are categorized as severe AA if two of the following are present: neutrophil count less than 0.5×10^9/L, platelet count less than 20×10^9/L, or reticulocyte count less than 20×10^9/L. Cases with an absolute neutrophil count of less than 0.2×10^9/L are categorized as very severe AA. Cases

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ACQUIRED APLASTIC ANEMIA—FACT SHEET

Definition
- Peripheral blood pancytopenia caused by marrow hypoplasia

Incidence
- Rare (annual incidence of two new cases per 1 million population)
- Fewer than 1000 cases per year in the United States

Morbidity and Mortality
- Severe AA is rapidly fatal without treatment
- Non severe cases may have a prolonged, mild clinical course

Gender, Race, and Age Distribution
- No major gender or racial difference in the United States
- Most patients present at 15 to 25 years of age, with second peak in incidence after 60 years of age
- More common in Asia than in the United States and Western Europe

Clinical Features
- AA may be secondary to toxic agents, but most cases are idiopathic and likely autoimmune mediated
- Symptoms reflect peripheral cytopenia: fatigue (anemia), bruising or bleeding (thrombocytopenia), or infections (neutropenia)
- Some patients eventually develop features of paroxysmal nocturnal hemoglobinuria

Prognosis and Therapy
- Prognosis relates to degree of cytopenia
- Bone marrow transplantation can be curative in many cases
- Immunosuppressive treatment is effective but with risk of relapse
- There is a long-term risk of MDS or AML

Box 5.1 Causes of Secondary Aplastic Anemia

Drugs and Toxins
Chloramphenicol
Nonsteroidal antiinflammatory drugs
Antiepileptics
Gold
Chemotherapy agents
Benzene
Insecticides

Infectious Process
Epstein-Barr virus
Hepatitis
Influenza
Parvovirus
Human immunodeficiency virus (HIV)

Others
Radiation therapy
Autoimmune disease

APLASTIC ANEMIA—PATHOLOGIC FEATURES

Microscopic Findings
- Peripheral blood pancytopenia without polychromasia, without myeloid left shift
- Markedly hypocellular aspirate and biopsy specimen, usually less than 10% cellular
- May have islands of residual hematopoiesis
- Dyspoietic changes, if any, limited to erythroid lineage

Immunohistochemistry
- $CD34^+$ blasts absent or rare and scattered

Cytogenetics and Molecular Studies
- Normal karyotype in most cases
- Acquired somatic mutations reported in approximately 25%

Differential Diagnosis
- Hypocellular MDS
- Congenital aplastic syndrome (e.g., FA)
- PNH
- Large granular lymphocyte leukemias

not fulfilling criteria for severe or very severe AA are classified as nonsevere AA.

Bone Marrow

The bone marrow aspirate in AA is hypocellular and, in many cases, is markedly hypocellular. The spicules consist predominantly of fatty tissue. The scant cellularity present is typically composed of lymphocytes, plasma cells, histiocytes, and other stromal elements (Fig. 5.2). Care should be taken not to misinterpret the residual lymphocytes and plasma cells as a lymphoproliferative disorder or plasma cell myeloma. Immunohistochemical stains or flow cytometric studies may be helpful. Some cases will display residual foci of hematopoiesis, but the overall cellularity remains greatly reduced. When residual hematopoietic elements are present, the erythroid elements may show dyspoietic changes. However, granulocytic and megakaryocytic dysplasias are not detected. The bone marrow core biopsy is also hypocellular (<30%; Fig. 5.3). Some cases may display residual islands of hematopoietic tissue, sometimes referred to as *hot pockets*. In most cases, however, the cellularity is markedly decreased, with less than 10% residual cellularity. The residual stromal elements and bone are normal.

Ancillary Studies

Flow Cytometry

Flow cytometric studies will demonstrate a normal phenotype in any residual hematopoietic elements. Such studies

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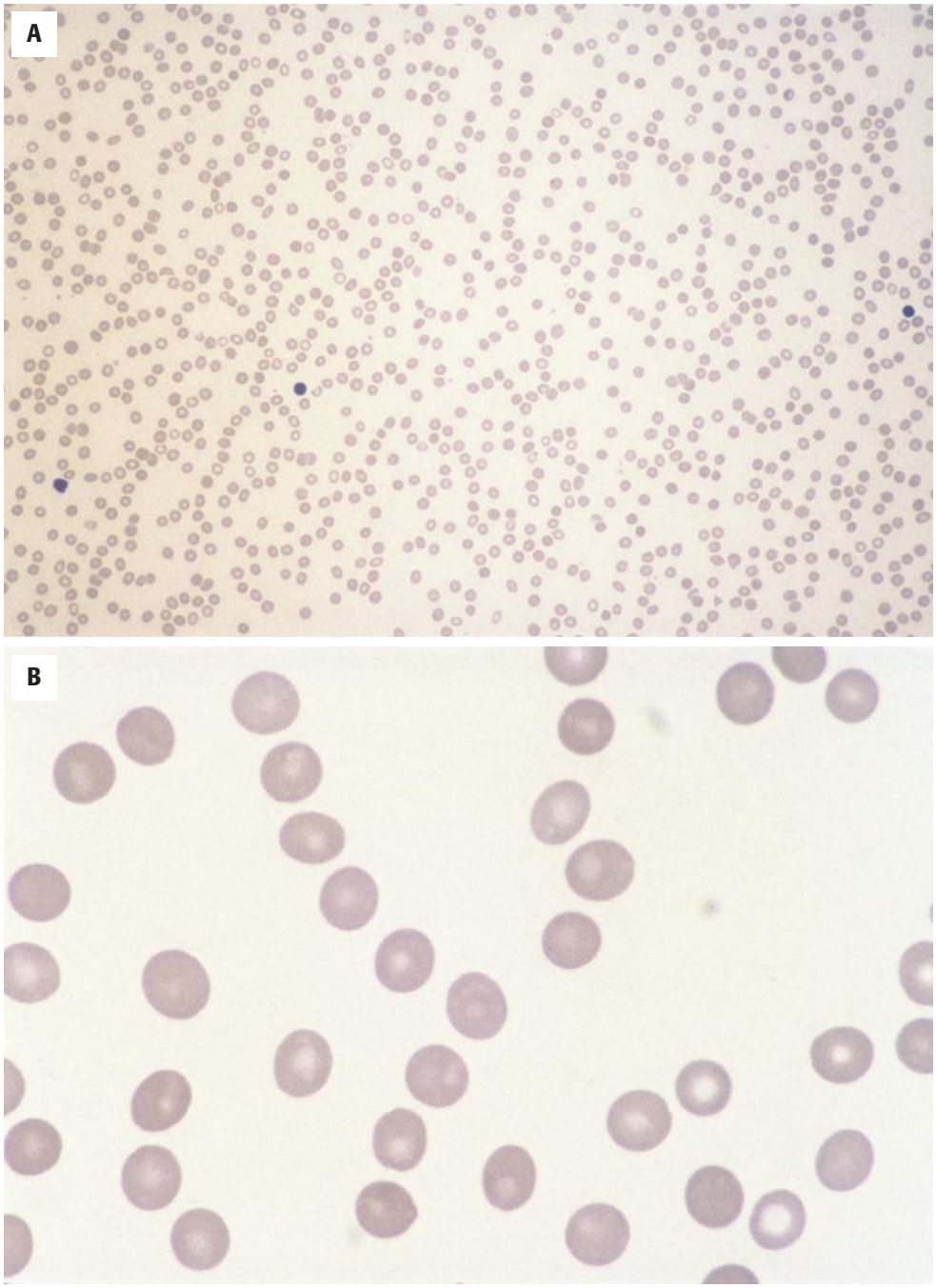


FIG. 5.1

Acquired aplastic anemia: peripheral blood. A, At low power, there is pancytopenia. The few remaining white blood cells consist of unremarkable small lymphocytes. B, At higher power, the red blood cells are macrocytic or normocytic and show no polychromasia.

are useful primarily to exclude the presence of an abnormal myeloid population, such as in a hypocellular myelodysplastic syndrome, or the presence of an underlying lymphoproliferative disorder.

Immunohistochemistry

If present, $CD34^+$ mononuclear cells are rare and scattered. The presence of increased numbers of $CD34^+$ blasts or clusters of $CD34^+$ cells is suggestive of a hypocellular myelodysplastic syndrome.

Cytogenetics

Classic cytogenetic studies are routinely performed on bone marrow aspirate specimens at most institutions. Cases of AA will generally display a normal karyotype. However, up to 12% of AA cases have been reported to show clonal cytogenetic abnormalities, including del(5q), trisomy 6, trisomy 8, and abnormalities of chromosomes 7 and 13. These abnormal clones tend to be present in only a small number of metaphases and are often transient or resolve with immunosuppressive therapy. In such cases,

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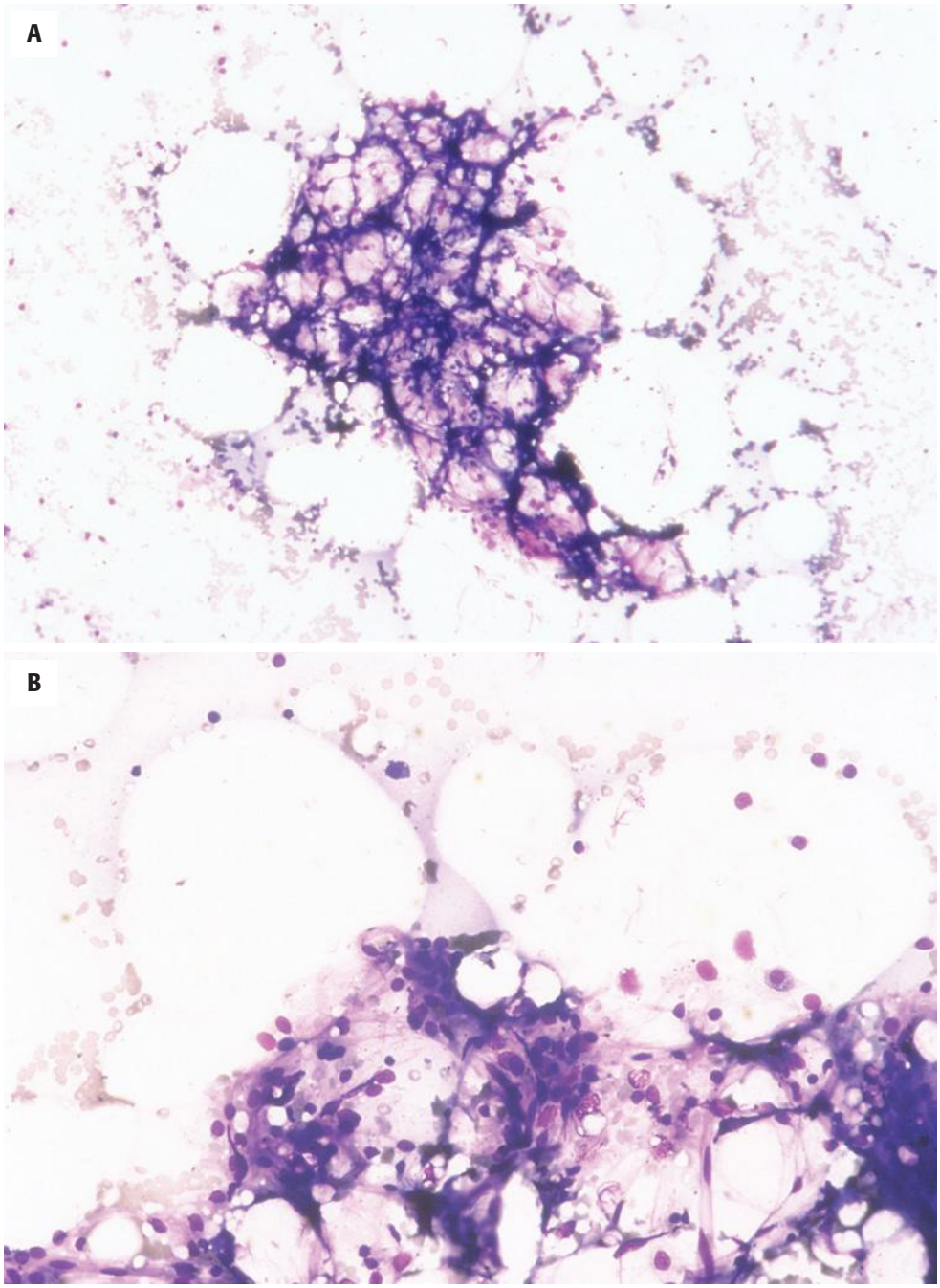


FIG. 5.2

Acquired aplastic anemia: bone marrow aspirate. A, The aspirate smear contains numerous markedly hypocellular spicules. B, At high power, there is little to no residual hematopoietic activity. The scant cellularity present consists of small lymphocytes, plasma cells, histiocytes, and other stromal elements.

results should be interpreted very cautiously, and a definitive myelodysplastic syndrome should be diagnosed only in the presence of accompanying morphologic dysplasia. The identification of monosomy 7 may be particularly associated with progression to a morphologic myelodysplastic syndrome.

Molecular Studies

Next-generation sequencing studies have shown that acquired clonal mutations may be found in approximately 25% of cases of AA. The mutations are often present at a low variant allele fraction (<10%) and may be transient, further suggesting that residual hematopoiesis in AA may represent dynamic oligoclonal populations. The most frequently mutated genes include *PIG-A* (mutated in PNH), *BCOR, BCORL1*, *ASXL1*, and *DNMT3A*. Mutations in *PIG-A*, *BCOR*, and *BCORL1* have been associated with better response to immunosuppressive therapy and favorable prognosis. Mutations in *ASXL1* and *DNMT3A* appear to be less frequent in AA than in myelodysplastic syndrome (MDS), but more frequent than in age-related clonal hematopoiesis, and

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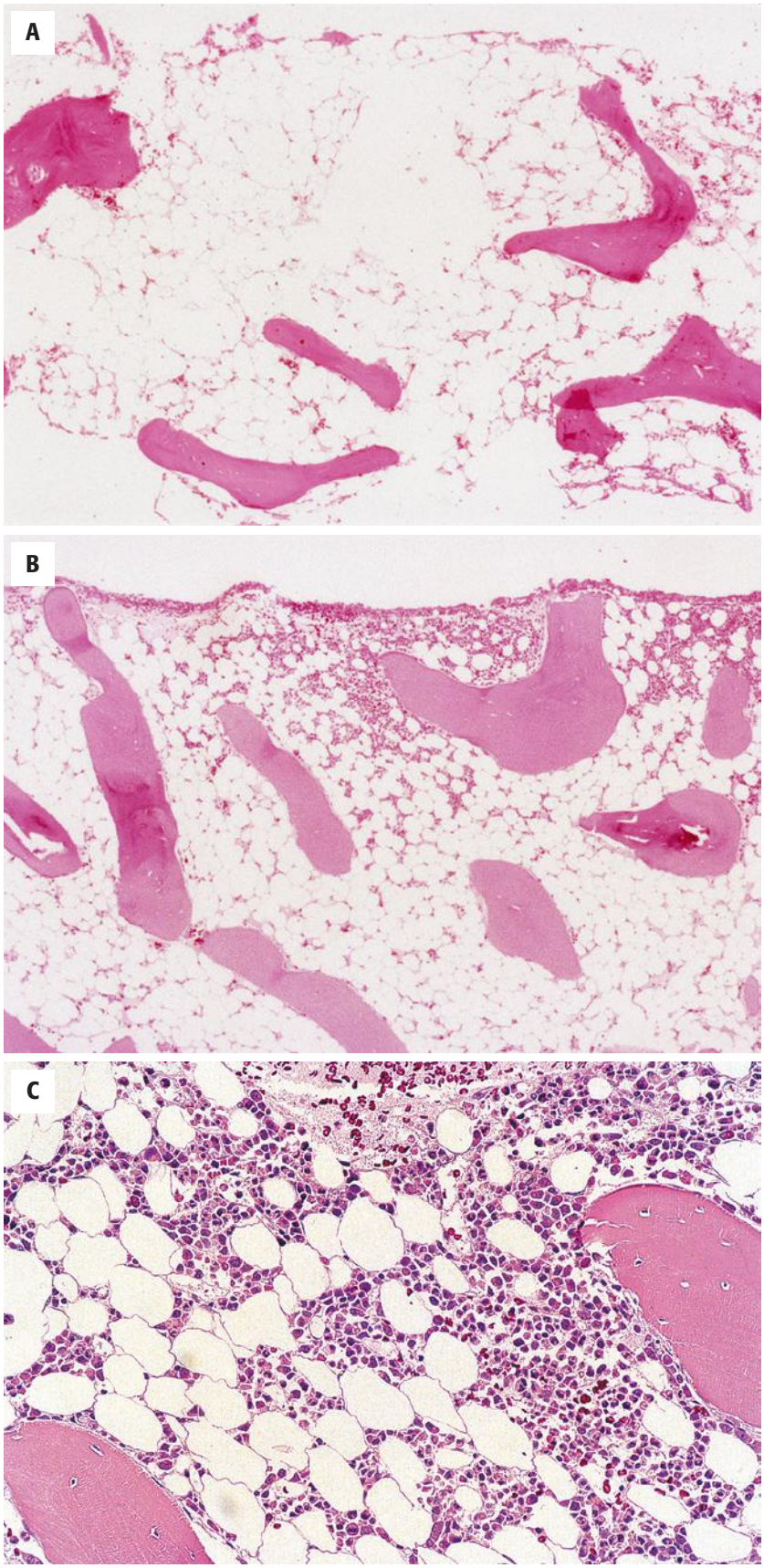


FIG. 5.3

Acquired aplastic anemia: bone marrow biopsy. A, The bone marrow core biopsy is markedly hypocellular with little to no hematopoietic activity. B, In some cases, residual foci of hematopoiesis are seen, known as *hot pockets*. Overall, however, the bone marrow biopsy is markedly hypocellular. C, Higher power view of small, cellular focus. The residual hematopoietic activity is morphologically unremarkable.

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the presence of mutated *ASXL1* and *DNMT3A* has been associated with an increased risk of progression to MDS or acute myeloid leukemia (AML) and an unfavorable prognosis.

Differential Diagnosis

The major differential diagnosis of acquired AA in adults is a hypocellular myelodysplastic syndrome. If significant dyspoietic changes are noted in the residual hematopoietic elements, the possibility of a hypocellular MDS should be considered. The lack of cytogenetic abnormalities and the presence of a normal immunophenotype favor a diagnosis of AA. Although cases of FA and other congenital aplasia syndromes typically present in childhood, some cases can manifest well into adulthood. As will be described later, chromosome breakage studies are essential for the recognition of FA. The presence of skeletal abnormalities, growth retardation, or other congenital abnormalities is also suggestive of a congenital disorder. Some cases of paroxysmal nocturnal hemoglobinuria will display marked hypocellularity. As discussed in the section on paroxysmal nocturnal hemoglobinuria (PNH), there is a fundamental interrelationship between AA and PNH. Flow cytometric analysis for glycosylphosphatidylinositol (GPI)-linked proteins is useful in distinguishing pure AA from PNH-AA overlap syndrome (see PNH). Last, marked hypocellularity may be apparent in some lymphoproliferative disorders, especially the large granular lymphocyte leukemias. Flow cytometric analysis in such cases will document the presence of increased numbers of cytotoxic lymphocytes (usually $CD57^{+}CD3^{+}$ T cells or $CD16/CD56^{+}CD3^{-}$ natural killer cells). Approximately 10% of patients with apparent idiopathic AA have been reported to show abnormally shortened telomeres and/or mutations in the telomerase complex and may represent part of the spectrum of dyskeratosis congenita. Assays for telomere length may also be helpful in evaluating apparent idiopathic AA.

Prognosis and Therapy

The prognosis in AA depends on the degree of the patient's pancytopenia. In cases that are not severe, the clinical course is prolonged, and some patients, especially those with only minimal cytopenia, may have spontaneous recoveries. In severe cases, the untreated disease is generally fatal. Patients may be treated successfully with immunosuppression, in keeping with an autoimmune cause in most cases. Allogeneic bone marrow transplantation is also curative for most patients. Patients who are treated successfully with either immunosuppression or transplantation, however, remain at risk for the development of secondary clonal disorders. Some cases will evolve into PNH, as discussed later in this chapter. Patients are also at long-term risk of MDS and overt acute myeloid leukemia (AML).

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Paroxysmal nocturnal hemoglobinuria is a syndrome of hemolysis, thrombosis, or bone marrow failure that is secondary to an acquired defect in the production of glycosylphosphatidylinositol (GPI)-linked proteins on the cell membranes of red blood cells, platelets, and other hematopoietic cells. GPI anchors are required for the membrane-bound expression of a wide variety of proteins, including important complement regulatory proteins such as CD55 and CD59. In cases of PNH, acquired somatic mutations occur in *PIG-A,* an X-linked gene whose protein product is required for the synthesis of GPI anchors. The subsequent lack of CD55 and CD59 at the cell surface of hematopoietic elements is thought to lead to complement-mediated lysis of red blood cells and complement-mediated activation of platelets. The former leads to the hemolytic complications, and the latter leads to the thrombotic complications seen in PNH. The loss of other GPI-anchored proteins may also contribute to the manifestations of this disease. Recently, a case of PNH due to mutation/deletion in *PIGT*, downstream of *PIGA* in the GPI anchor pathway, has also been reported.

Clinical Features

Paroxysmal nocturnal hemoglobinuria can occur at any age, although the median age at presentation is approximately 40 years. Males and females are equally affected. The clinical findings in PNH are related to the presence of hemolysis, thrombosis, or bone marrow failure. Patients with anemia may exhibit fatigue and lethargy, and jaundice may be present at times of ongoing hemolysis. In classic cases, there may be dark-colored urine secondary to hemoglobinuria, especially in the first morning urine; however, this "classic" presentation is actually uncommon. Venous thrombosis (especially in unusual anatomic sites) occurs in approximately 20% of patients and is an important source of morbidity and mortality in PNH. PNH is generally underrecognized and the diagnosis should be considered in the differential diagnosis of unprovoked thrombosis. In patients with pancytopenia, thrombocytopenia may lead to petechia and bleeding. There is also an intriguing, although incompletely understood, relationship to AA. Approximately one third of patients carry a prior diagnosis of AA before development of symptoms of PNH. In another one third of PNH cases, patients will eventually meet criteria for severe AA. The term *PNH-AA overlap syndrome* has been used to describe these patients. The final third of PNH cases display primarily hemolytic features, without development of other significant cytopenias.

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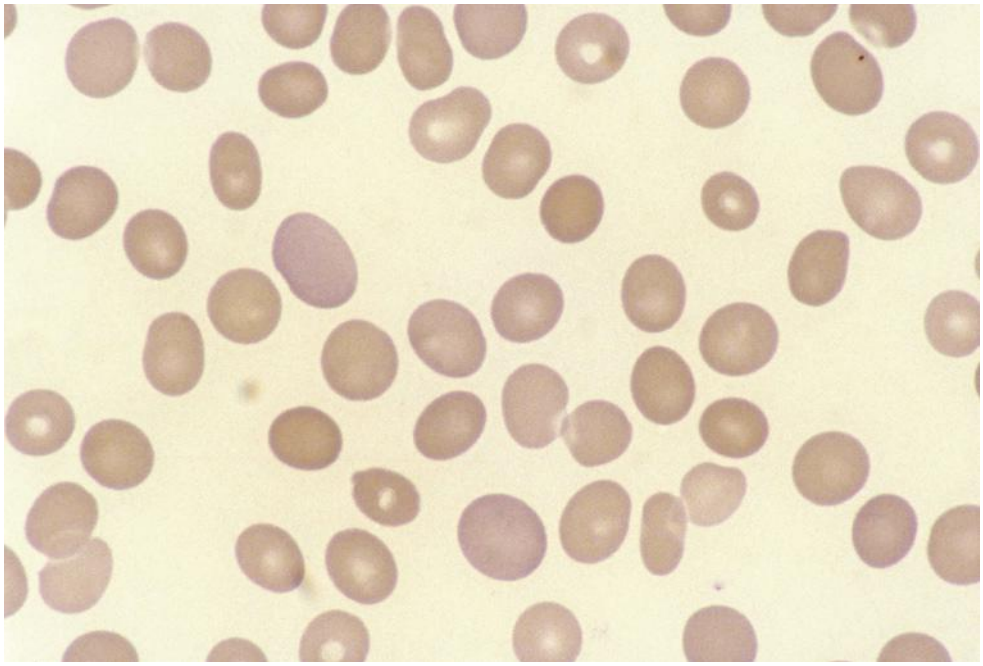


FIG. 5.4

Paroxysmal nocturnal hemoglobinuria: peripheral blood. In this case of paroxysmal nocturnal hemoglobinuria with prominent hemolysis, the red blood cells are macrocytic with polychromasia. In cases with more prominent marrow failure, polychromasia and reticulocytosis may be absent.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA—FACT SHEET

Definition

- An acquired, clonal disorder of hemolytic anemia, thrombosis, or bone marrow failure secondary to *PIG-A* mutation and loss of GPI-linked cell membrane proteins

Incidence

- Rare; estimated at 1 new case per year per 1,000,000 people

Morbidity and Mortality

- Major sources of mortality are thrombosis and bone marrow failure

Gender, Race, and Age Distribution

- Median age at onset: 40 years
- Males and females equally affected
- Many ethnic groups affected

Clinical Features

- Variable clinical presentation
- Anemia secondary to hemolysis, marrow failure, or both
- Increased thrombosis caused by platelet activation
- Risk of bleeding because of thrombocytopenia

Prognosis and Therapy

- Chronic clinical course
- Small risk of transformation to acute leukemia
- Treated by transfusion support, immunosuppression, and complement inhibiting monoclonal antibody (eculizumab)
- Bone marrow transplantation possibly curative

Pathologic Features

Peripheral Blood

The morphologic findings in the peripheral blood are highly variable. In some cases the predominant process is a hemolytic anemia. The red blood cells show polychromasia, and numbers of reticulocytes are increased (Fig. 5.4). In other patients, however, the predominant clinical picture is one of bone marrow failure. In such cases, the anemia is often macrocytic with a characteristic lack of polychromasia and reticulocytosis. Platelets are often mildly to moderately decreased. In patients with the full PNH-AA overlap syndrome, there will be pancytopenia.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA—PATHOLOGIC FEATURES

Microscopic Findings

- Variable peripheral blood and bone marrow morphology
- Hemolytic cases display polychromasia and reticulocytosis with bone marrow erythroid hyperplasia
- Other cases show pancytopenia with marrow aplasia

Ancillary Studies

- Flow cytometry for GPI-linked proteins (e.g., CD55, CD59, FLAER) allows for sensitive diagnosis
- Cytogenetic studies showing normal karyotype

Differential Diagnosis

- Autoimmune hemolytic anemia
- Aplastic anemia
- MDS

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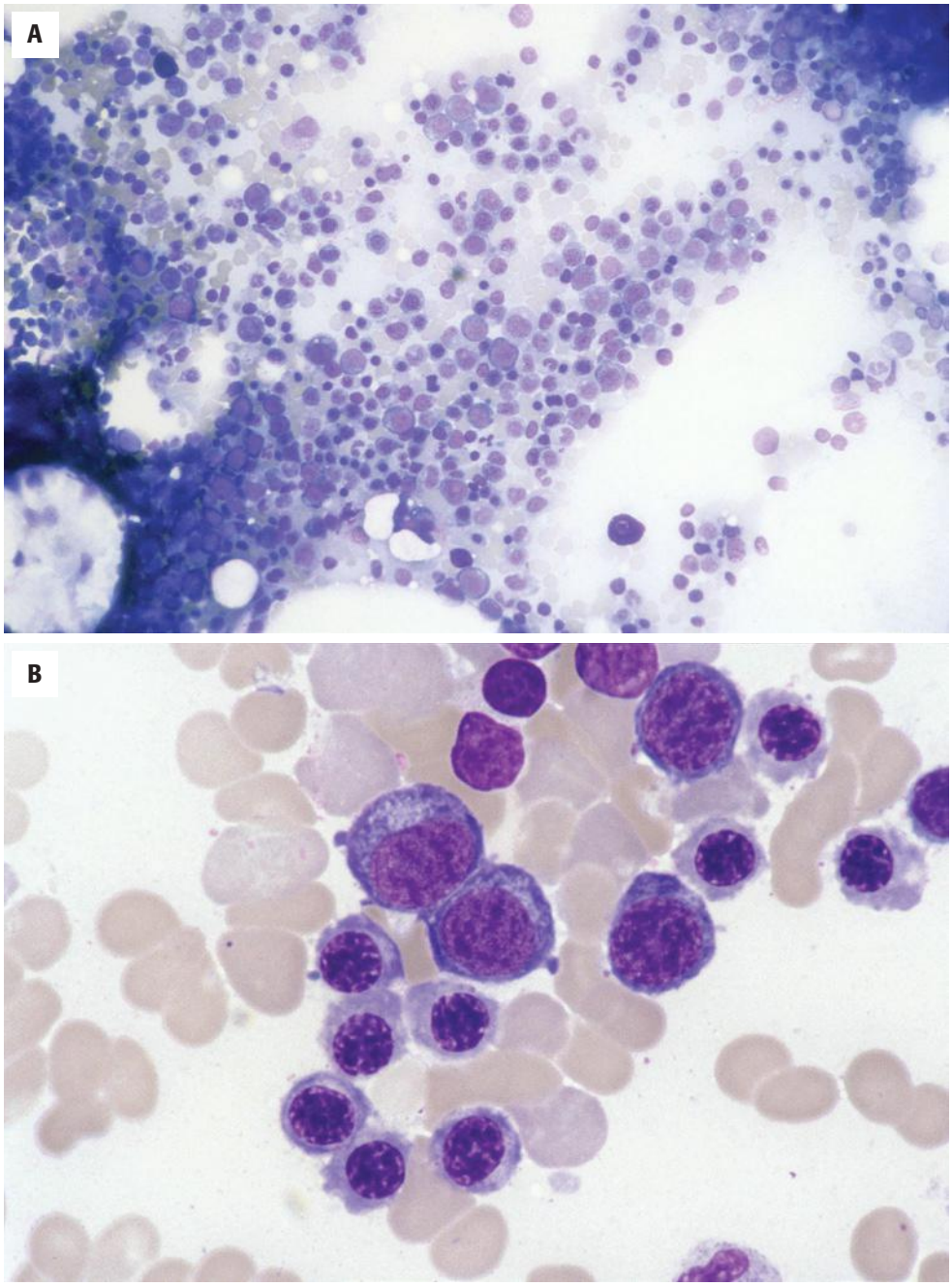


FIG. 5.5

Paroxysmal nocturnal hemoglobinuria: bone marrow aspirate. A, In cases with prominent hemolysis, the bone marrow aspirate is cellular with an erythroid predominance. B, High-power view of erythroid elements with an essentially unremarkable morphology. Dyspoietic changes may be identified in other cases with florid erythroid hyperplasia.

Bone Marrow

As with the peripheral blood, the morphologic findings in the bone marrow may also be highly variable in PNH (Figs. 5.5 and 5.6). In patients with anemia that is mainly secondary to hemolysis, the bone marrow will generally be hypercellular with an erythroid predominance. In cases in which bone marrow failure is the predominant feature, there is hypocellularity that may be severe. Dyspoietic changes can be seen in those cases with florid erythroid hyperplasia; however, dyspoietic changes in the myeloid or megakaryocytic series are generally not seen.

Ancillary Studies

Flow Cytometry

Flow cytometric studies can be used to detect the decreased expression of GPI-anchored proteins on granulocytes and red cells, and consensus guidelines have been established for the diagnosis and monitoring of PNH by this method (see Chapter 23). Commonly used antibodies include those against CD24, CD55, and CD59 as well as fluorescently labeled aerolysin (FLAER), a bacterial toxin that binds to GPI anchors (Fig. 5.7). Flow cytometric studies

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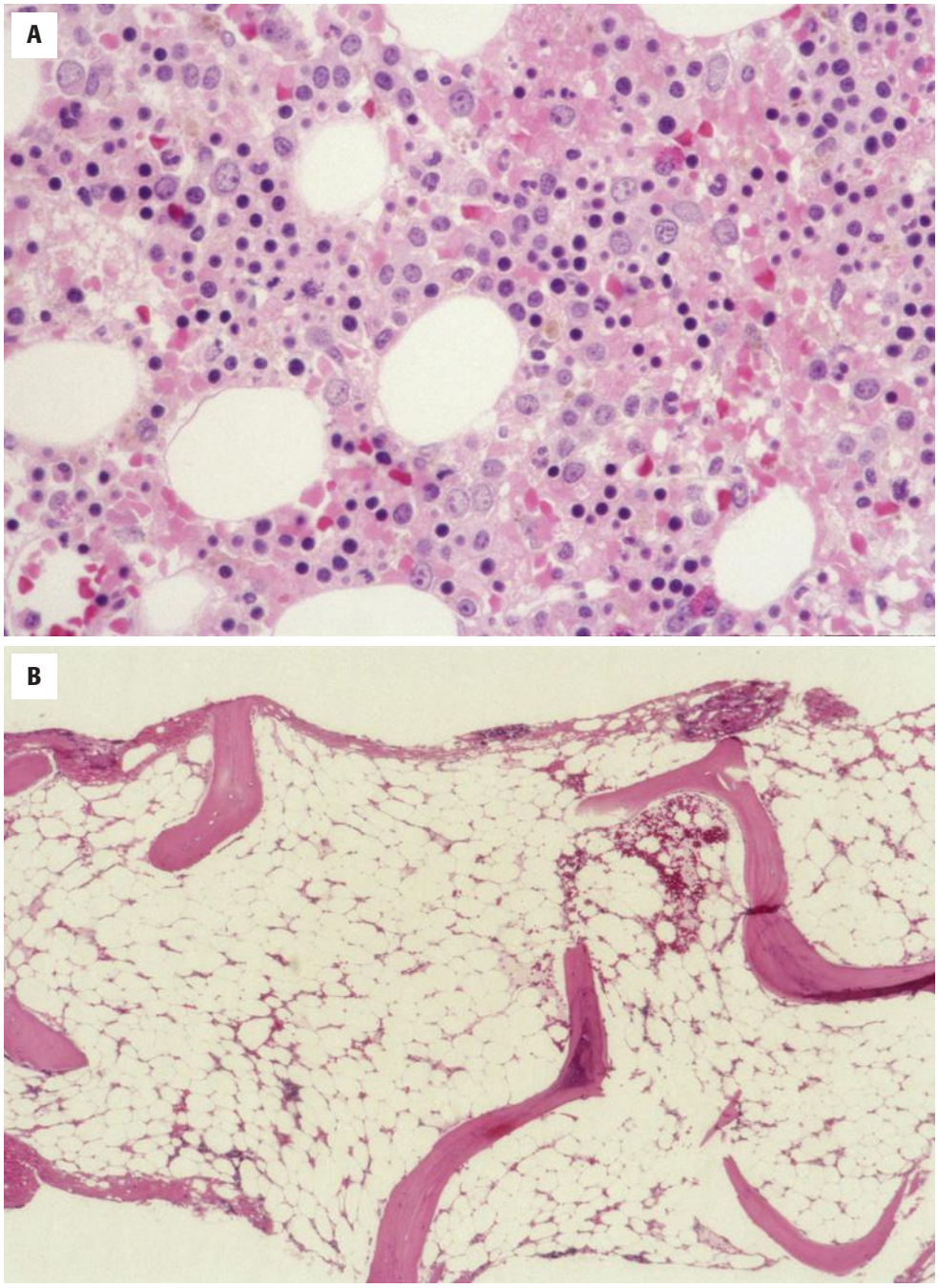


FIG. 5.6

Paroxysmal nocturnal hemoglobinuria: bone marrow biopsy. A, In this case of paroxysmal nocturnal hemoglobinuria with prominent hemolysis, the bone marrow is hypercellular with an erythroid predominance. B, In cases with features of the paroxysmal nocturnal hemoglobinuria–aplastic anemia overlap syndrome, the bone marrow may be markedly hypocellular.

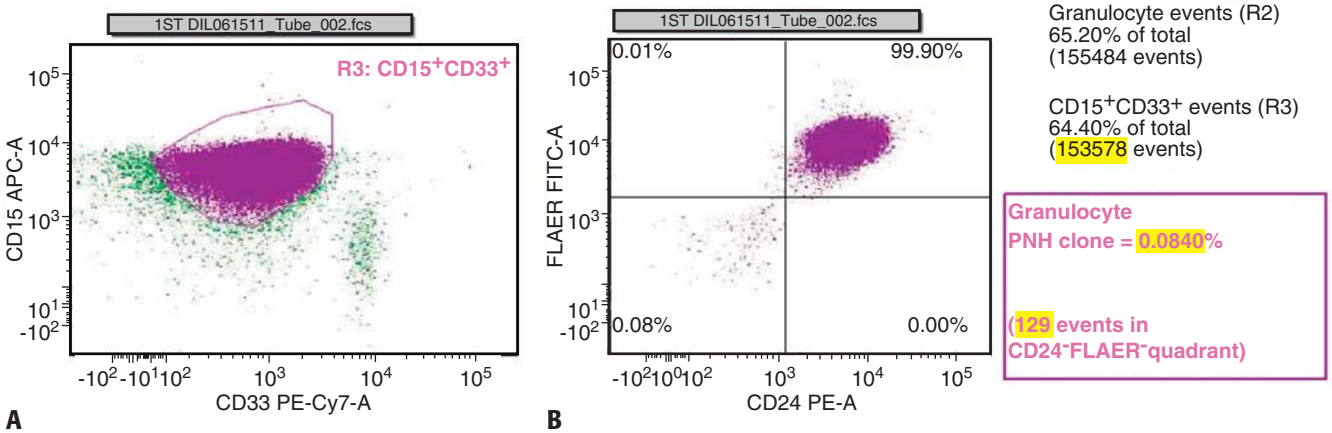


FIG. 5.7

Paroxysmal nocturnal hemoglobinuria (PNH): flow cytometry testing. A, Analysis of red blood cells. Normal red blood cells show coexpression of CD55 and CD59 *(upper right),* whereas PNH-type red cells lack expression of both markers *(lower left).* B, Analysis of granulocytes. Normal granulocytes expressing CD55 and CD59 are present *(upper right)* as well as PNH-type granulocytes lacking both CD55 and CD59 *(lower left).*

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have shown a high sensitivity for PNH and have now replaced older diagnostic assays such as the Ham test or sucrose lysis test. However, it should be noted that cells lacking GPI anchors can also be seen in other settings, especially when the percentage of abnormal cells is small. In particular, small numbers of PNH-type cells can be seen in cases of myelodysplasia or in patients with AA who lack any other features of PNH. The significance of these findings and the relationship of such cases to typical PNH are currently controversial.

Differential Diagnosis

In cases of PNH in which the dominant feature is bone marrow failure, the differential diagnosis includes myelodysplasia and AA. The presence of dyspoietic changes in the myeloid or megakaryocytic series or the finding of clonal cytogenetic abnormalities suggests an MDS. Cases of pure AA lack significant populations of PNH-type cells in flow cytometric studies. A low percentage of PNH-type cells (usually, <1%) can be seen in AA and myelodysplastic syndrome and does not imply a clinical diagnosis of PNH; however, such patients, particularly those with AA, may respond to immunosuppressive therapy. Serial measurements for PNH clone size is warranted because of the potential for subsequent clinical PNH. In cases dominated by the presence of hemolysis, the differential diagnosis includes other forms of intravascular hemolysis. A positive Coombs test result strongly suggests an autoimmune hemolytic anemia rather than PNH. Cases of drug-induced hemolysis or ABO-incompatible transfusion reactions are distinguished by the clinical history and the lack of PNH-type cells by flow cytometry.

Prognosis and Therapy

A median survival of 10 to 15 years following a diagnosis of PNH has been reported. However, with the advent of flow cytometric testing, many cases of PNH are being diagnosed at an earlier stage of disease, and the survival of patients diagnosed with modern flow cytometric studies may therefore be significantly longer. The current treatment approach to PNH employs the monoclonal antibody eculizumab, an anti-C5 complement inhibitor, which has been shown to decrease hemolysis, the need for transfusion, and possibly thrombosis. Management of thrombotyic risk is crucial because thrombotic complications appear to be a major source of mortality in PNH. Bone marrow transplantation may be curative but currently is reserved primarily for cases with severe marrow failure or life-threatening thrombosis and poor response to eculizumab. Finally, there is a relatively low long-term risk of evolution to acute leukemia (<3% of cases overall).

OTHER ACQUIRED BONE MARROW FAILURE SYNDROMES

Although AA and PNH are associated with pancytopenia, other syndromes are characterized by unilineage marrow failure. The features of these unilineage disorders are described briefly below.

PURE RED CELL APLASIA

Pure red cell aplasia (PRCA) is defined as an acquired anemia secondary to absence or near-absence of erythroid precursors in the bone marrow. PRCA may present at any age, but most cases are reported in adults and adolescents. PRCA represents an immune-mediated response against erythroid precursors that may arise as a drug reaction (including in response to exogenous erythropoietin administration) or in association with other autoimmune diseases. In some cases, PRCA may occur in immune dysregulation secondary to thymoma, lymphoma, or other neoplasm.

The peripheral blood displays an anemia without polychromasia. Bone marrow biopsy reveals a near absence of erythroid elements, often with only occasional basophilic erythroblasts (pronormoblast) identified. Granulocytes and megakaryocytes are unremarkable. The differential diagnosis includes congenital red cell aplasia (i.e., Diamond-Blackfan anemia) and parvovirus B19 infection. Red cell aplasia due to parvovirus is more common in patients with preexisting red cell abnormalities, such as sickle cell anemia or hereditary spherocytosis. The characteristic parvovirus inclusions may be morphologically recognized in routine bone marrow aspirate or biopsy preparations, and immunohistochemical stains for parovirus B19 are available. Therapy for PRCA includes immunosuppression and management of any underlying predisposing condition (such as resection of thymoma or treatment of associated lymphoid neoplasm).

AGRANULOCYTOSIS

Agranulocytosis may be defined as marked neutropenia (usually $<0.5 \times 10^9$/L) due to a marked decreased in granulopoiesis in the bone marrow. Agranulocytosis may present at any age but is most common in adulthood. Most cases represent drug reactions, with a very large number of drugs that have been implicated. Drugs may be divided into those agents that regularly and predictably show granulocyte toxicity (such as chemotherapy agents) and those that produce agranulocytosis in a sporadic fashion. Granulocyte toxicity may occur as a direct result of the drug on granulocytic cells, or the drug may induce an antineutrophil immune response.

Bone marrow biopsy shows decreased granulopoiesis, often with occasional remaining blasts and promyelocytes, but lacking more mature elements (so-called maturation

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arrest). The differential diagnosis includes myelodysplastic syndromes. Agranulocytosis may also be seen in the setting of T-cell large granular lymphocyte leukemias, and workup of unexplained neutropenias should include an assessment for abnormal T-cell infiltrates. Management of agranulocytosis includes elimination of the offending drug and support of neutrophil counts through growth factor therapy when required.

ACQUIRED AMEGAKARYOCYTIC THROMBOCYTOPENIA

Acquired amegakaryocytic thrombocytopenia (AAT) is a very rare entity, with only a small number of cases reported in the literature. In contrast to the far more common idiopathic thrombocytopenic purpura (ITP), which typically shows peripheral thrombocytopenia with normal or increased megakaryocytes in the bone marrow, AAT by definition shows markedly decreased to absent megakaryocytes. Some cases have been reported in association with viral infection or drug reaction, whereas others are idiopathic with a presumed autoimmune mechanism. Various immunosuppressive therapies have been employed to successfully treat AAT, providing further support for the argument that many cases may represent an autoimmune disorder.

FANCONI ANEMIA

Fanconi anemia is a multigenic disease, with 19 different genes being identified to date (Table 5.1). All the mutated genes appear to have role in a common pathway of DNA repair. Eighteen of the genes are inherited in an autosomal-recessive fashion, whereas one gene (*FANCB,* 2% of patients) is X-linked. A detailed discussion of the molecular biology of FA is beyond the scope of this chapter, and detailed reviews are listed in the Suggested Reading, available on the Expert Consult website.

Clinical Features

The clinical features are diverse, but approximately 90% of FA cases will display hematologic abnormalities, usually manifest as pancytopenia secondary to bone marrow aplasia. Cytopenia typically will manifest in the first decade of life, although in some cases diagnosis may be delayed until early adulthood. The most common nonhematologic abnormalities include hyperpigmentation and hypopigmentation of the skin, skeletal defects (especially anomalies of the thumbs), renal abnormalities, and growth retardation. Many other types of abnormalities may occur at lower frequency. Patients with FA are also at increased risk of neoplasia, including MDS and AML, as well as squamous cell carcinomas.

TABLE 5.1
Congenital Cytopenia Syndromes

Syndrome	Known Mutated Gene(s)
Anemia	
Diamond-Blackfan anemia	RPS19, RPS17, RPS24, RPL35A, RPL5, RPL11, RPS7, RPS26,RPS10
Congenital dyserythropoietic anemias	CDAN1 (Type 1), SEC23B (Type 2), KIF23 (Type 3)
Pearson syndrome	Various deletions in mtDNA
Cartilage-hair hypoplasia	RMRP
Neutropenia	
Severe congenital neutropenia (Kostmann syndrome)	ELANE, HAX1, G6PC3, *GFI1,* WAS
Cyclic neutropenia	ELANE
Schwachman-Diamond syndrome	SBDS
Myelokathexis (WHIM syndrome)	CXCR4
Reticular dysgenesis	AK2
Thrombocytopenia	
Congenital acquired megakaryocytopenia	MPL
Thrombocytopenia with absent radii	RBM8A
Pancytopenia	
Fanconi anemia	FANCA, FANCB, FANC, FANCD1/BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ/BRIP1, FANCL, FANCM, FANCN/PALB2, FANCO/RAD51C, FANCP/SLX4, FANCQ/ERCC4, FANCR/RAD51, FANCS/BRAC1, FANCT/UBE2T
Dyskeratosis congenita	*DKC1, TERT, TERC, TINF2, RTEL1, NOP10, NHP2, WRAP53, CTC1*

Pathologic Features

Peripheral Blood

Peripheral blood is typically normal at birth, and the first abnormality identified is usually a macrocytosis without anemia. As the child ages, cytopenia will eventually develop. In many cases, thrombocytopenia or leukopenia occurs first, followed by anemia and pancytopenia. The median age at onset of pancytopenia is 7 years. Minimal, if any, morphologic evidence of dysplasia is seen in the peripheral blood.

Bone Marrow

The bone marrow features also vary with the course of disease progression. Early in childhood the results of a bone marrow examination may be morphologically unremarkable. By the time peripheral blood pancytopenia has developed,

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FANCONI ANEMIA—FACT SHEET

Definition
- An inherited bone marrow failure syndrome caused by a defect in DNA repair

Incidence
- Approximately 3 per 1,000,000

Morbidity and Mortality
- Bone marrow failure causes increased risk of infection (neutropenia), bleeding (thrombocytopenia), and symptoms of fatigue (anemia)
- There is an increased risk of malignancy, including both hematologic and solid tumors

Gender, Race, and Age Distribution
- FA occurs equally in males and females and affects many ethnic groups
- Median age at onset of pancytopenia is 7 years, although there is substantial variation

Clinical Features
- Clinical findings are heterogeneous
- Approximately 30% display only hematologic manifestations
- 70% of patients have other abnormalities, including skin pigmentation anomalies, skeletal defects, renal malformations, and growth retardation

Prognosis and Therapy
- There is a long-term risk of MDS and AML
- Bone marrow transplantation can be curative for hematologic disease, but an increased risk of solid tumors remains

FANCONI ANEMIA—PATHOLOGIC FEATURES

Microscopic Findings
- Peripheral blood pancytopenia
- Bone marrow hypocellularity
- Little to no dysplastic changes in residual hematopoiesis

Cytogenetics
- Susceptibility to chromosome breakage identified by diepoxybutane or mitomycin C testing
- Clonal karyotypic abnormalities generally absent

Differential Diagnosis
- Acquired AA
- Other congenital marrow failure syndromes

however, the bone marrow features mirror those seen in patients with acquired AA. The bone marrow aspirate is typically hypocellular with numerous fatty spicules (Fig. 5.8). The residual cellularity often consists primarily of lymphocytes, plasma cells, and stromal cells. The bone marrow core biopsy will display marked hypocellularity. The aspirate and biopsy specimens, however, may each show foci of residual hematopoiesis, such that generous sampling of the bone marrow is needed for complete evaluation. Residual erythroid elements may show some degree of dyspoiesis, but the presence of increased numbers of blasts or dysplastic changes in granulocytes or megakaryocytes is suggestive of the development of an MDS.

Ancillary Studies

Chromosome Breakage Analysis

An increased susceptibility to chromosomal breakage is the defining feature of FA. When FA cells are treated with clastogenic agents, such as mitomycin C or diepoxybutane, the resulting DNA damage is not properly repaired, and breaks in the chromosomes are visible in metaphase spreads (Fig. 5.9). Testing for chromosomal breakage is therefore an essential component in the workup for children and young adults with hypocellular bone marrow.

Metaphase Cytogenetic Studies

In addition to chromosome breakage studies, metaphase cytogenetic analysis is important in the evaluation of suspected FA. Uncomplicated cases of FA are usually associated with a normal karyotype, although spontaneous chromosome breaks, similar to those induced by mitomycin C or diepoxybutane testing, can occur in some patients. Because patients with FA are at high risk of developing myelodysplasia, routine cytogenetic analysis is recommended. The presence of a clonal cytogenetic abnormality suggests development of an MDS. However, transient cytogenetic clones of uncertain clinical significance have been reported in some cases of FA. It is therefore important to carefully consider all the clinical, morphologic, and cytogenetic findings for final evaluation.

Molecular Studies

Using lymphoblast complementation studies, in which lymphoblast cell lines derived from patient cells are hybridized with cell lines known for carrying defects, a complementation group can be defined that is associated with a particular genetic abnormality. Sequencing studies can then be used to confirm a precise mutation or to screen family members for carrier status. Recently, next-generation sequencing panels have become available that contain known FA-associated genes as well as genes associated with other congenital bone marrow failure syndromes that may be in the differential diagnosis.

Differential Diagnosis

The differential diagnosis of FA includes acquired AA. Although the median age of onset of pancytopenia in FA is

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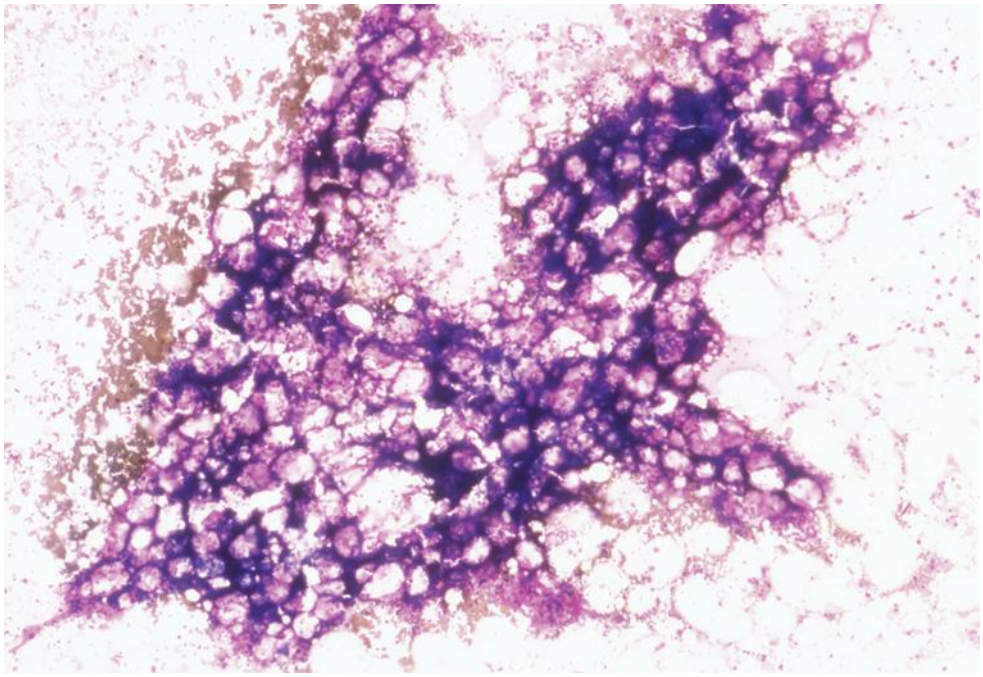


FIG. 5.8

Fanconi anemia: bone marrow aspirate. The aspirate smear contains numerous markedly hypocellular spicules. The morphology is similar to that seen in acquired aplastic anemia. *(Courtesy Steven H. Swerdlow, University of Pittsburgh.)*

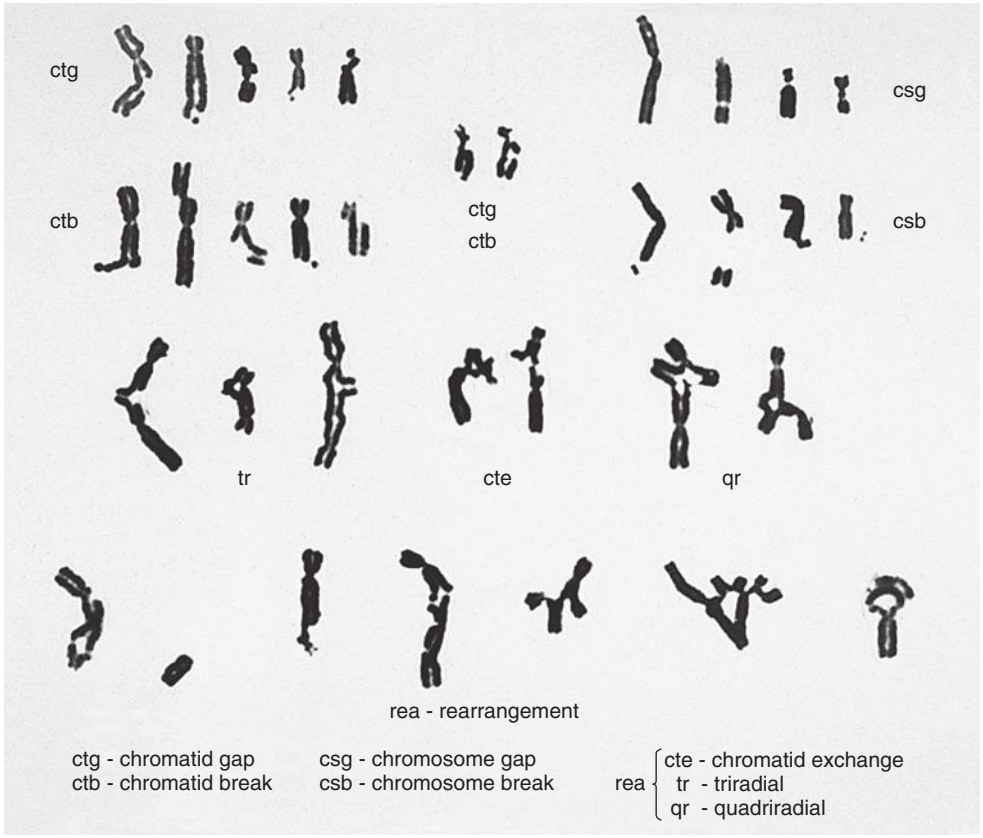


FIG. 5.9

Fanconi anemia: chromosome breakage studies. Karyotype from a case of Fanconi anemia stimulated with clastogenic agents. Numerous chromosomal breaks and other anomalies are present. *(From Wickramasinghe SN, McCullough J:* Blood and bone marrow pathology, *New York, 2003, Churchill Livingstone, p 259.)*

7 years, onset can occur in some patients in early adulthood. Considerable overlap is therefore seen in the age groups of patients with FA and acquired AA. The morphologic findings in FA and AA are also highly similar. In some patients with FA, the correct diagnosis is strongly suggested because of the presence of other congenital abnormalities. In other patients, however, only hematologic abnormalities are present, and chromosome breakage studies are required for definitive diagnosis. Other congenital syndromes associated with bone marrow failure must also be considered, as discussed later. FA can be distinguished from each of these other congenital disorders, however, by chromosomal breakage studies. Establishing a correct diagnosis of FA is extremely important, because conditioning regimens used in preparation for bone

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marrow transplant must be altered since patients with FA are highly sensitive to the DNA-damaging agents that are typically used.

Prognosis and Therapy

Patients may be treated supportively with transfusions and growth factor therapy. However, the only definitive treatment for the hematologic features of FA is bone marrow transplantation. Importantly, successful transplantation does not ameliorate the increased risk of solid tumors in patients with FA. Patients undergoing bone marrow transplantation are also at risk of therapy-related malignancies, which can occur at a higher frequency in FA than in other conditions treated with bone marrow transplantation. An analysis of 20 years of data from the International Fanconi Anemia Registry demonstrated a median survival of 24 years. Approximately 5% to 10% of patients will eventually develop MDS or AML, with an actuarial risk as high as 50% by 40 years of age.

DYSKERATOSIS CONGENITA

DKC is a rare inherited bone marrow failure syndrome caused by genetic defects in telomere maintenance. To date, nine genes have been identified as mutated in DKC that account for approximately 70% of cases. The most frequently mutated gene (≈30% of cases) is the X-linked *DKC1*. Autosomal-dominant forms of the disease are associated with mutations of *TINF2*, *TERC*, *TERT*, and *RTEL1*, and autosomal-recessive forms have been reported with mutations of *NOP10*, *NHP2*, *WRAP53*, *RTEL1*, *TERT*, and *CTC1*.

Clinical Features

DKC was originally defined by the clinical triad of reticulated hyperpigmentation of the skin, dystrophic nails, and oral leukoplakia. It is now recognized that the classic diagnostic triad is not present in many cases, and a broad spectrum of additional congenital abnormalities may also be present. Patients with DKC are at risk for pulmonary fibrosis, liver disease, MDS/AML, and solid tumors, especially squamous cell carcinomas of the head and neck.

Pathologic Features

Peripheral Blood

Peripheral blood is normal at birth. Pancytopenia develops over time, with a reported mean age of 10 years. There are no known characteristic morphologic features in the peripheral blood. The presence of significant dysplastic changes should raise concern for progression to MDS.

DYSKERATOSIS CONGENITA—FACT SHEET

Definition
- Inherited bone marrow failure syndrome due to defects in telomere maintenance

Incidence
- Approximately 1 per 1,000,000

Morbidity and Mortality
- Bone marrow failure
- Pulmonary fibrosis
- Liver disease
- Malignancies including MDS/AML and squamous cell carcinomas

Gender, Race, and Age Distribution
- Median age of 10 years at pancytopenia onset

Clinical Features
- Classic triad of cutaneous hyperpigmentation, dystrophic nails, and mucosal leukoplakia
- Heterogeneous clinical presentation and many patients lack classic triad
- May mimic idiopathic AA

Prognosis and Therapy
- There is a long-term risk of MDS and AML
- Bone marrow transplantation can be curative for hematologic disease, but an increased risk of solid tumors remains

Bone Marrow

Bone marrow findings are nonspecific and mimic both idiopathic aplastic anemia and other congenital bone marrow failure syndromes. Depending on the stage of disease, the marrow may vary from normocellular to markedly hypocellular.

Ancillary Studies

Telomere Assessment

Screening for DKC can be accomplished through assays measuring telomere length. The most widely used technique is a combined flow cytometry/fluorescence in situ hybridization (flow-FISH) assay. The finding of telomeres less than 1% of age-matched controls is highly sensitive and specific for DKC.

Metaphase Cytogenetic Studies

Cases are expected to display a normal karyotype, and the finding of cytogenetic abnormalities raises concern for

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DYSKERATOSIS CONGENITA—PATHOLOGIC FEATURES

Microscopic Findings
- Peripheral blood pancytopenia
- Variable bone marrow cellularity
- Few to no dysplastic changes in residual hematopoiesis

Ancillary Studies
- Telomere length by flow-FISH assay <1% of age-matched controls
- Cytogenetic studies normal
- Chromosomal breakage analysis normal

Differential Diagnosis
- Acquired AA
- FA and other congenital marrow failure syndromes
- MDS

progression to MDS/AML. Unlike in FA, chromosomal breakage studies are normal.

Molecular Studies

The demonstration of mutations in the known DKC-associated genes assists in confirming the diagnosis. With the rapid growth of multigene next-generation sequencing assays, it is becoming possible to simultaneously test for DKC and numerous other congenital bone marrow failure syndromes that may be in the differential diagnosis.

Differential Diagnosis

The differential diagnosis includes FA and other congenital bone marrow syndromes. FA and DKC can be distinguished on the basis of chromosomal breakage analysis and telomere length testing. Multigene sequencing panels, where available, may also be helpful in distinguishing DKC from other mimics. The differential diagnosis also includes idiopathic AA. As some patients with DKC have *de novo* mutations, a family history of disease may not always be present, and some patients have only very subtle or no appreciable nonhematopoietic abnormalities. In such cases, distinguishing DKC from idiopathic AA requires specific DKC testing.

Prognosis and Therapy

Bone marrow failure develops in up to 90% of patients and represents the major source of morbidity and mortality. The incidence of MDS/AML is 40% by age 50. Patients may be responsive to androgen therapy and growth factor support, but bone marrow transplant is the only curative treatment for the hematologic manifestations of DKC.

OTHER CONGENITAL CYTOPENIA SYNDROMES

Briefly summarized in this section are the features of other rare inherited disorders leading to peripheral cytopenia resulting from bone marrow failure (see Table 5.1). The bone marrow morphologic findings in some of these diseases have not been extensively characterized. In several of these disorders, there may be pancytopenia with bone marrow hypocellularity that produces a differential diagnosis including FA, DKC, and idiopathic AA. In many cases, the correct diagnosis may be suggested based on the constellation of congenital abnormalities. Next-generation sequencing panels containing known genes associated with many of these disorders are increasingly available.

DIAMOND-BLACKFAN ANEMIA

Diamond-Blackfan anemia (DBA) is an autosomal-dominant disorder characterized by macrocytic anemia and reticulocytopenia that manifests within the first year of age. Other somatic findings, including short stature, abnormal thumbs, craniofacial abnormalities, and cardiac or urogenital anomalies, are seen in approximately 25% of cases. There is an increased risk for malignancies including MDS, AML, and osteosarcoma. The bone marrow shows a near complete absence of erythroid precursors. The neutrophilic and megakaryocytic series are generally unremarkable. The first gene to be identified in patients with DBA was *RPS19,* a ribosomal protein mutated in approximately 25% of patients. To date, eight additional genes have been identified in DBA, all encoding ribosomal proteins and accounting for approximately 50% of DBA cases in aggregate. These genetic findings have strongly suggested that DBA is due to ribosome gene haploinsufficiency, although it remains unclear why these abnormalities lead to selective loss of the erythroid lineage. DBA may be treated by steroid therapy and transfusion support. Stem cell transplant may correct the anemia, but the increased risk of sarcoma remains.

CONGENITAL DYSERYTHROPOIETIC ANEMIAS

The congenital dyserythropoietic anemias (CDAs) are a heterogeneous group of disorders characterized by anemia manifesting in childhood with bone marrow erythroid hyperplasia and marked dyserythropoiesis. Three main types have been described (types I, II, and III), and other minor forms have also been reported in a small number of patients. In type I or type III CDA, anemia is typically macrocytic, whereas type II CDA manifests with normocytic anemia. The dyserythropoiesis noted on bone marrow biopsy is striking and highly characteristic, with frequent internuclear chromatin bridges noted in type I CDA, prominent binuclearity noted in type II, and numerous multinucleate erythroid precursors

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found in type III CDA. Electron microscopic features specific for each type have also been described. In type II CDA there is abnormal glycosylation of membrane proteins, leading to positivity for the Ham test (acidified serum lysis test). The degree of anemia in CDA is variable, and patients may be supported by transfusions, when needed. Iron chelation is frequently employed to prevent or treat iron overload, which is common. Stem cell transplant is the only definitive therapy for CDA.

PEARSON SYNDROME

Pearson syndrome is a rare congenital disorder of refractory anemia with ringed sideroblasts associated with pancreatic exocrine deficiency and metabolic acidosis. The syndrome is caused by variably sized deletions in mitochondrial DNA. Essentially all cases occur in sporadic fashion, although several cases have been reported in which the patient's mother had another mitochondrial disorder. Patients typically exhibit a macrocytic anemia during the first 6 months of life. Neutropenia and thrombocytopenia may also be present to varying degrees. Bone marrow examination reveals erythroid and myeloid precursors with cytoplasmic vacuoles. There is increased hemosiderin with ring sideroblasts. Bone marrow examination and confirmatory mitochondrial DNA testing establish the diagnosis. Survival is typically poor, with death generally attributed to metabolic acidosis or hematologic complications.

CARTILAGE-HAIR HYPOPLASIA

Cartilage-hair hypoplasia is an autosomal-recessive form of dwarfism associated with metaphyseal dysostosis, hypoplasia of cartilage, and fine, lightly colored hair. Most patients display a macrocytic, reticulocytopenic anemia, which may be accompanied by neutropenia in a subset of patients. Anemia is most severe during early childhood and, in many cases, resolves by adolescence. In vitro assays of the bone marrow demonstrate marked defects in formation of erythroid colonies and granulocyte-macrophage colonies, similar to other bone marrow failure syndromes. Cartilage-hair hypoplasia was initially described in Old Order Amish families and subsequently also found in families of Finnish background. The disorder is caused by mutations in the RNA component of the mitochondrial RNA-processing endoribonuclease (*RMRP* gene). There appears to be an increased risk of malignancy, including both hematologic and nonhematologic neoplasms.

SEVERE CONGENITAL NEUTROPENIA (KOSTMANN SYNDROME)

Patients with severe congenital neutropenia (SCN), or Kostmann syndrome, develop recurrent infections secondary to absolute neutropenia in infancy. The peripheral red blood cells and platelets are generally normal. Bone marrow examination reveals a characteristic maturation arrest of the granulocytic series at the promyelocyte-myelocyte stage. Genetic studies have shown that SCN is molecularly heterogeneous. Approximately 50% of patients show an autosomal-dominant inheritance with mutations in the *ELANE* gene, which encodes neutrophil elastase, a serine protease found in neutrophil primary granules. The same gene is mutated in cyclic neutropenia (see the following section), and these conditions must be distinguished by serial measurements of the absolute neutrophil counts. An autosomal-recessive form of the disease is associated with a mutation in *HAX1*, a mitochondrial protein. Rare cases have been associated with mutations in *G6PC3*, *GFI1,* and *WAS* genes, whereas approximately one third of cases have yet to be characterized at the molecular level. Growth factor therapy is effective in raising neutrophil counts and preventing infection in most patients. Patients with SCN have an increased risk of progression to MDS and AML, with an incidence of 36% and 25%, respectively, after 15 years of growth factor therapy.

CYCLIC NEUTROPENIA

Cyclic neutropenia is an uncommon hereditary disorder that, like SCN, manifests in infancy with an isolated neutropenia. The two conditions are distinguished by the fact that in cyclic neutropenia the peripheral blood absolute neutrophil counts oscillate on an approximately 21-day cycle from within the normal range to markedly decreased. Serial measurements of the absolute neutrophil count two to three times per week for 6 to 8 weeks should demonstrate the typical periodicity. Bone marrow findings may vary with the course of the peripheral blood neutrophil counts, from a virtual absence of granulocytic cells beyond the promyelocyte stage at the nadir to granulocytic hyperplasia with complete maturation during the recovery phase. Cyclic neutropenia is caused by mutations in the *ELANE* gene, which is also mutated in severe congenital neutropenia. Mutated *ELANE* is thought to give rise to a defective protein that leads to shortened neutrophil lifespan, producing the periodic neutropenia. Growth factor therapy reduces periods of neutropenia and the risk of infection. Symptoms are typically less severe in adults than in children, and in contrast to SCN, there does not appear to be a significantly increased risk of progression to MDS and AML.

SHWACHMAN-DIAMOND SYNDROME

Shwachman-Diamond syndrome is a rare autosomal-recessive disorder characterized by pancreatic exocrine insufficiency and neutropenia. Other congenital abnormalities are also often present. Many patients develop anemia or thrombocytopenia, and pancytopenia is present in a smaller subset. Neutropenia is typically present at birth or develops in early infancy. When

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pancytopenia develops, the average age is approximately 3 years. Bone marrow examination typically reveals a hypoplastic marrow. In more than 90% of patients, Shwachman-Diamond syndrome is caused by biallelic mutations in the *SBDS* gene, located on chromosome 7. The results of chromosome breakage studies are negative. The diagnosis can be confirmed by DNA analysis of the *SBDS* gene. Treatment consists of pancreatic enzyme replacement and growth factor therapy. There is an increased risk of development of myelodysplasia and AML. Stem cell transplantation is considered for patients with severe disease or development of malignancy.

MYELOKATHEXIS (WHIM SYNDROME)

In myelokathexis, there is peripheral neutropenia because of prolonged retention of maturing granulocytes within the bone marrow. Bone marrow examination reveals granulocytic hyperplasia with numerous myeloid cells that display hypermature chromatin, frequently with irregular nuclear folding such as bilobed or bizarre forms. Myelokathexis usually manifests in infancy as part of the warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome. WHIM syndrome is caused by mutations in the *CXCR4* gene, which encodes a chemokine that regulates neutrophil migration. Myelokathexis is also described in Chapter 3.

RETICULAR DYSGENESIS

Reticular dysgenesis is a rare, severe immunodeficiency syndrome of congenital agranulocytosis and lymphopenia (severe combined immunodeficiency, or SCID). Both cytotoxic and humoral immune responses are absent, and there is little if any development of lymph nodes and thymus. Most cases also show anemia, and some patients will also have thrombocytopenia. Bone marrow examination shows a hypocellular marrow with a virtual absence of myeloid and lymphoid precursors. The presence of abnormalities involving both myeloid and lymphoid lineages suggests an abnormality of an early hematopoietic stem cell. Mutations in the mitochondrial gene *AK2* have been identified as the cause of reticular dysgenesis in most patients. Fatal infection typically occurs in infancy. Bone marrow transplantation has been curative in some patients.

CONGENITAL AMEGAKARYOCYTIC THROMBOCYTOPENIA

Congenital amegakaryocytic thrombocytopenia is a rare disorder of marked thrombocytopenia and absent megakaryocytes. There are no other associated congenital abnormalities. Patients typically develop thrombocytopenia during infancy or early childhood, and bone marrow examination at this stage discloses normocellular bone marrow with absent or markedly decreased megakaryocytes. Approximately half of patients will develop pancytopenia with a mean age at onset of pancytopenia of 3 years. Bone marrow examination at this stage displays a hypocellular marrow similar to that seen in idiopathic AA. Most cases appear to be caused by homozygous or compound heterozygous mutations in the receptor for thrombopoietin, *MPL*. There is an increased risk of MDS and AML. Without bone marrow transplantation, which can be curative, the average survival is reported to be 9 years.

THROMBOCYTOPENIA WITH ABSENT RADII

Thrombocytopenia with absent radii (TAR) manifests neonatally with thrombocytopenia, bilateral absent radii, and other heterogeneous abnormalities. The condition can be distinguished from FA, which can also manifest with radial defects, by chromosome breakage studies. Bone marrow examination in TAR displays absent or decreased and abnormally small megakaryocytes. Most cases display an autosomal-recessive pattern of inheritance. Biallelic mutations in the *RBM8A* gene have been described in TAR, usually consisting of one allele with a deletion on chromosome 1q21.1, including the *RBM8A* locus and one allele containing a mutation in the noncoding region of *RBM8A* that leads to decreased protein function. Clinical management includes platelet transfusions. Cow's milk allergy is common in patients with TAR, and exposure to cow's milk can exacerbate thrombocytopenia. Thrombocytopenia typically improves over time, with platelet counts usually normalizing by adulthood.

FAMILIAL APLASTIC ANEMIA

A number of kindreds have been described with familial bone marrow failure syndromes that do not clearly correspond to one of those described previously. Cases have been reported with autosomal-dominant, autosomal-recessive, and X-linked inheritance patterns, with and without other associated congenital abnormalities. These cases are distinguished from FA by the absence of chromosomal breakage in clastogen-stimulated analysis. Bone marrow examination in such cases is reported to show variable degrees of bone marrow hypoplasia. Proper diagnosis and classification of such cases are difficult, and without knowledge of the familial inheritance pattern, many cases would likely be classified as idiopathic AA.

NONHEMATOLOGIC SYNDROMES

Hematologic complications, including AA, have been reported in some patients with a variety of other syndromes that are conventionally considered nonhematologic, including Down syndrome, Dubowitz syndrome, Seckel syndrome, and Noonan syndrome. Correlation of the hematologic findings with the other clinical and constitutional cytogenetic findings is required for proper diagnosis.

The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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6

Benign Causes of Bone Marrow Abnormalities Including Infections, Storage Diseases, Systemic Disorders, and Stromal Changes

■ Dennis P. O'Malley, MD ■ Lauren Smith, MD ■ Yuri Fedoriw, MD

■ INTRODUCTION

A wide variety of benign conditions can be encountered in the bone marrow, and their clinical and morphologic findings are quite broad. A bone marrow biopsy may provide diagnostic answers to hematologic or systemic problems that have not been diagnosed previously. Often the differential diagnosis of these conditions includes both primary hematopoietic and non-hematopoietic malignancies. This chapter gives the reader an overview of this wide array of disorders and important features for their diagnosis.

■ INFECTIONS

Clinical Features

The clinical features of infections of the hematopoietic system are broad. Exact incidences are difficult to estimate, but in general infections involving the bone marrow are rare. The frequency is increased in individuals who are immunosuppressed. There is no specific gender predilection for infection. The clinical signs and symptoms vary depending on the specific infection; however, when marrow involvement is present in a systemic infection, it is typically associated with a worse prognosis.

Pathologic Features

The pathologic features of marrow infections are highly variable depending on the specific infectious agent. In general viral, bacterial, fungal, and parasitic or protozoal infections can all involve the marrow. Select causative agents are discussed in the following sections. In addition, macrophage activation syndromes/hemophagocytic disorders, often associated with infections, are also discussed.

INFECTIONS—FACT SHEET

Definition
- A wide variety of infections can be identified in peripheral blood and bone marrow

Clinical Features
- Feature are variable, depending on the type and severity of infection
- Immunosuppressed individuals (after chemotherapy, after transplant, human immunodeficiency virus or acquired immunodeficiency syndrome [HIV/AIDS], some medications) have an increased incidence of infections

Laboratory Features
- If infectious etiology is suspected, appropriate microbiologic studies (culture, serology, polymerase chain reaction) should be performed on peripheral blood or bone marrow before initiating therapy

VIRAL

Among the viruses, parvovirus B19, Epstein-Barr virus (EBV), HIV, and other viruses can have significant peripheral blood and bone marrow effects. Because it is often associated with viral infections, macrophage activation syndrome/hemophagocytic lymphohistiocytosis (MAS/HLH) will also be discussed in this section.

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INFECTIONS—PATHOLOGIC FEATURES

Peripheral Blood

- Nonspecific changes: leukocytosis, eosinophilia, toxic changes (granulation, Döhle bodies), lymphocytosis with atypical forms, monocytosis, anemia, thrombocytopenia
- Circulating organisms rarely seen
- Notable intracellular organisms: bacteria (ingested by white blood cells [WBCs]), malaria, histoplasmosis, *Candida* species. Extracellular organisms: *Filaria, Borrelia* species

Aspirate Smear

- Most often nonspecific changes, including left shift, toxic changes, increased myeloid-to-erythroid (M:E) ratio, lymphocytosis
- Rarely organisms may be seen within macrophages, such as leishmaniasis or histoplasmosis

Core Biopsy and Clot Section

- Nonspecific changes include granulomas, fibrosis, increased M:E ratio, lymphocytosis, plasmacytosis, eosinophilia
- Rarely organisms may be identified on hematoxylin and eosin (H&E) stain

Special Stains

- AFB/GMS/PAS stains are beneficial for mycobacterial or fungal infections
- Occasionally, tissue Gram stain or Warthin-Starry stain may be useful

Flow Cytometry, Immunohistochemistry, Molecular, Cytogenetic

- Many immunohistochemical stains are available for detection of viral and other infectious agents
- Molecular methods for detection of organisms may be useful in confirming cases with equivocal morphologic findings

EPSTEIN-BARR VIRUS

An acute EBV infection in an immunocompetent host (i.e., infectious mononucleosis [IM]) is typically characterized by the presence of atypical lymphocytosis in association with sore throat, fever, and lymphadenopathy. Laboratory findings include the presence of IgM antibody to EBV. More commonly the heterophile antibody, or monospot, test is performed to confirm acute infection. The peripheral blood usually has lymphocytosis with 10% or more large, atypical lymphocytes (Fig. 6.1). These large lymphocytes appear approximately 3 to 4 days after the appearance of symptoms. The lymphocytosis may persist for several months.

The bone marrow is not typically examined in cases of IM. The changes in bone marrow associated with acute EBV infection are generally nonspecific and include lymphoid aggregates; increased CD8+ lymphocytes, including atypical forms; increased M:E ratio; and a left shift in myeloid. maturation. Confirmation of the diagnosis on a bone marrow sample can be accomplished with various immunohistochemical stains or in situ techniques for EBV in acute infections.

Chronic active EBV infection is a systemic lymphoproliferative disorder occurring primarily in children and young adults of Asian or Latin American descent, although rare older individuals may be affected. Patients exhibit IM-like symptoms (fevers, lymphadenopathy, and organomegaly) that occur at least 3 months after primary infection and with no known immunodeficiency. The bone marrow shows subtle interstitial and/or sinusoidal infiltrates of small mature T or natural killer cells that are EBV positive by in situ hybridization. These cells might not be apparent on H&E staining and become apparent only on staining for EBV; therefore a high index of suspicion is required. High-titer EBV viral capsid antigen IgG and early antigen IgG can be demonstrated in serum as can EBV DNA by polymerase chain reaction. By definition, T-cell receptor gene rearrangement studies are polyclonal; if monoclonal, a diagnosis of EBV-positive T-cell lymphoma of childhood (World Health Organization [WHO] 2016) is warranted. This bona fide T-cell malignancy is covered in Chapter 9, Peripheral T-cell Lymphomas. Prognosis is guarded with indolent behavior in some patients, whereas others may die of the disease. Evolution to monoclonal disease, overt T-cell lymphoma, or macrophage activation syndrome or hemophagocytic lymphohistiocytosis may occur.

MACROPHAGE ACTIVATION SYNDROME/ HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS/ HEMOPHAGOCYTOSIS

Hemophagocytic lymphocytic histiocytosis (HLH) clinically present with high fever, myalgia, fatigue and myalgia. Accompanying physical findings and laboratory abnormalities include hepatosplenomegaly, jaundice, severe cytopenias, abnormal liver function tests, hypertriglyceridemia, hyperferritinemia, and coagulopathy. It has many causes and can be divided into primary and secondary forms. Primary forms of HLH (also termed familial HLH) are rare and can occur in familial patterns or as sporadic cases. While in many cases the genetic defect may be unknown, primary HLH is often due to mutation of genes that affect cytotoxic functions in T and NK cells (such as perforin *[PRF1] UNC13D, STX11,* and *STXBP2*) or genes that result in lysosomal trafficking disorders that also impair cytotoxic function. These include syndromic immunologic disorders Chediak-Higashi syndrome *(LYST),* Griscelli syndrome *(RAB27A),* and Hermansky-Pudlak syndrome *(AP3B1).*

Secondary HLH can be seen in an association with rheumatologic/inflammatory disorder associated macrophage activation syndrome (MAS), malignancy, or infection. MAS has an associated mortality rate of about 20% and criteria for MAS in systemic juvenile arthritis are presented in Box 6.1. Associated secondary etiologies for infection and malignancy associated (secondary) HLH are shown in Box 6.2. Malignancy-associated HPS can be seen in conjunction with peripheral T-cell lymphomas, B-cell lymphomas, Hodgkin lymphoma, epithelial malignancies, and leukemias, in roughly that order of frequency. In cases of T-cell lymphomas, the

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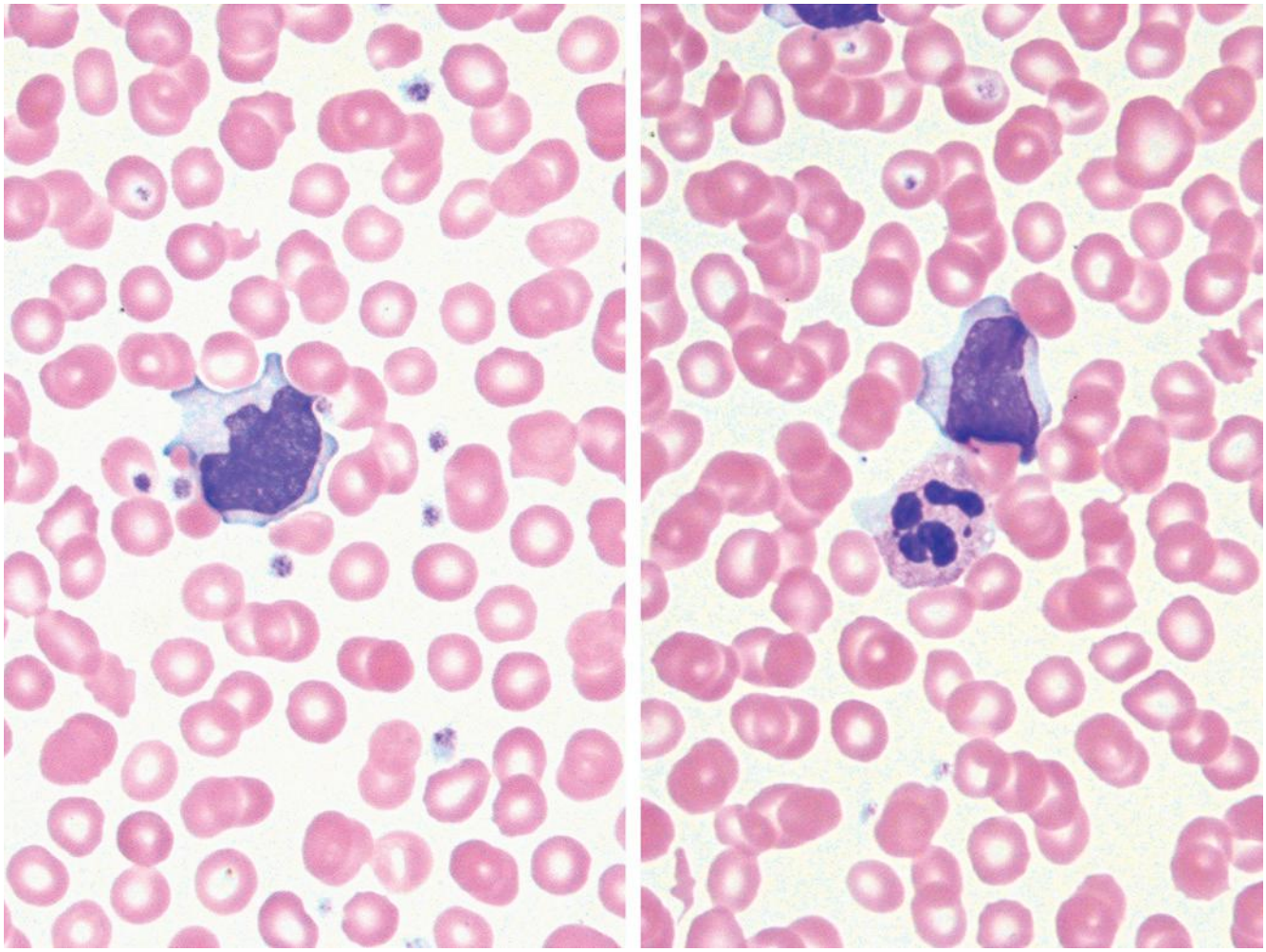


FIG. 6.1

Examples of reactive lymphocytes seen in the peripheral blood in infectious mononucleosis. These findings can also be seen in other viral infections and are not specific to Epstein-Barr virus infection.

Box 6.1 Diagnostic Criteria for Distinguishing Macrophage Activation Syndrome (MAS) from Systemic Juvenile Idiopathic Arthritis (SJIA)[a]

Laboratory Criteria

Decreased WBC count (<4.0 K/μL)
Decreased platelet count (262 K/μL)
Hypofibrinogenemia (≤2.5 g/L)
Increased AST (>59 U/L)

Clinical Criteria

CNS dysfunction
Hepatomegaly
Hemorrhages
Hemophagocytosis in bone marrow aspirate[b]

[a]At least two laboratory criteria or at least one laboratory and one clinical criterion would confirm a diagnosis of MAS in SJIA.
[b]Bone marrow biopsy may only be necessary in cases that are equivocal.

Box 6.2 Etiologic Agents Associated With Hemophagocytosis or Hemophagocytic Lymphohistiocytosis

Epstein-Barr virus
Hepatitis viruses
Cytomegalovirus
Other viruses
Congenital (±Epstein-Barr virus infection): familial hemophagocytic syndrome (perforin gene and other related gene mutations)
T-cell lymphoma–associated
Germ cell tumor–associated
Other malignancies
Immunosuppression: often infection-related

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MACROPHAGE ACTIVATION SYNDROME/HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS/HEMOPHAGOCYTOSIS—FACT SHEET

Definition

- Hemophagocytosis is a morphologic finding with several possible etiologies characterized by prominent destruction of marrow elements within macrophages
- Macrophage activation syndrome (MAS) is a clinical disorder associated with systemic symptoms caused by overwhelming inflammation caused by immune dysregulation (often in the setting of rheumatologic disease) with marked increases in circulating cytokines. Although hemophagocytosis may be seen in MAS, it is not required for the diagnosis

Incidence

- Rare (see Box 6.1)

Clinical Features

- Systemic symptoms (fever, night sweats, weight loss) common

HEMOPHAGOCYTOSIS—PATHOLOGIC FEATURES

Peripheral Blood

- Pancytopenia common

Aspirate Smear

- Hypocellular aspirate smears, with prominent increase in macrophages with ingested cellular elements and debris

Core Biopsy and Clot Section

- Reduction in cellularity and variably prominent macrophages with ingested cellular elements
- Lymphocytosis, fibrosis, and plasmacytosis common

Flow Cytometry, Immunohistochemistry, Molecular, Cytogenetic

- Stains for EBV-LMP, Epstein-Barr nuclear antigens, or in situ hybridization for EB-encoded RNA (EBER) may be informative
- Polymerase chain reaction for T-cell receptor gene rearrangement may be beneficial in T-cell lymphoma–associated types

Differential Diagnosis

- Infection-associated (EBV most common); malignancy-associated (especially T-cell lymphoma); familial (primary) hemophagocytic lymphohistiocytosis (*PRF1, MUNC13-4, STX11, STXBP2,* etc. mutations); idiopathic

EBV, Latent membrane protein.

malignant cells can be inconspicuous relative to the background of hemophagocytosis.

Hemophagocytosis describes the presence of ingestion of hematologic cells by macrophages. The morphologic appearance of hemophagocytosis, regardless of the underlying etiology, of all types is similar. Core biopsy specimens and aspirate smears show macrophages with ingested cellular elements (Fig. 6.2), most often erythrocytes; however, other hematopoietic cells can also be seen within the macrophages. The macrophages may also contain cell fragments or amorphous debris, which are remnants of hematopoietic components. The macrophages are benign and lack cytologic atypia. In core biopsy specimens, the marrow may have an unusual appearance. The intertrabecular space is filled with cells (hemophagocytic macrophages), but there is an overall decrease in hematopoiesis. In all cases of hemophagocytosis, the marrow should be assessed carefully for signs of viral inclusions and of atypical cells that may represent an associated malignancy. The differential diagnosis also includes true histiocytic malignancies that, although rare, may also phagocytize other cellular elements. More details on MAS/HLH are presented in Chapter 19.

OTHER VIRUSES

Parvovirus B19 is a DNA viral infection seen predominantly in young or immunosuppressed patients, leading to severe anemias caused by red blood cell aplasia. In acute parvoviral infection the virus preferentially infects red blood cell precursors; in disorders with a shortened red blood cell life span (e.g., hereditary spherocytosis), this causes a severe symptomatic anemia. The marrow findings are characteristic (Fig. 6.3). If the marrow is biopsied at the appropriate stage, giant pronormoblasts and intranuclear viral inclusions can be seen. Chronic infections by parvovirus, seen in immunocompromised patients, more often have pancytopenia. Occasionally these cases will have erythroid hyperplasia with numerous virally infected erythroid cells and prominent viral inclusions. An association with HLH has also been reported. An immunostain is available with approximately 80% sensitivity, but serologic testing for the virus is considered the gold standard.

Hepatitis C virus–infected patients can develop an atypical lymphoproliferative disorder in the setting of essential mixed (type II) cryoglobulinemia. This type of cryoglobulin is composed of a monoclonal immunoglobulin (Ig) M and polyclonal IgG components. Although patients do not manifest overt lymphoma, subtle monoclonal lymphoid components may be detected in the bone marrow of some patients when detailed immunophenotyping is performed. Patients may then develop lymphoma (e.g., lymphoplasmacytic lymphoma) at some point, often many years later, in the disease course.

HIV/AIDS

A wide variety of hematologic changes are seen in patients with HIV/AIDS (Table 6.1). It is important to remember that although these findings are common in HIV/AIDS, they are also not entirely specific.

Macrocytosis with or without anemia is a common finding in HIV/AIDS peripheral blood. These effects are multifactorial, and causes include direct effects of the virus, medications, and secondary dietary deficiencies. The macrocytosis is

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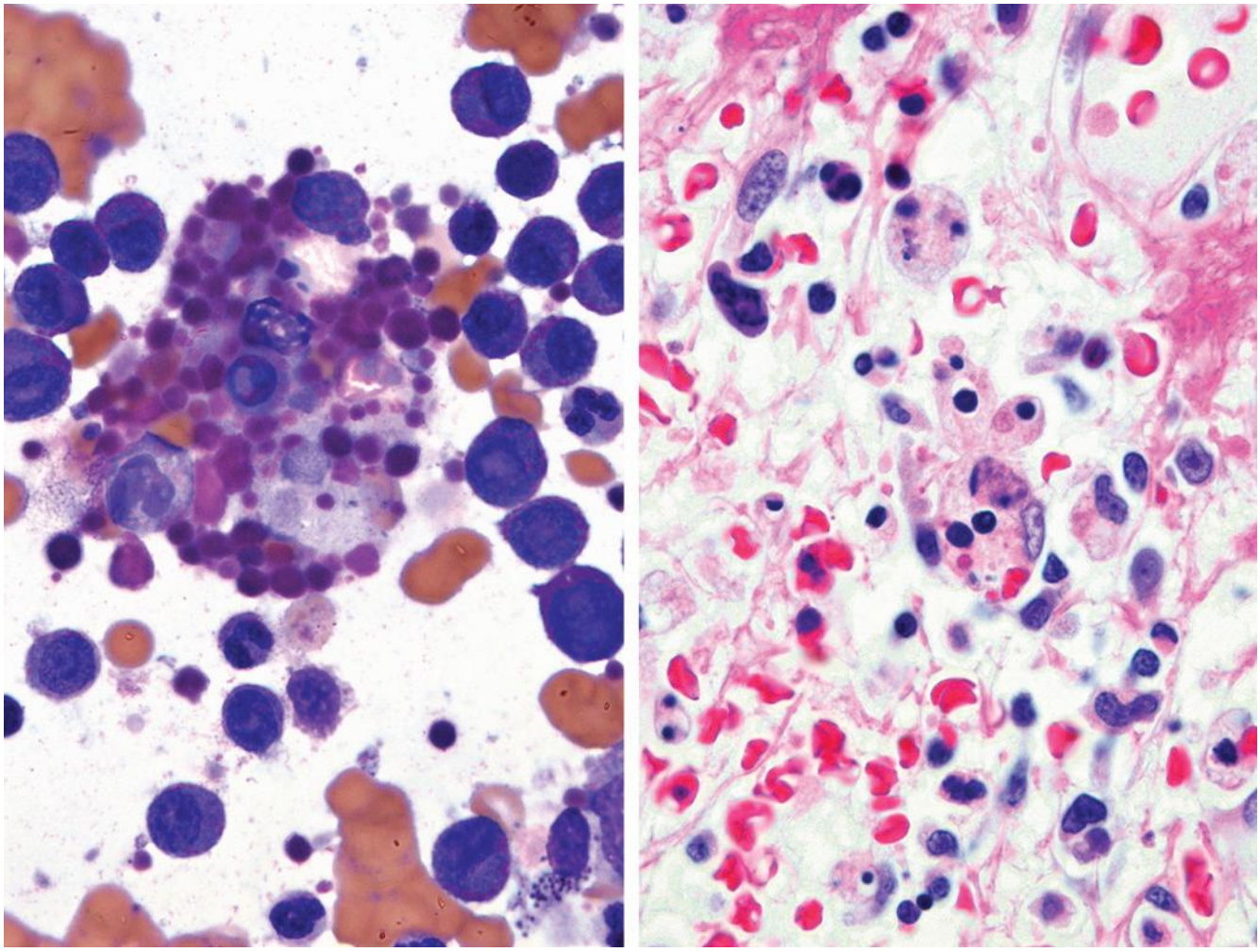


FIG. 6.2

Example of aspirate smear and core biopsy findings in hemophagocytic syndrome. Although this case is due to Epstein-Barr virus infection, other causes would show similar morphologic findings.

HIV/AIDS MYELOPATHY—FACT SHEET

Definition

- *HIV myelopathy* refers to the constellation of bone marrow findings seen in association with HIV/AIDS

Incidence

- Essentially all patients with HIV/AIDS will experience hematologic and bone marrow changes throughout the course of their disease

Morbidity and Mortality

- Hematologic disease may contribute to morbidity in HIV/AIDS

Clinical Features

- Features are variable depending on disease severity
- Clinical indication of severity is often based on CD4+ lymphocyte count

Prognosis and Therapy

- Newer antiretroviral therapies improve quality of life and survival in HIV/AIDS. However, no cure is available and resistance to therapy is increasingly common

generally mild to moderate. Rouleaux resulting from hypergammaglobulinemia, hyperviscosity, and amyloidosis have been rarely associated with HIV infection.

The most common finding in peripheral blood is lymphopenia, as a result of reduced numbers of CD4+ T cells. Occasionally there may be leukocytosis in patients with HIV/AIDS. If lymphocytosis is present, it may be composed of large granular lymphocytes, which are either CD8+ T cells or natural killer cells, or B cells with lymphoplasmacytic features. Increases in granulocytes, often with toxic changes (e.g., vacuolation, toxic granules, and Döhle bodies), may be due to bacterial infections. Nuclear fragmentation or other

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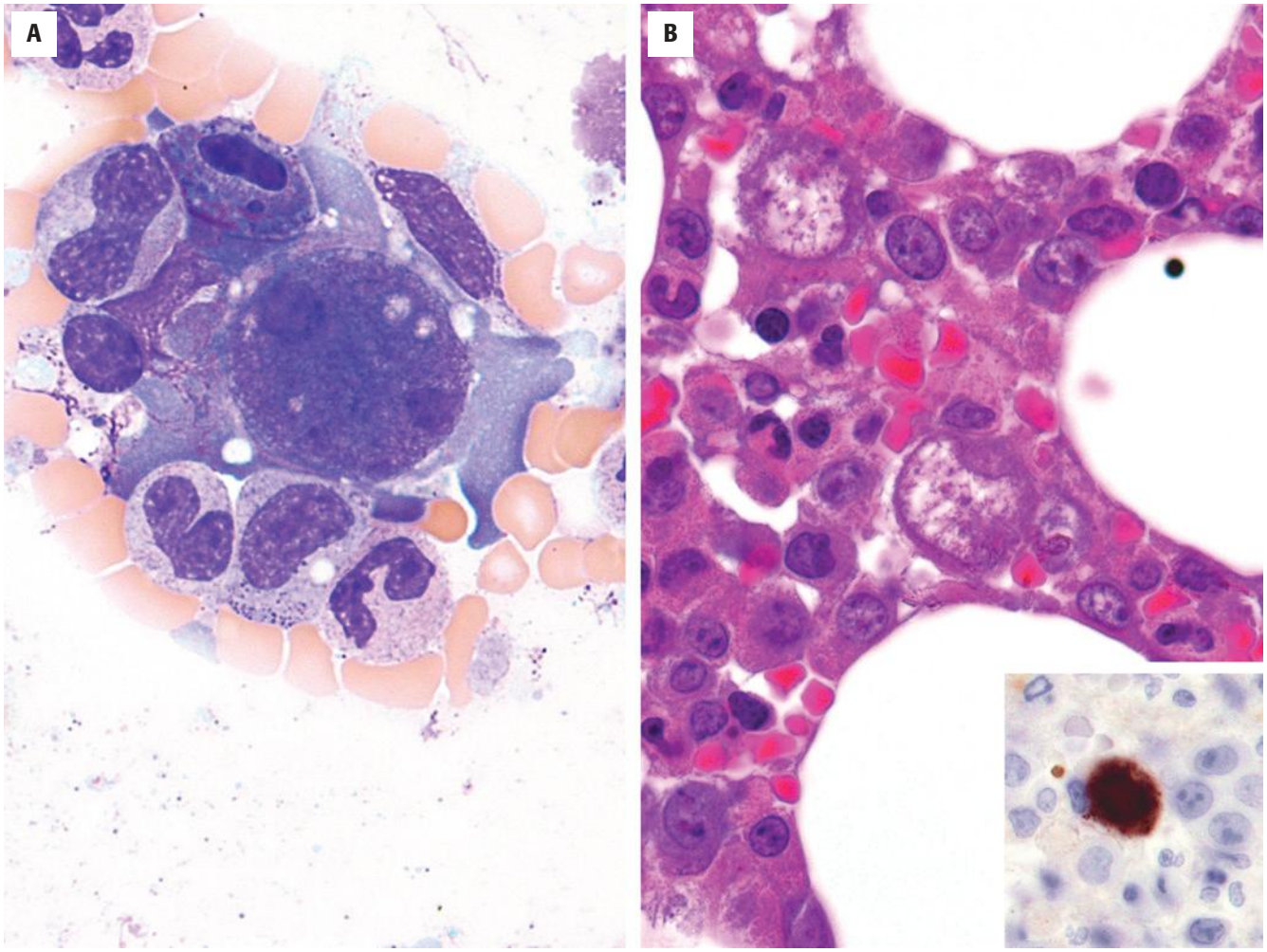


FIG. 6.3

Parvovirus infection in bone marrow. A, Aspirate smear showing giant pronormoblasts characteristic of parvovirus infection. B, Biopsy showing large infected erythroid elements with open, cleared nuclei. *Inset,* An example of a parvoviral immunohistochemical stain.

HIV/AIDS MYELOPATHY—PATHOLOGIC FEATURES

Peripheral Blood

- Macrocytosis (result of medication effect and nutritional effects), anemia (multifactorial), poikilocytosis, thrombocytopenia, lymphopenia, variable increases in large granular lymphocytes

Aspirate Smear

- Erythroid megaloblastic changes, increased lymphocytes, increased plasma cells, increased megakaryocytes, including naked nuclei

Core Biopsy and Clot Section

- Hypercellularity common, atypical megakaryocytes with naked and hyperchromatic nuclei, lymphoid aggregates, granulomas, plasmacytosis (polyclonal), fibrosis

Special Stains

- Stains for fungal and mycobacterial infections (GMS, AFB) should be performed routinely
- Some patients with HIV/AIDS may have chronic parvoviral infection; immunohistochemistry for parvovirus may be of benefit

Differential Diagnosis

- HIV/AIDS myelopathy may mimic primary myelodysplastic syndromes or myeloproliferative neoplasms
- Increased incidence of infections and of malignancies can complicate diagnosis

GMS, Gomori methenamine silver; *AFB,* acid fast bacillus.

degenerative changes of white blood cells (WBCs) can be seen and may be due to infections or drug effects. Eosinophilia is often seen in these patients.

Autoimmune cytopenias (i.e., immune thrombocytopenic purpura [ITP], autoimmune hemolytic anemia, autoimmune neutropenia) can be seen in association with HIV/AIDS infection and can have a significant effect on the peripheral blood findings. Thrombocytopenia may be one of the earliest findings in HIV infection. The two main causes of thrombocytopenia are immune-mediated destruction and decreased

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production. Hypersplenism and drug effects may also contribute to low platelets.

The bone marrow findings in HIV/AIDS are protean (Fig. 6.4). A wide variety of relatively specific and numerous nonspecific findings are found in the bone marrow (see Box 6.2). The constellation of findings is referred to as HIV myelopathy. Some of the more common findings include hypercellularity, granulomas, poorly formed histiocytic aggregates, lymphoid aggregates, plasmacytosis (with occasional Russell or Dutcher bodies), focal serous fat atrophy (gelatinous transformation), dilated sinuses, increased iron, and marrow fibrosis. Some of the less common findings include hypocellularity, infection without granuloma (low CD4 count), ring sideroblasts, hemorrhage, and diffuse serous fat atrophy or gelatinous transformation. Megakaryocytes can be infected directly by the HIV virus and consequently show several changes in HIV/AIDS. The megakaryocytes often have distinctive morphologic findings, the most pathognomonic of which is the presence of naked nuclei. Other changes include an absolute increase in number, clustering (mimicking myeloproliferative neoplasms), unilobated megakaryocytes (mimicking myelodysplastic syndromes), large forms, and abnormal nuclear chromatin.

Infectious agents of a variety of types may be seen in HIV/AIDS marrows; they may present confusing or unusual marrow findings that can mimic lymphomas, sarcomas, or epithelial malignancies. Clinical suspicion, culture results, and the use of organism stains can be beneficial. Relatively common infections in HIV/AIDS include cryptococcosis, histoplasmosis, coccidioidomycosis, pneumocystosis, HHV-8, cytomegalovirus (CMV), adenovirus, EBV, toxoplasmosis, and leishmaniasis.

Lymphoma (including Hodgkin and non-Hodgkin types), plasma cell dyscrasia, and spindle cell lesions (including Kaposi sarcoma, inflammatory pseudotumor, and mast cell disease) can also be discovered initially in the bone marrow.

TABLE 6.1

Marrow Changes Associated With HIV/AIDS

Morphology	Cellularity changes (most often hypercellular) Megakaryocyte changes (e.g., naked nuclei, clustering) Fibrosis Lymphoid aggregates Plasmacytosis Histiocyte: aggregates, hemophagocytosis Dyspoiesis simulating myelodysplastic syndrome Gelatinous transformation, serous fat atrophy
Infections	Fungal: cryptococcosis, histoplasmosis, coccidioidomycosis, blastomycosis, other fungi Viral: direct HIV effects, Epstein-Barr virus–associated, human herpesvirus 8, cytomegalovirus, other viruses Parasitic or protozoal: leishmania, toxoplasmosis, others Bacterial: mycobacteria, rickettsia, others
Hematologic and autoimmune complications	Thrombotic thrombocytopenic purpura, immune thrombocytopenic purpura, autoimmune anemia, autoimmune neutropenia, coagulopathies
Malignancy	Hematologic: Burkitt lymphoma, diffuse large B-cell lymphoma, large granular lymphocytosis, Hodgkin lymphoma Nonhematologic: Kaposi sarcoma, other carcinomas, sarcoma

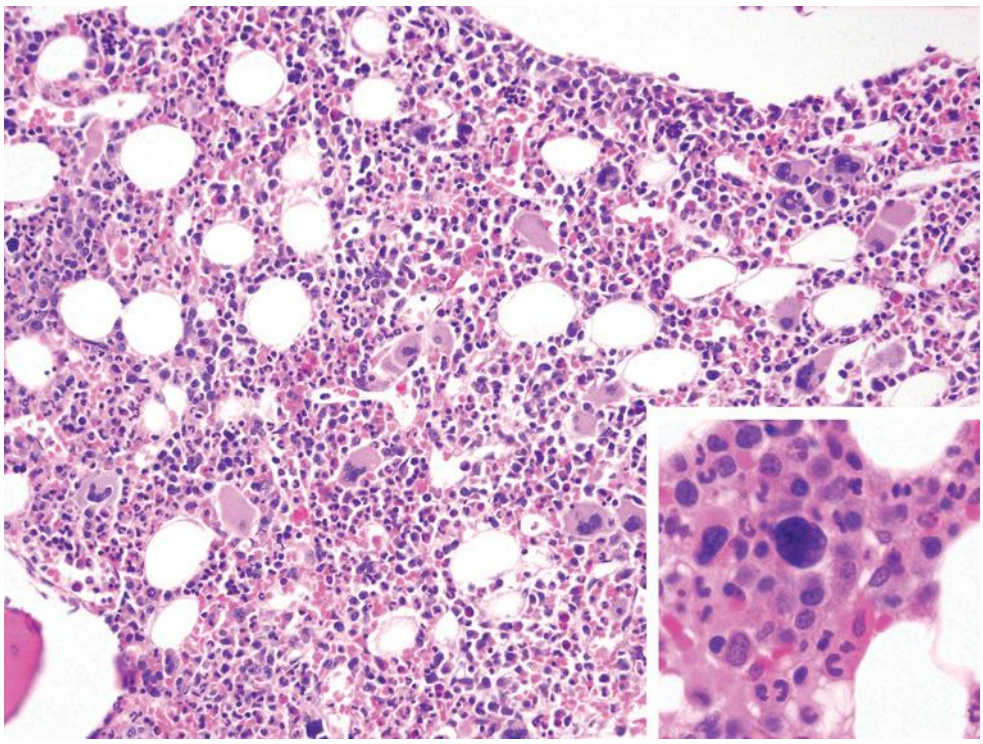


FIG. 6.4

Bone marrow findings in HIV/AIDS. A variety of features can be seen, but common findings include hypercellularity, increases in megakaryocytes, lymphoid aggregates, and plasmacytosis. *Inset,* A naked megakaryocyte nucleus, a common and relatively specific bone marrow finding in HIV/AIDS.

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BACTERIAL

Bone marrow findings in bacterial sepsis are nonspecific. In fact, most bacterial infections produce no significant marrow findings. Most cases manifest only as an increase in granulocytes, a left shift in maturation, toxic changes, or other nonspecific changes as outlined previously. Some of the organisms with identifiable changes are discussed in the following sections.

Whipple disease *(Tropheryma whippelii),* which may be seen in the bone marrow, produces similar morphologic findings to those seen in other sites. Rickettsial infections may show the presence of ring (or donut) granulomas. This finding is suggestive, but by no means specific, for Q fever *(Coxiella burnetii).*

MYCOBACTERIAL

A number of mycobacterial species can involve the bone marrow. Although not the most common in the United States, marrow involvement by *Mycobacterium tuberculosis* can be rarely associated with a diagnosis of myelophthisis (i.e., marrow failure resulting from replacement of hematopoietic marrow by an abnormal tissue causing a leukoerythroblastic blood picture). In typical cases of marrow involvement by tuberculosis, the marrow will show granulomatous inflammation, occasionally with central, caseous necrosis. The degree of involvement is variable, but immunocompetent hosts will typically form granulomas. In severely immunosuppressed patients, granulomas may be poorly formed or absent. Instead small, indistinct histiocytic aggregates may be seen. In either case, organisms are usually rare when visualized with an acid-fast stain. Immunohistochemical staining may allow visualization of more organisms.

Mycobacterium avium-intracellulare is more commonly seen in marrows of immunosuppressed patients. In the most obvious cases, there are aggregates of histiocytes filled with granular cytoplasm (Fig. 6.5). Acid-fast (AFB) staining usually reveals numerous organisms that, in contrast with *M. tuberculosis,* stain positively with periodic acid-Schiff (PAS).

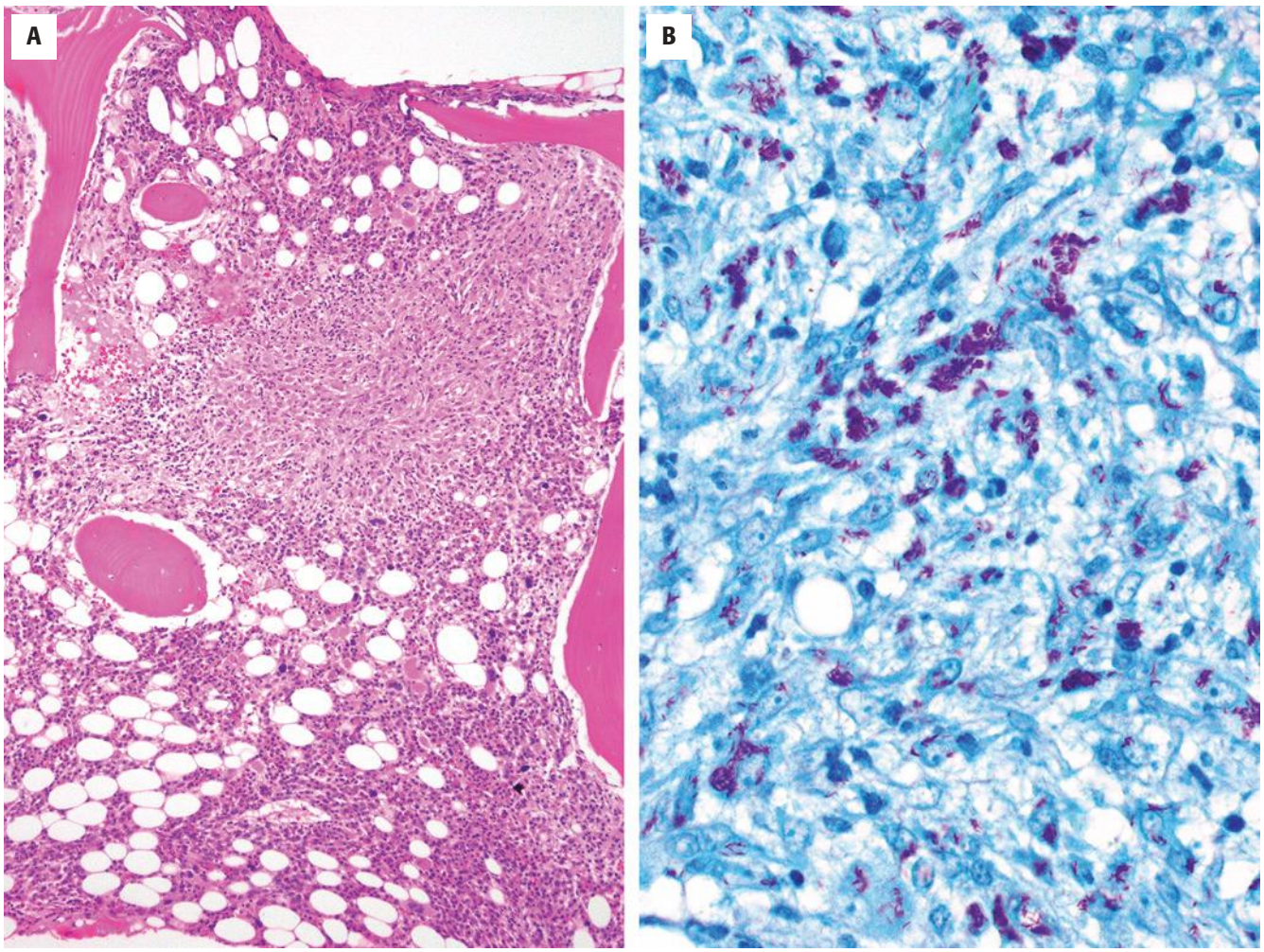


FIG. 6.5

Mycobacterium avium-intracellulare in bone marrow. A, Biopsy showing large granuloma composed of expanded, pink macrophages. B, An acid-fast bacillus stain that highlights numerous positive elongated mycobacteria.

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FUNGAL

A variety of fungi can be found in bone marrow. Marrow involvement is associated with disseminated infections and is most often seen in immunocompromised patients. Some infections are more or less common based on geographic location. The fungal infections that are more commonly seen in the United States include histoplasmosis, blastomycosis, and coccidioidomycosis. Other fungi that are worldwide in their distribution include *Cryptococcus* species, *Candida* species, and *Aspergillus* species. Other more rare types of fungi have also been reported in the bone marrow.

Histoplasmosis *(Histoplasma capsulatum)* is a dimorphic fungus that is endemic in the central United States, particularly in the Ohio Valley region. It consists of small (2 to 4 μm) budding yeast forms usually seen within macrophages (Fig. 6.6). They can be better visualized by Giemsa and Gomori methenamine silver (GMS) stains.

Blastomycosis (*Blastomyces dermatitidis*) is another dimorphic fungus (Fig. 6.7). It typically infects the skin or respiratory tract. Disseminated infection can eventually lead to the presence of the organisms in the bone marrow. The yeast forms are generally spherical with thick walls, are up to 40 μm in diameter, and have characteristic broad-based budding of daughter forms. They are easily seen with a GMS stain.

Cryptococcus *(Cryptococcus neoformans)* is the most common systemic fungal infection occurring in patients with HIV (see Fig. 6.7); however, marrow involvement is unusual. Typically the yeast forms are easy to see, with thick, gelatinous capsules. The thickness of the capsule depends on the strain and host conditions. Bone marrow biopsies can also occasionally have associated granulomatous inflammation or hemophagocytosis. Histochemical stains, most notably GMS and mucicarmine, are useful for identification.

Coccidioidomycosis *(Coccidioides immitis)* is a fungal infection that typically involves the lung. The most common geographic distribution is the western United States and Mexico. Certain racial groups have an increased sensitivity to infection. Rare disseminated cases may have bone marrow involvement. The organisms are large spherules (10–80 μm), roughly the size of a megakaryocyte, packed with smaller

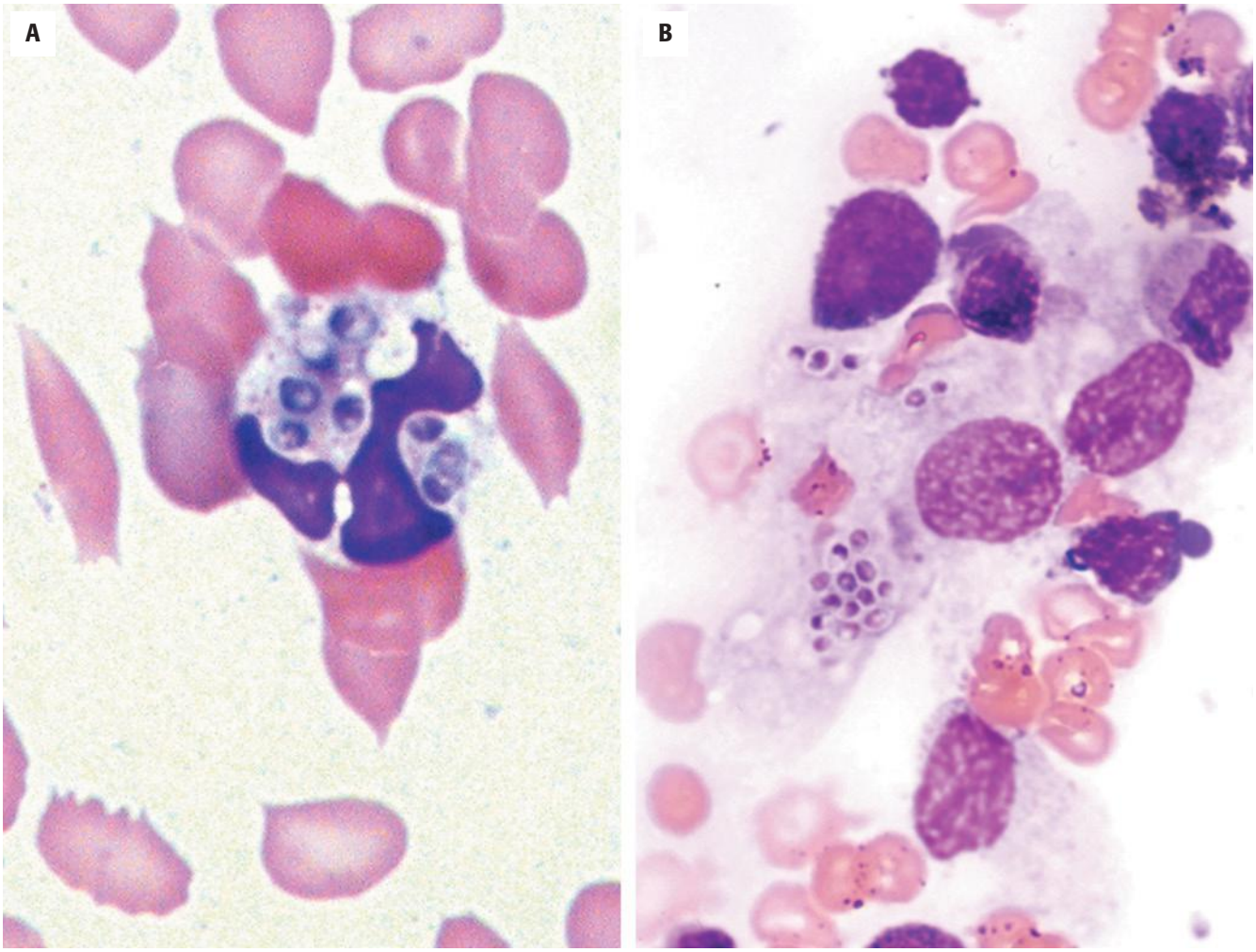


FIG. 6.6
Histoplasmosis yeast forms in (A) peripheral blood and (B) bone marrow aspirate.

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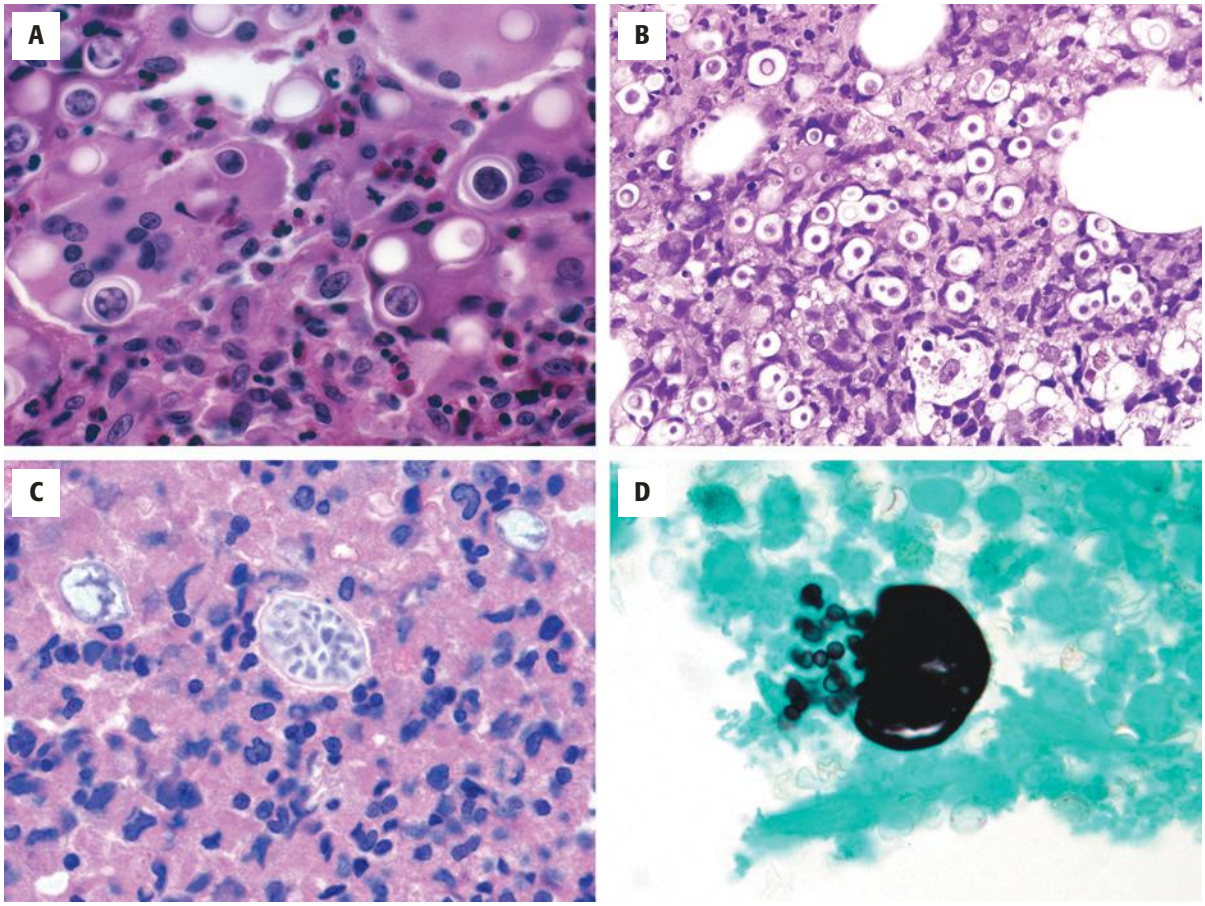


FIG. 6.7

Fungi in bone marrow. A, Blastomycosis. B, Cryptococcus. C, Coccidioidomycosis in core biopsy. D, Rupturing coccidiomycosis endospores highlighted by Gomori methenamine silver stain.

(2–5 μm) endospores. Often the organisms are associated with lymphoid aggregates and granulomas. They will stain well with fungal stains (GMS, PAS) (see Fig. 6.7).

The classification of pneumocystis (*Pneumocystis jirovecii,* formerly *P. carinii*) is uncertain, but currently it is classified as a fungus. It causes pneumonia in HIV+ patients but may also rarely involve the bone marrow in disseminated disease. The organisms may be seen in association with granulomas and histiocytic aggregates. The cysts are small and often intracellular within macrophages. Occasionally there is a frothy extracellular material, similar to that seen in lung (Fig. 6.8). The organisms can be visualized with Giemsa, GMS, and immunohistochemical stains.

PARASITIC AND PROTOZOAL

Most parasitic and protozoal infections are more frequent in peripheral blood (e.g., malaria, babesiosis, filaria); however, they can be seen rarely in bone marrow. In contrast, other rare protozoal infections may be seen more commonly in bone marrow.

Leishmaniasis is a relatively common protozoal infection worldwide. The most prevalent species causing disseminated leishmaniasis is *Leishmania donovani*. It requires two hosts to complete its life cycle, and humans are a primary host and the sand fly is a secondary host. The organisms are small, intracellular, and typically seen within macrophages (Fig. 6.9). They can sometimes be confused with histoplasmosis or, less commonly, *Cryptococcus* species. These organisms are PAS, GMS, and mucicarmine negative. They are best identified with a Giemsa stain, which allows better visualization of the nucleus and kinetoplast, a small rod-shaped structure seen opposite the nucleus.

In toxoplasmosis, bone marrow involvement is seen rarely. Toxoplasma species are obligate intracellular parasites, with cats as the typical host. Humans are infected by exposure to feces from infected cats. Less commonly, exposure occurs through ingestion of undercooked meat from infected animals. Clinical presentation of disseminated disease includes pancytopenia and central nervous system infection with lung and heart involvement. The bone marrow may show only small histiocytic aggregates or well-formed granulomas less frequently. Less common findings include necrosis, edema, and fibrin deposition. Cysts with organisms are seen in histiocytes and megakaryocytes or in extracellular locations; more rarely free trophozoites are seen in the interstitium.

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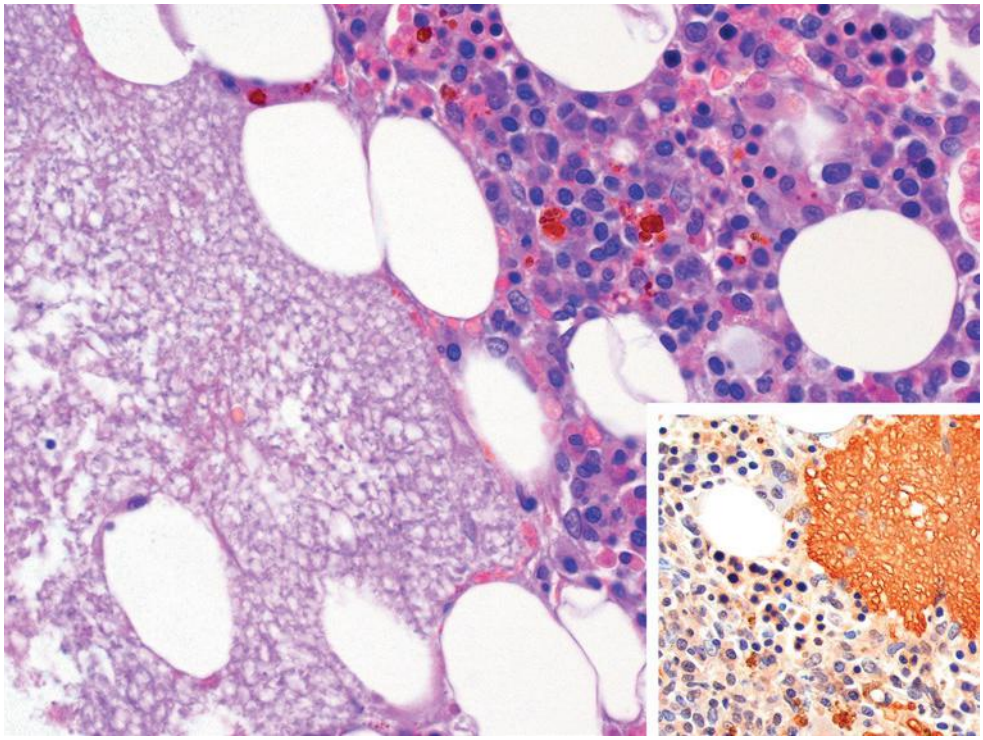


FIG. 6.8
Pneumocystis involving bone marrow. Note the frothy material, similar to that seen in lung. *Inset,* Immunohistochemical staining for pneumocystis.

GRANULOMAS

Clinical Features

Bone marrow granulomas are rare and are reported in approximately 1% to 2% of all marrows. Several types of granulomas are seen in marrow, and these can be associated with specific etiologies, although no type of granuloma is entirely specific for a particular diagnosis. Granulomas have been associated with a variety of conditions, including neoplasms, viral, bacterial and fungal infections, autoimmune disorders, medications, and sarcoidosis (Box 6.3; Fig. 6.10).

GRANULOMAS—FACT SHEET

Definition
- Organized clusters of macrophages as a response to a variety of marrow insults

Incidence
- 1% to 2% of all marrows

Clinical Features
- Variable depending on underlying etiology

Pathologic Features

Granulomas are, at their most basic level, an aggregate of macrophages displaying variable degrees of epithelioid features (rounded cell morphology with abundant cytoplasm). There are often surrounding or intermixed lymphocytes and other inflammatory cells. Multinucleated giant histiocytic cells can be seen in almost any type of granuloma. The evaluation of granulomas in bone marrow, except perhaps lipogranulomas, should include special stains (i.e., AFB, GMS, PAS) to evaluate for the presence of microorganisms.

Lipogranulomas, which consist of small amounts of fat surrounded or ingested by macrophages and lymphocytes, are seen in approximately 10% of bone marrow biopsy specimens. A suggested cause in some cases is local damage to adipocytes, inciting macrophages and an immunologic response. Rarely, lipogranulomas may be caused by intravascular administration of fatty substances. Rarely, foreign body granulomas may simulate lipogranulomas because of the presence, in both conditions, of vacuolated macrophages. Examination under polarized light may make identification of the foreign material easier.

Necrotizing granulomas are most often associated with infectious etiologies. These are typically well-defined nodules of epithelioid macrophages surrounding an area of necrotic, fragmented, unstructured debris. Any necrotizing granuloma should be investigated for the presence of infectious organisms, such as tuberculosis or fungal infections (discussed previously). Rarely, systemic disorders associated with granulomas can manifest with central necrosis.

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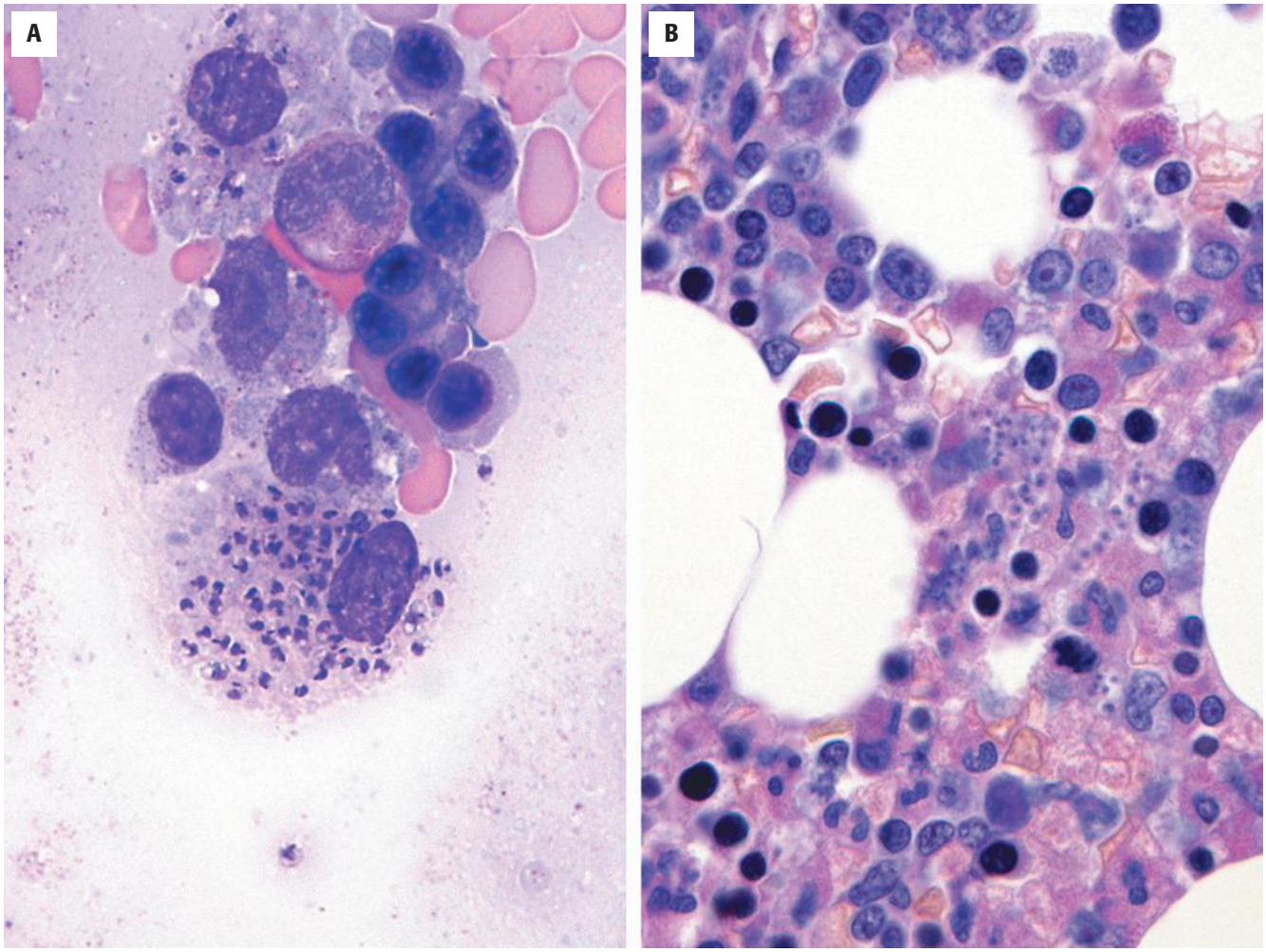


FIG. 6.9
Leishmaniasis in (A) aspirate smear and (B) core biopsy specimen.

GRANULOMAS—PATHOLOGIC FEATURES

Peripheral Blood
- No specific findings seen

Aspirate Smear
- May be seen as cohesive aggregates or clusters of macrophages often associated with lymphocytes

Core Biopsy and Clot Section
- Varying-sized nodules of enlarged, pink macrophages (e.g., epithelioid) with varying numbers of infiltrating or surrounding lymphocytes

Special Stains
- AFB, GMS, PAS staining should be performed in all cases with granulomas to rule out mycobacterial and fungal organisms

Differential Diagnosis
- Infectious, malignancy-associated (e.g., Hodgkin lymphoma), sarcoid, numerous other etiologies

Several medications may be associated with granuloma formation. The most common of these are procainamide and sulfonamide. Other medications associated with marrow granulomas include chlorpropamide, phenylbutazone, phenytoin, methyldopa, allopurinol, ibuprofen, penicillamine, and tolmetin sodium. Some lymphomas, notably Hodgkin lymphoma and periphera T-cell lymphoma, may be associated with granuloma formation.

SYSTEMIC DISORDERS

Clinical Features

Many systemic disorders have effects on the blood and marrow that can be evaluated and correlated with clinical findings. The clinical features depend on the specific entity in question. Several groups of systemic diseases are discussed in the following sections, including chronic alcoholism, gastrointestinal disorders, renal diseases, liver diseases, and autoimmune diseases.

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Box 6.3 Causes of Granulomas in Bone Marrow

Infectious
Viral infections
HIV/AIDS
Epstein-Barr virus, cytomegalovirus
Hepatitis C virus
Fungal infections
Histoplasmosis
Cryptococcus
Coccidioidomycosis
Aspergillosis
Blastomycosis
Bacterial, mycobacterial, rickettsial infections
Tuberculosis
Mycobacterium avium-intracellulare
Tularemia
Typhoid
Brucellosis
Borreliosis
Q fever
Mycoplasma species infections
Bacillus Calmette-Guerin
Mycobacterium leprae (leprosy)
Syphilis
Toxoplasmosis
Other

Malignancies
Hematologic
Hodgkin lymphoma
Non-Hodgkin lymphomas
Myeloma
Acute myeloid leukemia
Acute lymphoblastic leukemia
Chronic myelogenous leukemia
Myelodysplastic syndromes
Nonhematopoietic
Colon carcinoma
Lung carcinoma
Ovarian carcinoma
Neuroblastoma
Sarcomas

Medication-Induced
Nonsteroidal antiinflammatory drugs
Chemotherapy
Analgesics
Cytokines
Antibiotics
Antiepileptic
Antiarrhythmics

Autoimmune Disorders
Rheumatoid arthritis
Felty syndrome
Primary biliary cirrhosis
Sjögren syndrome
Pulmonary fibrosis
Systemic lupus erythematosus
Addison disease
Behçet disease
Erythema nodosum
Temporal arteritis
Posttransplantation

Foreign Bodies
Pneumoconiosis, silicosis
Intravenous drug abuse (foreign body; e.g., talc)

Pathologic Features

Chronic Alcoholism

One disorder associated with significant hematologic problems is chronic alcoholism. Ethanol has a direct deleterious effect on the development of hematopoietic cells; however, hematologic disorders seen in alcoholics can be caused by other mechanisms. Peripheral blood changes include anemia, stomatocytosis, acanthocytosis, macrocytosis (secondary to folate deficiency), vacuolation of WBCs, hypersegmentation of granulocytes (secondary to folate deficiency). These changes are due to a variety of effects including liver disease, nutritional deficiencies, and direct toxic effects of ethanol on hematopoietic cell development. Marrow findings include decreases in cellularity, ring sideroblasts, megaloblastic changes, mild dyspoiesis of erythroid precursors, excessive cytoplasmic granularity or vacuolation of myeloid cells (or both), and occasionally a left shift in WBC precursors. A mild polyclonal plasmacytosis of up to 10% often accompanies chronic alcoholism. A relatively specific but rare finding is the presence of cytoplasmic iron within plasma cells.

CHRONIC ALCOHOLISM—FACT SHEET

Definition
- Marrow changes are common in association with chronic ethanol abuse

Clinical Features
- Variable, depending on severity and length of abuse

Laboratory Features
- Abnormal liver function tests (alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase) commonly present

Gastrointestinal Disorders

Most of the hematologic changes due to gastrointestinal disorders are due to specific nutritional deficiencies, such as vitamin B12, folate, or iron deficiencies. Malabsorption or liver disease can also lead to other hematologic problems, most specifically red blood cell disorders and hemolysis.

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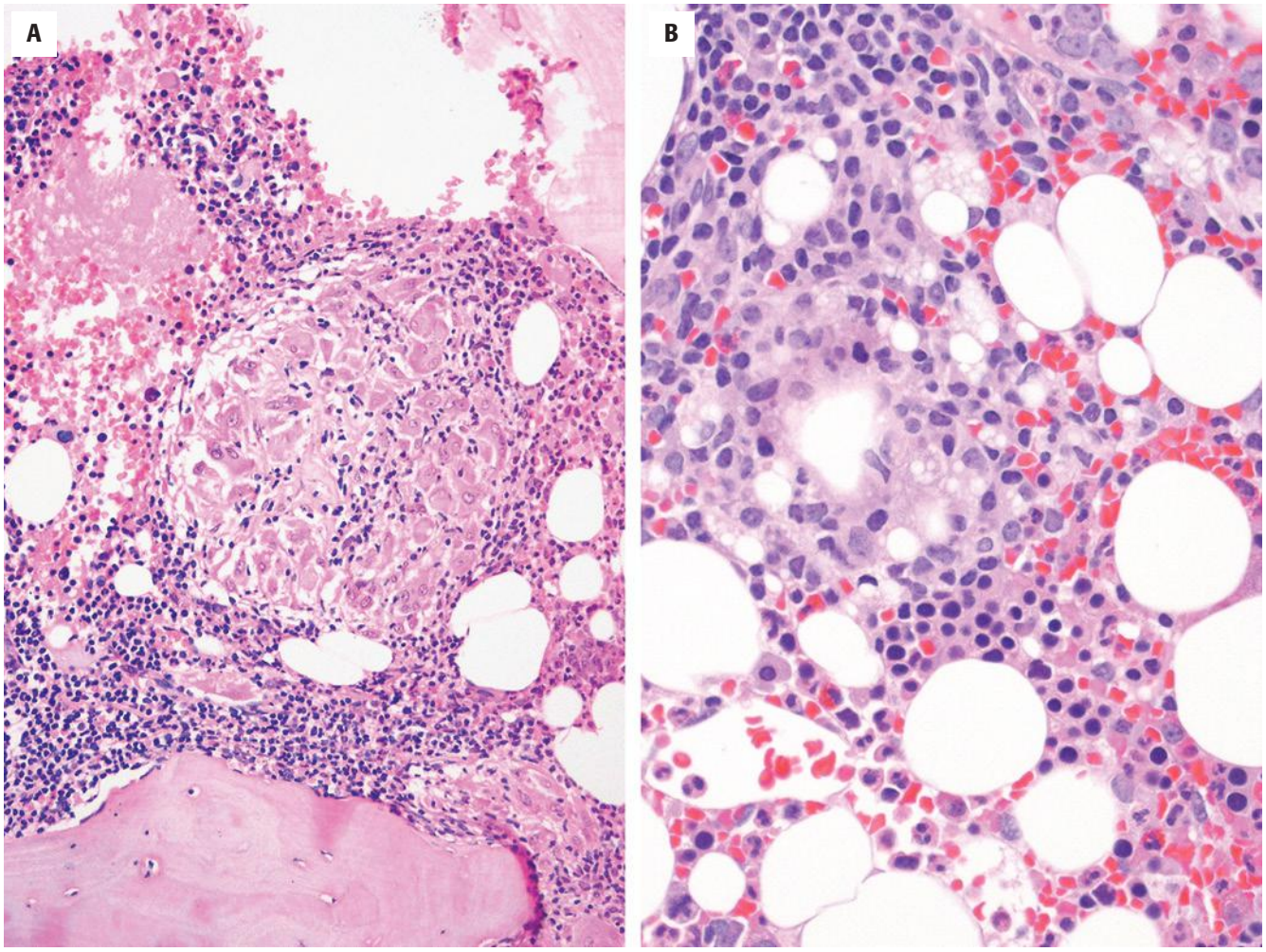


FIG. 6.10

Granulomas. A, A sarcoidal granuloma in marrow. Note the well-defined nature of the granuloma, with sharp edges. B, A lipogranuloma, with central fat and associated lymphocytes.

CHRONIC ALCOHOLISM—PATHOLOGIC FEATURES

Peripheral Blood

- Red blood cells: macrocytosis, stomatocytosis, acanthocytosis
- WBCs: hypersegmented neutrophils, toxic changes, vacuolated granulocytes
- Platelets: mild thrombocytopenia common

Aspirate Smear

- Cytoplasmic vacuoles may be seen; megaloblastic changes in erythroid series; toxic granulation of WBC precursors

Core Biopsy and Clot Section

- Often hypocellular
- Polyclonal plasmacytosis often present

Special Stains

- Iron stain may show ring sideroblasts
- Plasma cell iron is a rare finding

Differential Diagnosis

- Myelodysplastic syndromes (because of red blood cell changes and ring sideroblasts)
- Monoclonal gammopathies of undetermined significance/myeloma (plasmacytosis)

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Inflammatory bowel disorders, such as Crohn disease, may uncommonly be associated with the formation of bone marrow granulomas, with a lack of identifiable organisms. As mentioned previously, in Whipple disease the causative gram-positive organism can be found in the bone marrow in many cases.

Renal Diseases

Disorders of the kidneys are often associated with hematologic disorders. In chronic renal failure, there is a profound anemia resulting from a lack of erythropoietin production. Although the anemia is multifactorial, erythropoietin supplementation in these patients is often effective in ameliorating the anemia. Peripheral blood changes seen in chronic renal failure include increased numbers of echinocytes (burr cells). Bone marrow changes are those seen in anemia of chronic disease. A characteristic finding in renal disease includes the presence of diffuse changes of bone due to renal osteodystrophy. In these cases the intertrabecular spaces show myelofibrosis and increased vascularity. There is also increased activity of osteoblasts and osteoclasts with abnormally mineralized bone trabeculae. Hematopoietic elements are often reduced in number. Hemosiderin-laden macrophages can also be seen.

Autoimmune Diseases

A variety of autoimmune disorders have nonspecific effects on the blood and marrow. In the peripheral blood, the presence of lymphocytosis and activated or plasmacytoid forms is common. Anemia and megaloblastic changes in the erythroid series are common to almost all autoimmune diseases. In the bone marrow, lymphocytosis, lymphoid aggregates, and plasmacytosis are also common. Occasionally reticulin fibrosis may be observed (see autoimmune myelofibrosis later in this section).

Autoimmune disorders associated with decreased bone marrow cellularity include systemic lupus erythematosus (SLE), Sjögren syndrome, and rheumatoid arthritis (RA). Another autoimmune disorder associated with marrow changes is Felty syndrome, which is the association of RA with neutropenia and splenomegaly. Both RA alone and Felty syndrome, and to a lesser extent other autoimmune diseases, have an association with large granular lymphocytosis and large granular lymphocytic leukemias. SLE is associated with a variety of hematologic complications including leukopenia, anemias (including rare cases of pure red blood cell aplasia), and thrombocytopenia. Almost all the phenomena in SLE are autoimmune-mediated destruction of cellular elements. Thyroid disease can be associated with anemia, often resulting from erythroid hypoplasia. Macrocytosis and acanthocytes may be seen commonly in the peripheral blood. Rarely, in hypothyroidism, there may be serous atrophy in the bone marrow.

One of the more common autoimmune disorders seen in bone marrow evaluations is ITP. ITP is associated with in vivo destruction of platelets, most often caused by antibodies against common platelet antigens. There are few specific findings in the peripheral blood, other than thrombocytopenia, of varying severity. In the marrow, the most common finding is an increase in megakaryocytes, including smaller, more immature forms and senescent forms. Rarely, there are lymphocytes surrounding the megakaryocytes, in a rosette formation. These findings are not specific for ITP and can be found in a variety of causes of peripheral platelet destruction including infections, medication-related platelet destruction, and hypersplenism.

Other autoimmune cytopenias can occur. In autoimmune hemolytic anemia, peripheral blood findings include reticulocytosis, with an increase in spherocytes and occasionally poikilocytosis. Hemolysis is classically extravascular, although intravascular hemolysis can occur, particularly in fulminant cases. Laboratory findings include decreased haptoglobin and an increase in lactate dehydrogenase (LDH) and bilirubin. The direct antiglobulin test is usually positive and an IgG panagglutinin antibody ± complement C3. Bone marrow changes include a compensatory increase in erythroid elements. Autoimmune hemolytic anemia has several secondary causes (malignancy, including underlying lymphoproliferative disorders), systemic autoimmune disease, infections, and drug exposures. Autoimmune neutropenia is considerably less common than ITP or autoimmune hemolytic anemia. As anticipated, the peripheral blood shows an absolute decrease in neutrophils. The marrow most commonly shows a compensatory increase in myeloid precursors, an increased M:E ratio, and a left shift in maturation.

One disorder that deserves special mention is autoimmune myelofibrosis. This rare disorder is usually found in young women with a history of other autoimmune phenomena, such as SLE or RA. It can manifest with anemia or other cytopenias. The marrows are not aspirable. A review of the core biopsy morphology reveals relatively normal-appearing marrow, without significant dyspoiesis, except for general changes seen in autoimmune disorders (e.g., plasma cells, lymphocytes). However, reticulin staining of the marrow reveals moderate to severe fibrosis (Fig. 6.11). The importance in recognizing this disorder is not to confuse it with other neoplastic causes of myelofibrosis, such as idiopathic myelofibrosis.

IgG4-related disease (IgG4RD), a poorly understood but likely autoimmune disorder, may show manifestations in bone marrow. Polytypic IgG4-positive plasma cells may be present, but well-defined criteria for bone marrow involvement are lacking. With clinical suspicion, correlation with other possible sites of involvement with increased IgG4-positive plasma cells, serum IgG4 levels, and the presence of other autoimmune or systemic disorders should be considered.

STORAGE DISEASES

Clinical Features

The diagnoses seen in bone marrow are most commonly secondary to a congenital metabolic disorder that causes

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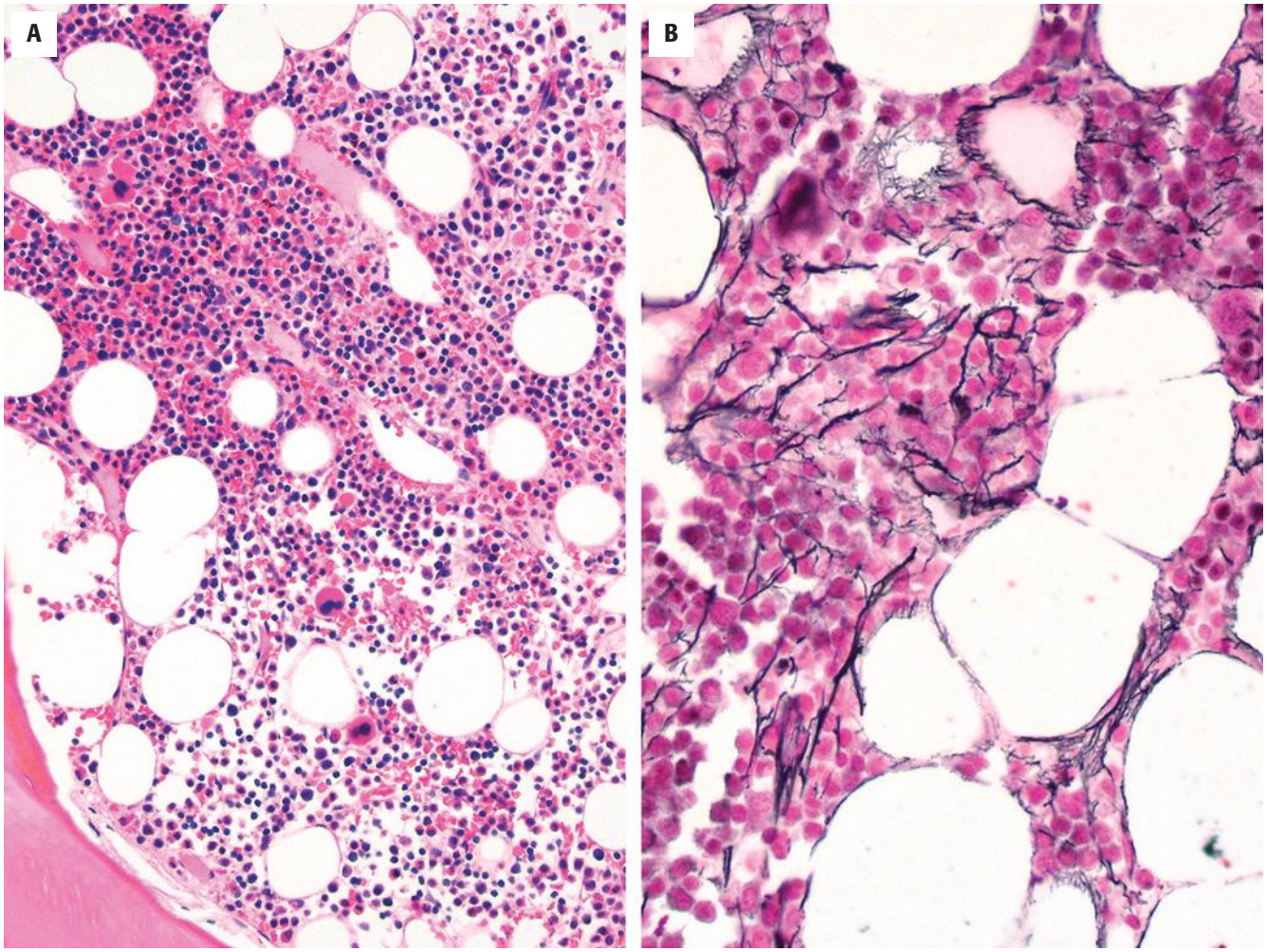


FIG. 6.11

Autoimmune myelofibrosis. A relatively normal-appearing bone marrow that is not aspirable (A) may be due to reticulin fibrosis (B), as in this case. Autoimmune myelofibrosis is most often identified in women with other autoimmune manifestations.

STORAGE DISEASES—FACT SHEET

Definition

- Accumulation of metabolic product, typically because of inherited abnormality (often autosomal-recessive), leading to varying physiologic dysfunctions

Incidence

- Rare

Clinical Features

- Variable depending on specific disorder and presentation
- Many present in infancy and childhood and may be associated with hepatosplenomegaly

Laboratory Features

- Specific defects lead to metabolic derangements
- May have anemias or thrombocytopenia due to marrow involvement
- Liver involvement may lead to liver function abnormalities

accumulation of a biochemical intermediate substance (Box 6.4). Occasionally, similar findings can be seen in both abnormal physiologic states or in association with neoplasms. Most of the metabolic diseases manifest in pediatric age groups, although some disorders manifest in adulthood. Many of these storage disorders are of autosomal-recessive inheritance. In the case of Gaucher and Niemann-Pick diseases, the incidence is increased in Ashkenazi Jews.

Pathologic Features

The two most commonly encountered storage disorders include Gaucher disease and Niemann-Pick disease. Gaucher disease is caused by an inborn error in metabolism, leading to accumulation of glucocerebroside in macrophages. There are several clinical variants of this disorder, with different degrees of clinical severity. In the bone marrow, there is an

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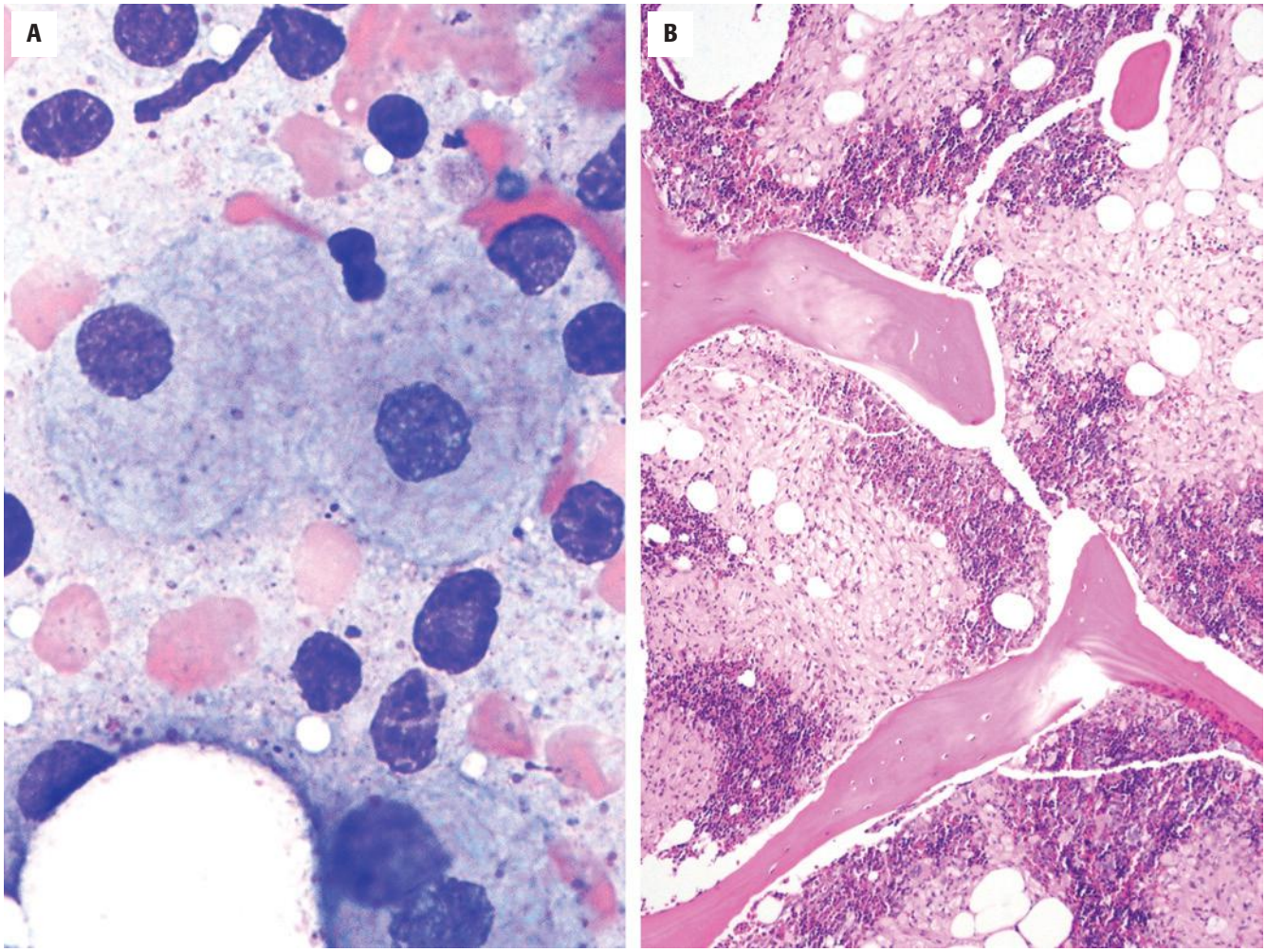


FIG. 6.12

Gaucher disease in bone marrow. A, Macrophages filled with excess metabolic product with "folded tissue paper" appearance. B, The same macrophages in a marrow core biopsy, showing large clusters and aggregates.

Box 6.4 Storage Disorders With Marrow Involvement

Neimann-Pick disease (types A, B, C)
Gaucher disease
Wolman disease
Cholesterol ester storage disease
GM_1 gangliosidoses
Tangier disease
Mannosidosis
Fucosidosis
Neuraminidase deficiency

accumulation of macrophages engorged with the excess metabolic product (Fig. 6.12). These macrophages have a distinctive appearance, with a pale blue cytoplasm in the standard Wright-Giemsa stain and a delicate "folded tissue paper" appearance of the cytoplasm. In core biopsies with H&E stains, the macrophages are large and in clusters, with dense pink cytoplasm. The cells are strongly PAS positive and diastase resistant. Similar cells can be seen in other disorders with rapid cell turnover, such as chronic myelogenous leukemia. These cells, termed *pseudo-Gaucher cells*, have a similar appearance, although they are usually much less numerous than in the metabolic disorder.

Niemann-Pick is a disorder of sphingomyelin metabolism. The macrophages are filled with numerous small vacuoles that contain a fatty metabolic product (Fig. 6.13). The cells usually stain only faintly with PAS but may be positive with Sudan Black B stains. In aspirate smears and core biopsy specimens, these cells may be seen singly or in clusters.

Storage disorders can mimic neoplasms on occasion. Most commonly seen are forms of Niemann-Pick and Gaucher disease. These can mimic unusual lymphomas or, more rarely, true histiocytic malignancies. Histologically, these clusters of macrophages are benign. The cells lack atypical features and have large amounts of cytoplasm. Depending on the product and the type of staining, they may appear pale and vacuolated.

Fabry disease is an X-linked storage disorder caused by a mutation in α-galactosidase-A and build-up of globotriaosylceramide and related glycosphingolipids in involved tissues

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STORAGE DISEASES—PATHOLOGIC FEATURES

Peripheral Blood
- Rare disorders may have prominent peripheral blood findings, such as prominent toxic-like granulation seen in mucopolysaccharidoses (e.g., Alder-Reilly anomaly)

Aspirate Smear
- Macrophages distended with abnormal metabolic product
- Gaucher disease: pale blue, folded tissue paper appearance
- Niemann-Pick disease: pale, with multiple small vacuoles

Core Biopsy and Clot Section
- Enlarged macrophages possibly present in clusters or sheets
- Often pale, granuloma-like appearance of H&E

Flow Cytometry, Immunohistochemistry, Molecular, Cytogenetic
- Molecular genetic studies are available to evaluate many inherited storage diseases

Differential Diagnosis
- More common types: Gaucher disease (glucocerebrosidase deficiency) and Niemann-Pick disease (sphingomyelinase deficiency); occasionally mimic malignancy or infectious etiologies

such as the heart, kidney, and nerves. Characteristic lamellar lysosomal inclusions are seen by electron microscopy. Macrophages in bone marrow contain globular inclusions in Romanowsky-stained smears. Sea blue histiocytes are macrophages containing blue-pigmented lipid on Romanowsky-stained smears and can be seen in several disorders including storage disease (Niemann-Pick); neoplastic processes, such as chronic myelogenous leukemia or myelodysplastic syndrome; iatrogenic settings, such as total parenteral nutrition; and reactive conditions, such as ITP.

BONE CHANGES

Clinical Features

The clinical features of bone abnormalities are variable and depend on underlying pathologic processes.

Pathologic Features

Evaluation for the presence of bone abnormalities is worthwhile and can lead to an unexpected diagnosis or support a clinical diagnosis. Changes, such as thickening or thinning of bone trabeculae, should be noted. Abnormal osteoid seams may indicate a bone disorder. Osteoblasts and osteoclasts are not prominent in adult bone, although they are common in pediatric specimens. An increase in osteoblastic or osteoclastic activity can be seen in diseases such as myeloma, metastases, or primary bone disorders, such as Paget disease. Sites of previous biopsy and metabolic diseases can also cause significant bone changes. Osteoblasts are seen more frequently in children with active bone formation. These cells may be confused with plasma cells and have a similar size and cytoplasmic color (pale blue in the Wright-Giemsa stain). They also have a small, round eccentric nucleus. One

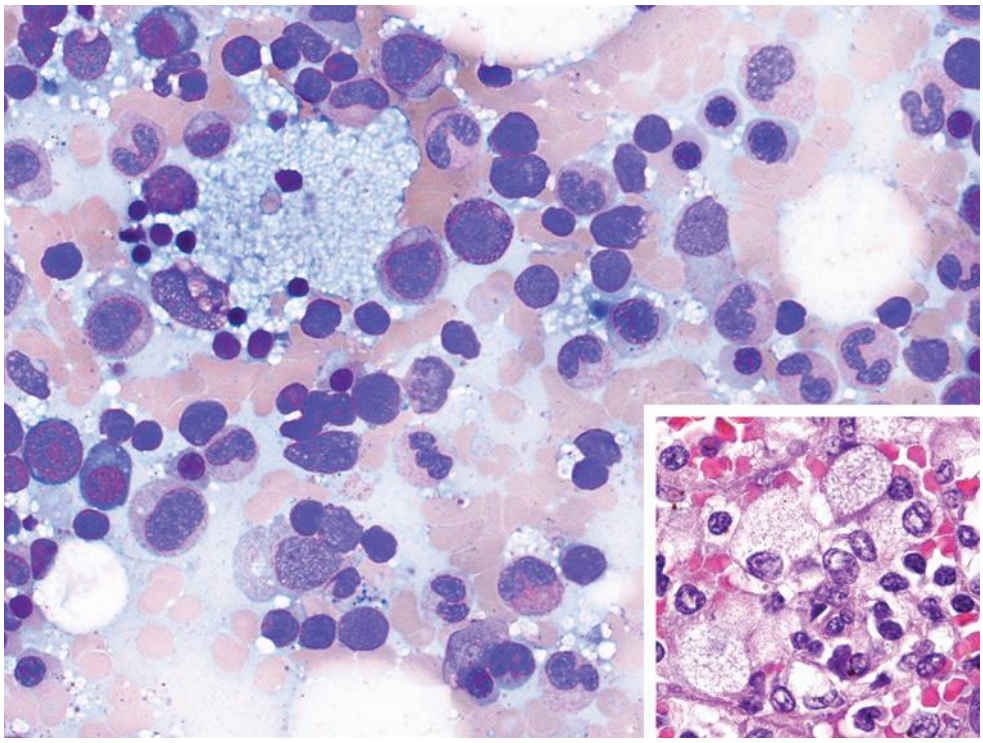


FIG. 6.13
Niemann-Pick disease in bone marrow. Macrophages filled with excess metabolic product have a multivacuolated appearance, in contrast to the appearance of Gaucher disease. *Inset,* Hematoxylin and eosin stain of Niemann-Pick macrophages, in this case in a spleen.

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BONE MARROW FIBROSIS—FACT SHEET

Definition
- Formation of reticulin/collagen fibrosis induced by a variety of neoplastic and non-neoplastic etiologies

Clinical Features
- Severe marrow fibrosis may be associated with splenomegaly

Prognosis
- Variable and dependent on underlying cause of fibrosis

BONE MARROW FIBROSIS—PATHOLOGIC FEATURES

Peripheral Blood
- In severe fibrosis, leukoerythroblastic blood may be seen
- Teardrop red blood cells may be seen

Aspirate Smear
- In severe fibrosis, bone marrow aspiration may not be possible

Core Biopsy and Clot Section
- Core biopsy may show minimal changes or severe streaming of cellularity between bands of collagen fibrosis, depending on severity and etiology

Special Stains
- Reticulin stain should be performed for evaluation of fibrosis. Trichrome stain is positive in severe (mature collagen) fibrosis (MF-2, MF-3)

Differential Diagnosis
- Chronic idiopathic myelofibrosis, other myeloproliferative neoplasms, acute megakaryoblastic leukemia, acute panmyelosis with myelofibrosis, HIV myelopathy, and autoimmune myelofibrosis

BONE MARROW NECROSIS—FACT SHEET

Definition
- Varying numbers of necrotic cells seen in bone marrow

Incidence
- Variable depending on the associated etiology

Clinical Features
- Features depend on underlying causes

difference from plasma cells is the location of the Golgi. In osteoblasts the Golgi is located near the cytoplasm edge, as opposed to the paranuclear location in plasma cells. Osteoclasts can be confused with megakaryocytes. They are large in size, but contrary to megakaryocytes, they have multiple nuclei rather than lobated nuclei.

BONE MARROW NECROSIS—PATHOLOGIC FEATURES

Peripheral Blood
- Often no abnormalities specific for bone marrow necrosis

Aspirate Smear
- Necrotic debris may be seen, with a lack of intact cells
- Debris-laden macrophages may be numerous

Core Biopsy and Clot Section
- Variably sized areas of nonviable cells or amorphous necrotic material are seen

Differential Diagnosis
- Etiology is variable and includes hematopoietic malignancies (especially acute lymphoblastic leukemia), nonhematopoietic malignancies (neuroblastomas), coagulation disorders (antiphospholipid syndrome, disseminated intravascular coagulation), red blood cell disorders (sickle cell), medications or chemotherapy, and other causes

One of the more commonly encountered primary bone disorders is osteoporosis. The finding typically encountered is thinning of the bone trabeculae. More rarely, disorders such as Paget disease are seen. Bone changes resulting from primary hyperparathyroidism include increased bone formation, fibrosis, and cyst formation (osteitis fibrosa cystica). Chronic renal disease also results in parathyroid hormone (PTH) excess, and the bone marrow changes associated with renal disease are similar (Fig. 6.14). Thickened bone trabeculae with prominent osteoid seams from osteoblastic and osteoclastic activity are seen. Stromal fibrosis develops around the trabeculae.

STROMAL DISORDERS

Clinical Features

The clinical features of stromal abnormalities are variable and depend on underlying pathologic processes.

Pathologic Features

Bone marrow stroma is often underappreciated in the evaluation of marrows. The stroma is a complex microenvironment that both nurtures and supports the development of hematopoietic cells. The stroma cells mostly consist of adventitial reticulum cells that are fibroblastic in nature and the precursors of myofibroblasts and smooth muscle cells that are found in the marrow. It is the adventitial reticulum cells and their derivatives that form the structural framework of the marrow. Other elements include fat cells, vascular elements, and bone. Additional components include undifferentiated stromal elements, which are typically inconspicuous cells in the marrow.

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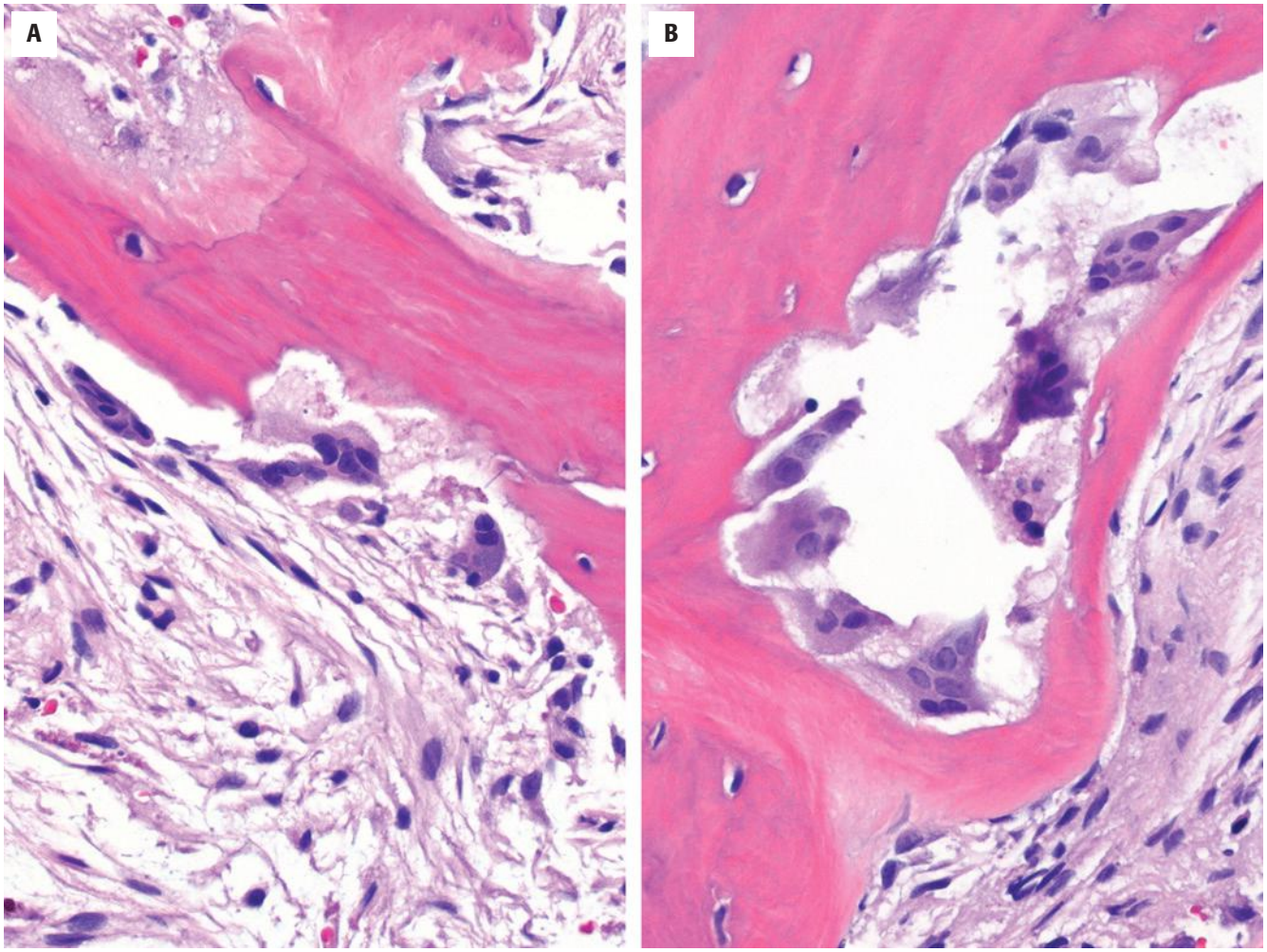


FIG. 6.14

Bone and marrow changes seen in renal disease. Interstitial fibrosis and irregular bone trabeculae (A) are common, as well as increased osteoclastic and osteoblastic activity (B).

A variety of stromal changes can make these elements more apparent. Fibrosis is a common reactive change in marrows (Fig. 6.15). Fibrosis in the marrow is typically secondary to other marrow changes. Reticulin, as assessed by the Gomori silver stain, is thought to represent largely immature collagen, presumably type III collagen; however, the stain identifies also type IV collagen. Fibrosis of the marrow is routinely measured on a scale of 0 to 3 (Table 6.2), as used in the current WHO classification. An important theoretical note is that fibrosis is not neoplastic; it is derived from non-neoplastic stromal cells in response to a variety of conditions.

Changes in fat are only rarely significant in the marrow. Lipogranulomas (discussed previously) are probably due to localized disruptions of fat cells. Serous fat atrophy, also called gelatinous transformation of bone marrow, is characterized by a reduction in hematopoietic cells and the loss of normal marrow fat cells (Fig. 6.16) replaced by adipocytes with a granular, pinkish cytoplasm. The hypocellular areas contain an amorphous extracellular gelatinous substance that is composed of mucopolysaccharides rich in hyaluronic acid.

TABLE 6.2

Grading of Myelofibrosis

MF-0	Scattered linear reticulin fibrosis with no intersections or cross-overs of fibers, similar to that seen in normal bone marrow. No significant staining of collagen staining with trichrome.
MF-1	Loose network of reticulin-positive fibers with many intersections, especially in perivascular areas. No significant collagen staining with trichrome.
MF-2	Diffuse and dense increase in reticulin fibers with many intersections, with focal bundle of thick fibers. These thick fibers are collagen and will be focally positive with trichrome stain. Focal osteosclerosis may be present, with or without trichrome-positive collagen.
MF-3	Diffuse and dense increase in reticulin fibers with extensive intersections and coarse bundles of fibers. Extensive collagen deposition can be identified by trichrome staining. Osteosclerosis is often present.

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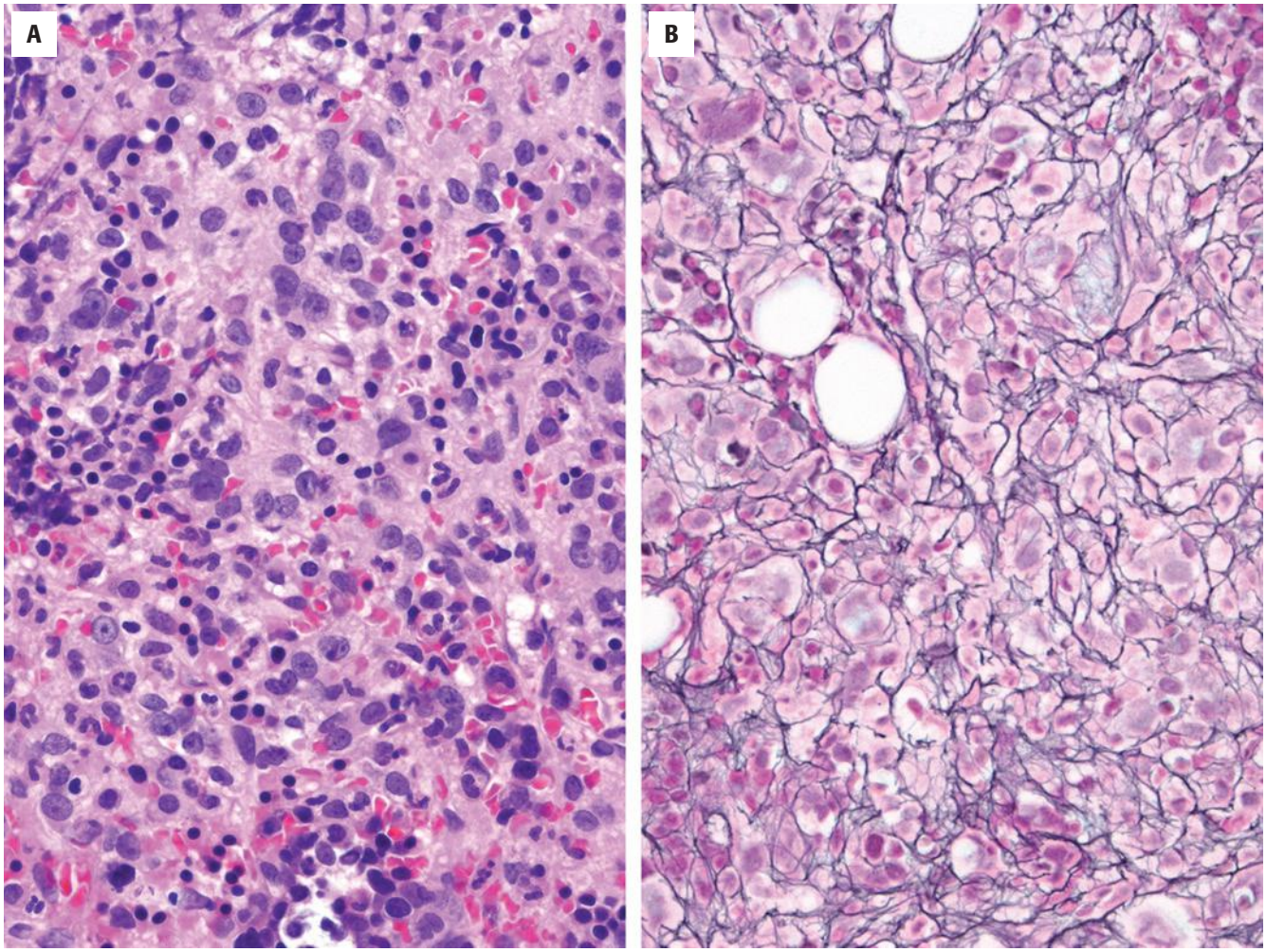


FIG. 6.15

Bone marrow fibrosis. A, This case of acute myeloid leukemia developing from a previous myeloproliferative disorder has extensive reticulin fibrosis. B, The stromal elements that produce the fibrosis are not part of the neoplastic clone but are in response to it.

This change is seen most often in states of severe metabolic compromise, such as cachexia or anorexia. However, it can be seen uncommonly in a wide variety of settings, including HIV infection, malignancies, alcoholism, acute febrile illnesses, and autoimmune disorders such as SLE.

Necrosis, an uncommon finding in the bone marrow, can be associated with a variety of causes (Box 6.5). Focal necrosis can be seen in a variety of infections and can be associated with granulomas. In addition, disorders of abnormal coagulation can be associated with ischemic marrow necrosis. These disorders include disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, and antiphospholipid antibody syndrome. A relatively common cause of significant marrow necrosis is sickle cell disease. Subsequent embolization of the necrotic marrow is thought to contribute to the acute chest syndrome. Malignancies, both as initial presentations or after treatment, can manifest as areas of necrosis or sheets of "ghost" cells (Fig. 6.17). In general, it is difficult to determine the etiology and cell type based on histologic findings alone. If there is viable

Box 6.5 Disorders Associated With Bone Marrow Necrosis

Sickle cell disease
Severe infections
Disseminated intravascular coagulation
Thrombotic thrombocytopenic purpura
Hemolytic-uremic syndrome
Antiphospholipid syndrome
Acute graft-versus-host disease after bone marrow transplant
Megaloblastic/sideroblastic anemias
Malignancies
Hematologic (e.g., acute lymphoblastic leukemia, acute myeloid leukemia, Hodgkin lymphoma, chronic myelogenous leukemia, myeloma)
Carcinomas
Neuroblastoma and other "small blue cell" tumors

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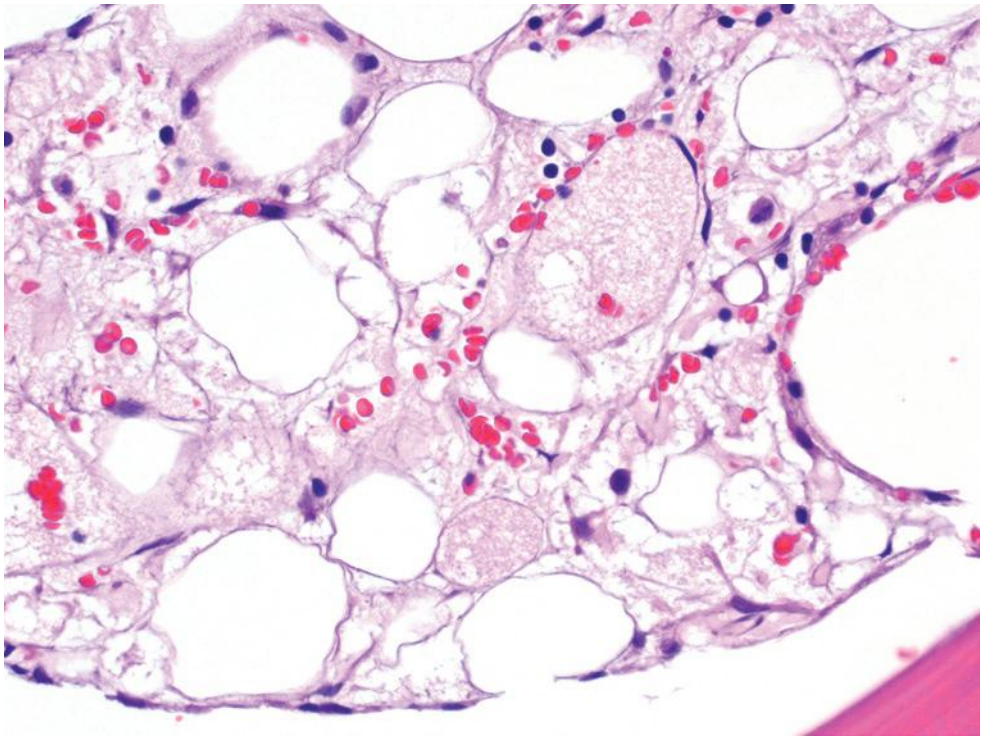


FIG. 6.16

Serous atrophy, also known as *gelatinous transformation of bone marrow,* can be associated with a variety of causes, such as anorexia nervosa, chemotherapy, HIV/AIDS, and malnutrition states.

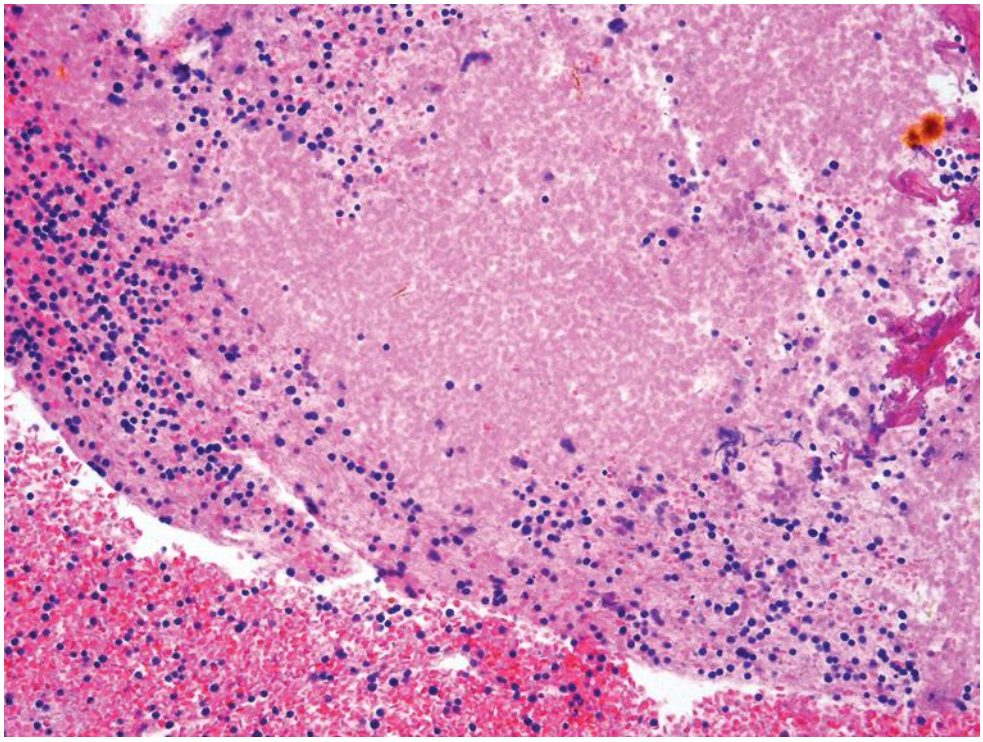


FIG. 6.17

Bone marrow infarct can be associated with both benign and neoplastic causes. This case illustrates necrosis in a case of acute lymphoblastic leukemia, with rare viable tumor cells present.

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marrow present, it will often provide clues to the diagnosis. Correlation with clinical history, and less commonly repeat biopsies, often yield a diagnosis.

LYMPHOCYTES AND LYMPHOID AGGREGATES

Clinical Features

Many different clinical conditions can be associated with the presence of an increased number of lymphocytes, lymphoid aggregates, and lymphoid precursors (hematogones) in the bone marrow. Lymphocytes represent approximately 20% of the normal marrow cellularity in adults and typically consist predominantly of T cells (T:B ratio approximately 4:1). Unlike in blood, CD8+ T-cells outnumber CD4+ T-cells. B cells are more numerous in children, where the marrow is the site of formation and early development of B cell precursors, or hematogones. Increases in lymphocytes, hematogones, or lymphoid aggregates may be seen in both reactive and neoplastic conditions.

Pathologic Features

Hematogones

Hematogones are benign, immature lymphoid precursor cells that are more numerous in the bone marrow of children. They are often increased after chemotherapy and in reactive marrow conditions. They are usually small, but can be of intermediate size, overlapping the size range of lymphoblasts (Fig. 6.18); when increased, they can be confused with acute lymphoblastic leukemia (ALL) or lymphoblastic lymphoma. In contrast to leukemic blasts, hematogones typically have a more condensed, smudged chromatin, and only rarely have nucleoli. Hematogones tend to be dispersed throughout the marrow, with no tendency to cluster, in contrast to leukemic blasts. The immunophenotype largely overlaps that of blasts seen in precursor B-ALL. For example, hematogones are positive for CD19 and CD10. Careful flow cytometric analysis of hematogones can usually distinguish them from precursor B-ALL. Hematogone populations often express a spectrum of CD10, CD20, CD34, and surface immunoglobulin, reflecting their maturation from immature B cells to more mature stages. Leukemic populations tend to be more homogeneous in their expression pattern, often express abnormally high or low levels of one or more of these antigens, and may aberrantly express myeloid markers. This fact is often reflected in the immunohistochemical staining patterns. Using immunohistochemistry for CD34 and TdT, markers of precursor cells, hematogones tend to be more variably positive, and scattered in distribution (interstitial distribution with clusters <5 cells) whereas B-ALL cells are more uniformly positive and show a tendency to form clusters. CD20 usually stains hematogones more strongly and consistently than the lymphoblasts, which variably express CD20 within the same case. These features can be helpful in distinguishing relapsed B-ALL from regenerative hematogones.

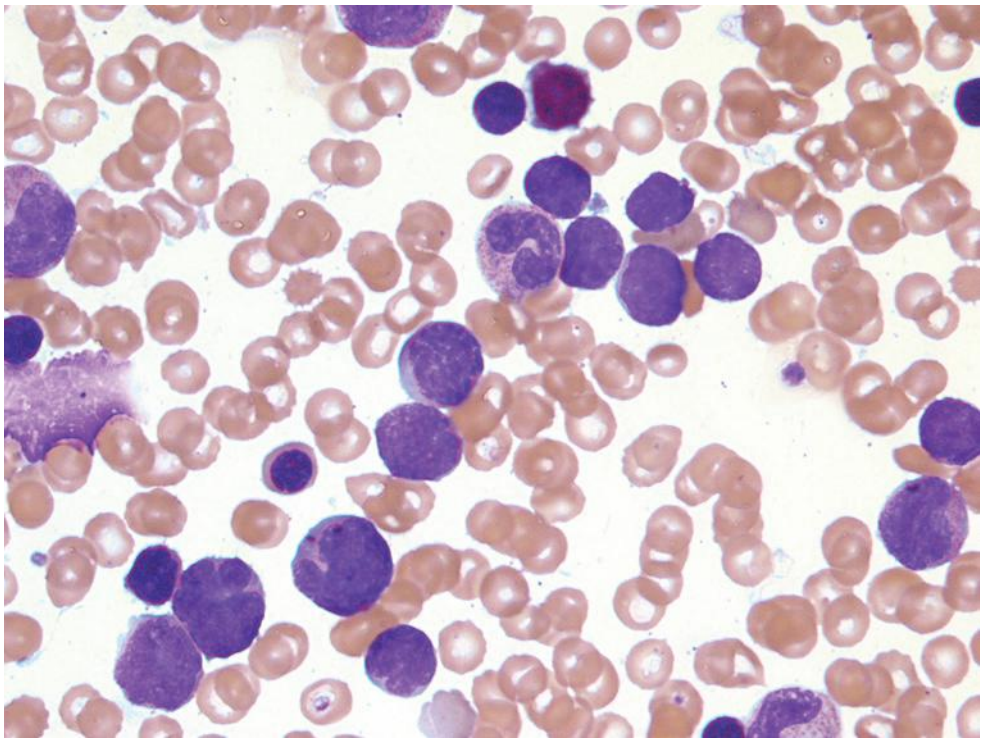


FIG. 6.18

A bone marrow aspirate showing numerous hematogones or B lymphocyte precursors. Hematogones are more common in the bone marrow of children, especially in association with other reactive conditions and after chemotherapy.

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Lymphoid Aggregates

Lymphoid aggregates (LAs) are frequently encountered in the bone marrow. They are not always pathologic, and their form, location, number, and most important, the clinical setting in which they are observed should be considered when evaluating them (Table 6.3). LAs tend to occur with more frequency as patients age. In addition, they are more common in patients with chronic disease and autoimmune conditions. Numerous or large lymphoid aggregates should always raise a concern for lymphoma. A paratrabecular location of lymphoid aggregates is suggestive of malignancy and should be evaluated by immunohistochemical stains. The composition of the LA is also important. Benign LAs are typically composed of predominantly small lymphocytes, with an intermixture of histiocytes and plasma cells with only rare larger lymphocytes. However, rare, well-formed germinal centers can be seen and are more often benign than malignant. If the cellular composition of the aggregate is homogeneous or there are numerous large lymphocytes, the possibility of lymphoma should be considered. Benign lymphoid aggregates are typically located adjacent to or associated with blood vessels. They typically have sharper, more well-defined borders than do lymphoma aggregates in marrow.

A difficulty in the assessment of posttreatment marrow is associated with the recent use of monoclonal antibody-based treatments. Both rituximab (anti-CD20 antibody) and alemtuzumab (anti-CD52 antibody), which are used in the treatment of lymphoid malignancy, are associated with the development of large reactive T-cell–rich LAs in posttreatment marrows that may be mistaken for residual lymphoma of leukemia. In the setting of B-cell malignancy, immunostaining can assist in distinguishing between reactive T-cell aggregates and B-cell lymphoproliferative disorders. This evaluation is further complicated by the fact that B cells can lose CD20 expression after treatment with anti-CD20 antibodies. In the context of prior anti-CD20 antibody therapy, a back-up B-cell stain (CD79a or PAX5) should be used to evaluate the composition of the lymphoid aggregates.

TABLE 6.3

Features of Bone Marrow Lymphoid Aggregates Suggesting Malignancy

Characteristic	*Benign*	*Malignant*
B-cell pattern[a]	+	++
T-cell pattern[b]	+++	-
Infiltrative edges	-	+++
Paratrabecular location	+	++
Large size	-	+
Cytologic atypia	-	++

[a]B-cell pattern: Composed predominantly of B cells or a central core of B cells surrounded by T cells.
[b]T-cell pattern: Composed of predominantly T cells, haphazard mixtures of T and B cells or a central core of T cells surrounded by B cells.
From Johnston A, Brynes RK, Naemi K, et al: *Arch Pathol Lab Med* 139:233–240, 2015.

CYTOKINE EFFECTS

Clinical Features

Cytokines are frequently used to treat a variety of cytopenic conditions. Most commonly, pathologists see marrows of patients who have received granulocyte–colony-stimulating factor (G-CSF), granulocyte macrophage–colony-stimulating factor (GM-CSF), or erythropoietin.

Pathologic Features

With all types of cytokine treatments, the marrow may show an increase in immature cells and cellularity, which can mimic neoplastic conditions. The effects of G-CSF or GM-CSF can mimic acute myeloid leukemia (AML), but occasionally myelodysplastic syndromes or myeloproliferative neoplasms can be simulated. The single most important factor in dealing with these situations is obtaining an appropriate clinical and treatment history; this can resolve most difficulties. GM-CSF and G-CSF will cause increases in immature cells in the peripheral blood. These cells typically include left-shifted myeloid elements in a variety of stages of maturation. In particular, myeloid blasts can occasionally be seen. Neutrophils and precursors usually display toxic granulation (Fig. 6.19). In the marrow, there will usually be an increase in the M:E ratio associated with left-shifted granulopoiesis with numerous promyelocytes and myelocytes. Promyelocytes may be increased to a degree to mimic acute promyelocytic leukemia. Again, toxic changes are characteristically present early in therapy. A slight increase in blasts may be seen. Concurrent flow cytometry on G-CSF-treated samples may show some dyspoietic changes in maturation and antigen expression and may mimic a myeloid

CYTOKINE EFFECTS—FACT SHEET

Definition
- Marrow changes associated with the pharmacologic use of cytokines for treatment
- Marrow changes may be predominant in individual cell lineages, depending on the cytokine used

Incidence
- Increases in use of cytokine therapies make these effects relatively common

Prognosis and Therapy
- G-CSF often used in stem cell mobilization
- Erythropoietin used in several types of chronic anemias

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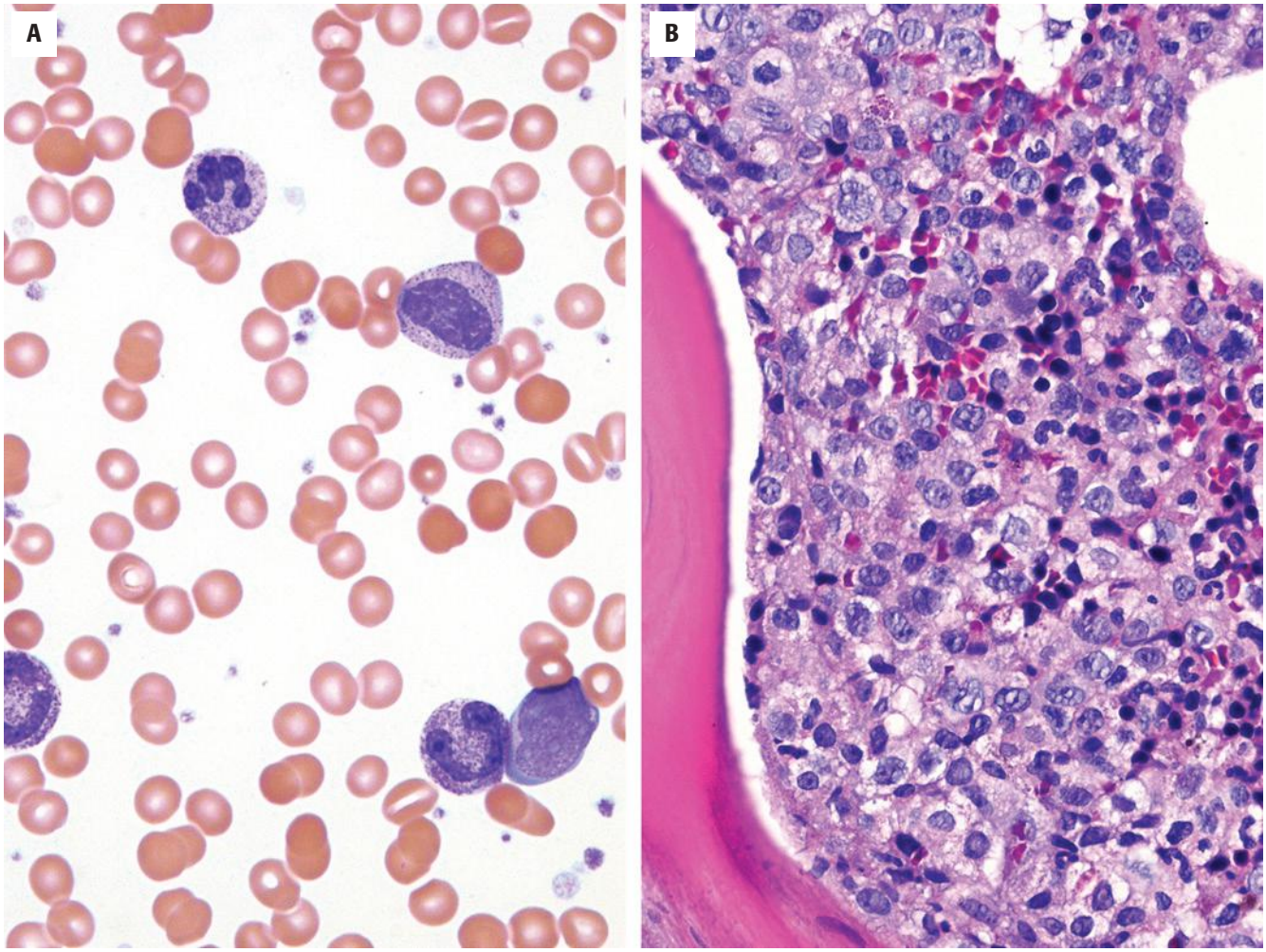


FIG. 6.19

Examples of peripheral blood (A) and marrow findings (B) in a case of granulocyte colony-stimulating factor therapy. The blood shows circulating blasts, but toxic changes in the more mature granulocytic cells suggest a reactive process such as growth factor effect. In the biopsy specimen, the pattern of development of granulocytic maturation from the bone trabeculae outward also points to a reactive process.

CYTOKINE EFFECTS—PATHOLOGIC FEATURES

Peripheral Blood

- G-CSF: increased in granulocytes, left shift with increased blasts, increased coarse toxic granulation

Aspirate Smear

- G-CSF: increased M:E ratio, left shift but overall blast percentage not significantly increased
- GM-CSF: similar to G-CSF with increases in monocytic elements
- Erythropoietin: decreased M:E ratio, megaloblastic changes, dysplastic features mimicking myelodysplastic syndrome (MDS) or acute erythroleukemia

Core Biopsy and Clot Section

- Often hypercellular; increases in individual cell lineages; often increase in immature elements

Differential Diagnosis

- Increased immature elements may be confused with leukemia
- Clinical history is important for evaluation
- Repeated biopsy may be necessary

stem cell disorder. With GM-CSF, in addition to the effect on granulopoiesis, there are often increased monocytes, monocytic precursors, and variably increased numbers of eosinophils. On more than one occasion changes of growth factor (G-CSF or GM-CSF) therapy have been misinterpreted as acute leukemia or myeloproliferative neoplasms. Presence of only modestly elevated blast counts, a spectrum of maturation in the granulocytic lineage (albeit concentrated in the early forms), toxic granulation, and a good history allow one to avoid mistaking growth factor effects for a myeloid malignancy. Patience will also clarify the matter because it is possible to follow the maturation of the granulocytic cells over the course of a few days or weeks. G-CSF and GM-CSF effects are also reviewed in Chapter 14 on acute myeloid leukemias.

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The effects of erythropoietin only rarely cause diagnostic difficulty. There is a predictable increase in erythroid precursors associated with a decrease in the M:E ratio. The increased immature erythroids may appear concerning on core biopsy specimens in which they may simulate early myeloid cells, but their erythroid nature is usually easily identifiable in aspirate smears. If difficulty remains, immunostaining with CD71, E-cadherin, or other erythroid markers can be used to confirm their erythroid derivation. At high levels, erythropoietin drives megakaryopoiesis, so that megakaryocytes may appear increased as well.

TOXINS

The effect of toxins on marrow depends on the agent. Benzene is well known as a cause of aplastic anemia and myelofibrosis. Pesticide exposure can also cause transient marrow aplasia. The aplastic marrows are not themselves difficult to diagnose. Rather, the recovery from a toxic insult can be troublesome. In these cases, the marrow tends to recover in waves showing a reactive hyperplasia of synchronized myeloid precursors. This finding can be concerning and raise a differential of AML or acute promyelocytic leukemia. In these cases, clinical correlation may be the key. Often the myeloid cells appeared to be morphologically normal promyelocytes. These cells do not have the morphologic abnormalities associated with acute promyelocytic leukemia (folded nuclei, Auer rods). Further investigation of clinical history for toxin or drug exposures, or repeated biopsy after an appropriate interval, typically resolve difficult cases.

Toxins and drug reactions may also mimic MDS. For example, arsenic poisoning can result in erythroid hyperplasia and severe dyserythropoiesis resembling MDS. Zinc overexposure, from excessive dietary supplements, results in copper deficiency, and a sideroblastic anemia mimicking myelodysplasia. Dyserythropoiesis with vacuolization, ring sideroblasts, and plasma cell iron granules are seen. Valproate therapy or toxicity can induce dysplastic changes that can be mistaken for MDS.

CHEMOTHERAPY

Clinical Features

Chemotherapy-related effects are seen in the bone marrow of patients undergoing systemic treatment for a variety of pathologic conditions, most often malignancies.

Pathologic Features

Antineoplastic chemotherapy can cause marrow suppression and a variety of dyshematopoietic changes. The major changes seen in myeloablative therapy are reviewed in Chapter 14.

CHEMOTHERAPY EFFECTS—FACT SHEET

Definition
- Marrow changes associated with cytotoxic chemotherapy

Clinical Features
- Variable depending on type, extent, and time since chemotherapy

CHEMOTHERAPY EFFECTS—PATHOLOGIC FEATURES

Peripheral Blood
- Cytopenias not uncommon

Aspirate Smear
- Hypocellularity is common
- Erythroid lineage shows dyspoiesis
- Increases in stromal elements are common

Core Biopsy and Clot Section
- Hypocellularity is common
- Clusters of immature-appearing erythroid and myeloid elements may be seen
- Mild fibrosis may be seen
- Stromal elements may be relatively increased with decreases in hematopoietic elements

Differential Diagnosis
- Evaluation for residual and recurrent disease is important

However, it is important to note that chemotherapy typically consists of antimetabolite drugs. Marrow, being metabolically active, can easily be affected by these medications. In any drug where anemia or myelosuppression can occur as a side effect, dysplasia-like changes can be seen in the marrow. In particular, the various dyserythropoietic changes seen in the myelodysplastic syndromes can be closely replicated by chemotherapy.

SUMMARY

A broad range of disorders affect the bone marrow that are not due to malignancy but rather are caused by infections, storage diseases, systemic disorders, or stromal changes. The findings in these disorders are highly variable and may be nonspecific; however, some show distinctive morphologic or immunophenotypic findings that make a diagnosis relatively straightforward. Because of the broad range of hematologic and clinical presentations, many of the conditions may be the primary or diagnostic manifestation of a disease, and their recognition and differentiation from other marrow disorders are important.

The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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SECTION 2

Lymphomas

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7

Small B-Cell Lymphomas

■ Rebecca L. King, MD ■ Paul J. Kurtin, MD

■ INTRODUCTION

This chapter covers the lymphomas of small B lymphocytes: small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL), mantle cell lymphoma (MCL), follicular lymphoma (FL), extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT), splenic marginal zone lymphoma (SMZL), nodal marginal zone lymphoma (NMZL), and LPL. Though morphologically somewhat similar to one another, these tumors are characterized by wide differences in clinical presentation, phenotype, genetic features, and outcome. Also discussed are the lesions of uncertain malignant potential related to follicular and mantle cell lymphomas: in situ follicular neoplasia (ISFN) and in situ mantle cell neoplasia (ISMCN).

In the past, the classification of the small lymphocytic lymphomas was based on the concept that there was a one-to-one correspondence between a lymphoma type and a corresponding normal cell counterpart. This is indeed true for FL and MALT lymphoma, which are thought to recapitulate the normal germinal center reaction (FL) or arise from normal mucosa-associated marginal zone B cells (MALT lymphoma). SLL/CLL and MCLs were thought to be the counterparts of naive B lymphocytes that had rearranged their immunoglobulin genes but that had not yet encountered antigen. Thus, one would predict that the cells of these two lymphoma types would have germline sequences of their immunoglobulin variable genes as would be characteristic for normal naive B lymphocytes. However, 50% of SLL/CLLs and 25% of MCLs have mutated immunoglobulin gene variable regions, indicating that they are neoplasms of post-follicular B cells that have encountered antigen. Currently, the World Health Organization (WHO) classification of tumors of lymphoid tissues does not solely consider the putative normal cell counterpart as the basis for classification of these tumors but attempts to define homogeneous entities based on a combination of clinical, morphologic, immunophenotypic, and genetic attributes. These are described in this chapter.

Three tasks confront the pathologist approaching the small B-cell lymphomas: distinguishing the lymphomas from reactive lymphoid hyperplasia, correctly classifying the lymphoma, and providing sufficient phenotypic and genetic information to the clinician so that he or she can select the most appropriate therapy. The cornerstone for accomplishing these tasks remains careful morphologic observations applied to well-fixed tissue sections on optimally prepared slides. Once one has reached the diagnosis of lymphoma, evaluating the tumor cell phenotype using a panel of antibodies to CD20, CD10, CD23, BCL6, BCL2, cyclin D1, CD3, CD5, and kappa and lambda immunoglobulin light chains (the latter two in paraffin sections to mark plasma cells), perhaps along with newer markers such as sex determining region Y-box 11 (SRY) and lymphoid enhancer binding factor-1 (LEF1), is usually sufficient to distinguish among all of the lymphomas discussed in this chapter. Genetic analysis is confined to the limited subset of cases that are not morphologically and phenotypically characteristic, as will be discussed later. Tables 7.1 through 7.4 summarize the morphologic, phenotypic, and genetic features that characterize the lymphomas discussed in this chapter.

■ SMALL LYMPHOCYTIC LYMPHOMA

Small lymphocytic lymphoma (SLL) is an indolent B-cell non-Hodgkin lymphoma that is treated in the World Health Organization classification as a single entity along with chronic lymphocytic leukemia (CLL). Historically, the term *SLL* was used for lymphomas that are morphologically and immunophenotypically indistinguishable from CLL but lacked lymphocytosis. However, most patients with "pure SLL" develop bone marrow involvement and lymphocytosis over the course of their disease. SLL in its "pure" form accounts for less than 10% of SLL/CLL and about 5% to 10% of all non-Hodgkin lymphomas. Many early studies on the features of SLL did not exclude blood involvement so that accurate information about the presentation, prognosis, and treatment of "pure" SLL is still lacking. This chapter reviews the pathologic features of lymph node–based SLL, whereas the features of CLL in blood and bone marrow are considered in Chapter 12 on mature B-cell leukemias.

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TABLE 7.1
Differential Diagnosis of Small B-Cell Lymphomas: Morphology

Disease	Lymph Node	Spleen	Bone Marrow	Cytology
CLL/SLL	Diffuse pattern, proliferation centers	Red pulp; cords, sinuses; later white pulp	Intertrabecular nodules, interstitial infiltrates; not paratrabecular	Small lymphocytes, prolymphocytes, and paraimmunoblasts
Mantle cell	Diffuse or nodular pattern, atrophic germinal centers	White pulp; atrophic germinal centers, obliterated marginal zones	Intertrabecular nodules, paratrabecular aggregates	Small lymphocytes with nuclear irregularity; NO large cells
MALT lymphoma	Paracortical infiltrates; surrounding germinal centers	White pulp marginal zones	Intertrabecular nodules, paratrabecular aggregates; intrasinusoidal infiltrates	Centrocyte-like cells, plasma cells, occasional large transformed lymphocytes; non-neoplastic germinal centers
Splenic marginal zone lymphoma	Nodular perifollicular pattern	White pulp nodules with dimorphic cytologic features	Intertrabecular nodules, paratrabecular aggregates, intrasinusoidal infiltrates	Dimorphic in spleen; mantle cell-like in center of nodules; medium size cells; irregular nuclei, abundant pale cytoplasm; occasional large transformed cells in perimeter of nodules
Nodal marginal zone	Perisinusoidal or surrounding benign germinal centers and mantle zones	White pulp; small germinal centers; residual non-neoplastic mantle cells	Intertrabecular nodules; paratrabecular aggregates; intrasinusoidal infiltrates	Medium size cells; irregular nuclei, abundant pale cytoplasm; occasional large transformed cells
Follicular lymphoma	True follicular nodularity	White pulp; germinal centers expanded by benign mantle and marginal zone cells	Intertrabecular nodules, paratrabecular aggregates	Small centrocytes and large centroblasts in varying proportions
Lymphoplasmacytic lymphoma	Paracortical and hilar infiltrates, open sinuses	Red pulp and occasionally white pulp	Intertrabecular nodules, paratrabecular aggregates, interstitial infiltrates	Small lymphocyte, plasmacytoid lymphocyte, plasma cell spectrum; varying large cells

CLL/SLL, Chronic lymphocytic leukemia/small lymphocytic lymphoma; *MALT,* mucosa-associated lymphoid tissue.

TABLE 7.2
Differential Diagnosis of Small B-Cell Lymphomas: Typical Phenotypes

	sIg	CD19	CD20	CD23	CD10/ BCL6	CD5	Cyclin D1	CD200	SOX11	LEF1
B-cell SLL/CLL	Monoclonal (dim)	+	+ (dim)	+	–	+	–	+	–	+
Mantle cell lymphoma	Monoclonal (bright)	+	+ (bright)	–	–	+	+	–	+	–/+
Marginal zone lymphoma (MALT, nodal and splenic)	Monoclonal (bright)	+	+ (bright)	–	–	±	–	–	–	–
Follicular lymphoma	Monoclonal (bright)	+	+ (bright)	±	+	–	–	–	–	–
Lymphoplasmacytic lymphoma	Monoclonal (also clg positive monoclonal plasma cells)	+	+	±	–	–	–	Dim/–	–	–

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TABLE 7.3
Differential Diagnosis of Small B-Cell Lymphomas: Cytogenetics

	Chromosome Abnormality	Genes Involved
B-cell SLL/CLL	+12; del(13q); del (11q), del (17p), del (6q)	Multiple
Mantle cell lymphoma	t(11;14)(q13;q32)	*CCND1-IGH*
MALT lymphoma	t(11;18)(q21;q32) t(14;18)(q32;q21) t(1;14)(p22;q32) t(3;14)(p13;q32) +3, +8, +18 del(6q23.3)	*BIRC3-MALT1* *IGH-MALT1* *BCL10-IGH* *FOXP1-IGH* ? *TNFAIP3*
Nodal marginal zone	+3	?
Splenic marginal zone lymphoma	del(7q)	?
Follicular lymphoma	t(14;18)(q32;q21)	*BCL2-IGH*
Lymphoplasmacytic lymphoma	—	-

TABLE 7.4
Common Mutations Present in Low-Grade B-Cell Lymphomas

	GENES AND APPROXIMATE FREQUENCY[a]					
Pathway	**Mantle Cell**	**Follicular**	**Extranodal Marginal Zone (MALT)**	**Splenic Marginal Zone**	**Nodal Marginal Zone**	**Lymphoplasmacytic**
NOTCH signaling	*NOTCH1, NOTCH2*	*NOTCH1, NOTCH2*		*NOTCH2, SWAP70, NOTCH1*	*NOTCH2*	
NF-κB/Inflammatory pathways	*TRAF2, BIRC3, NFKB1*	*CARD11, TNFAIP3, IRF4, FAS, TNFRSF14, IRF8, B2M,*	*MYD88*	*KLF2, TNFAIP3, MYD88, CARD11, TRAF3, BIRC3*	*KLF2*	*MYD88*
B-cell receptor signaling and differentiation						*CD79A/CD79B*
DNA damage and cell cycle control	*CCND1, TP53, RB1*	*MYC, FMN2, CCND3, PIM, TP53*		*TP53*		*TP53*
Chromatin modification	*ATM, SMARCA4, KMT2D, KMT2C, SALL3, UBR5*	*KMT2D, CREBBP, KMT2C, HIST1B-1E, EP300, ARID1A, MEF2B*		*ATM, CREBBP, KMT2D, EP300*	*KMT2D*	*ARID1, KMT2D*
MAPK-ERK		*EPHA7, SGK1*		*MAP3K14*		*CXCR4*
Cell adhesion and misc. cell signaling	*DLC1, FAT4, PCDHB2, SICOL11A1, COL16A1, GRIN2A, TBC1D26, PLXNB*	*SYNE1*			*PTPRD*	
Transcriptional regulation	*ZNF296, WHSC1*	*EZH2, BCL2, EBF1*		*SIN3A*		*MYBBPIA*
JAK-STAT signaling		*STAT6, SOCS1*				

[a]Frequency of mutations.
Red >90%; blue 50% to 89%; green 25% to 49%; orange 10% to 24%; purple 5% to 9%.

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Clinical Features

The median age at diagnosis is 55 to 65 years, with a male-to-female ratio of 2:1. In the international non-Hodgkin lymphoma classification project 91% of patients had advanced stage disease (stage III or IV), and 72% had bone marrow involvement. Almost all patients with SLL have generalized lymphadenopathy at presentation. At diagnosis, 30% have extranodal disease, usually due to spleen and/or liver involvement. Approximately 25% of primary splenic lymphomas are SLL. Although widely recognized as a precursor lesion to CLL, monoclonal B-cell lymphocytosis (MBL) has only recently been proposed to have a tissue counterpart.

Overall, the most common presenting complaint of SLL is asymptomatic generalized lymphadenopathy. Less than one third of patients suffer from B-symptoms. Anemia and thrombocytopenia are seen in up to one third of patients (hemoglobin <11 g/dL and <150,000 platelets/mm^3). By extension of the international consensus definition of CLL, absolute B-lymphocyte count should be less than 5000/μL. A small serum M component, less than 30 g/L, is detectable in 20% of cases. These antibodies are of the IgM type and can have specificity against self-antigens. A subset of patients with SLL will present with or develop autoimmune hemolytic anemia and/or thrombocytopenia. Of SLL patients, 40% have hypogammaglobulinemia, making them vulnerable to infection.

Pathological Features

Morphology

In approximately 80% of cases, SLL completely effaces the node architecture, often with invasion of the capsule and extension into the pericapsular fat (Fig. 7.1). In the remainder, neoplastic cells infiltrate the interfollicular areas, sparing small atrophic lymphoid follicles and leaving the sinuses patent. The neoplastic cells in SLL are small (6–12 μm) lymphocytes with round nuclear contours, distinctly condensed chromatin, inconspicuous nucleoli, and sparse agranular cytoplasm (Fig. 7.2). Although small lymphocytes predominate, almost all cases of SLL involving lymph nodes contain admixed intermediate sized cells termed *paraimmunoblasts*. They are approximately 1.5 times the size of the neoplastic small lymphocytes, have partially condensed chromatin, and small, distinct, central nucleoli (Fig. 7.3). Paraimmunoblasts can be distributed singly but, more often (90% of cases), they form ill-defined clusters imparting a pale, vaguely nodular pattern at low magnification. The collections of paraimmunoblasts are termed *proliferation centers*, *growth centers*, or *pseudofollicles*. Proliferation centers are not present in reactive conditions, and, among the small B-cell lymphomas discussed in this chapter, they only occur in CLL/SLL. In some instances, proliferation centers can be prominent and even begin to fuse (Fig. 7.4). Expansion of proliferation centers should not be

SMALL LYMPHOCYTIC LYMPHOMA—FACT SHEET

Clinical Features
- Median age 55–65 years
- Male predominance
- Asymptomatic generalized lymphadenopathy
- Of patients, 80% present with Ann Arbor stage IV disease due to bone marrow involvement
- Progresses to leukemic phase, indistinguishable from B-cell chronic lymphocytic leukemia in a high percentage of patients

Morphology
- Diffuse growth pattern with complete (80%) or partial (20%) involvement
- Small lymphocytes with round nuclei, condensed chromatin, sparse cytoplasm
- Proliferation centers composed of medium-large cells with vesicular chromatin and single central nucleoli (prolymphocytes/paraimmunoblasts).
- Transformation to diffuse large B-cell lymphoma (Richter syndrome) or Hodgkin lymphoma in 5%

Immunophenotype
- CD19+, CD20+(dim), sIg+(dim), IgM with or without IgD
- CD5+, CD23+ LEF1+ CD200+
- CD38 and ZAP-70 expression associated with adverse outcome

Genetics
- +12, del(13q), del(11q), del(17p), del(6q)
- Point mutations in Ig genes present in 50%

Prognosis and Therapy
- Indolent, incurable disease
- Watch and wait approach for asymptomatic stage III and IV patients
- Low-intensity single or multi-agent chemotherapy for symptomatic patients
- 8- to 10-year median survival
- Aggressive disease may be indicated by prominent proliferation centers
- Additional biological predictors of high risk for adverse outcome: del(17p), del(6q), germline immunoglobulin genes, zap-70 and/or CD38-positive tumor cells
- Transformation to diffuse large B-cell lymphoma in 20% heralds aggressive disease

Differential Diagnosis
- Reactive lymphoid hyperplasia
- Mantle cell lymphoma
- Follicular lymphoma
- Nodal marginal zone B-cell lymphoma
- Splenic marginal zone B-cell lymphoma
- Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue
- Lymphoplasmacytic lymphoma
- Lymphocyte-predominant Hodgkin lymphoma
- Lymphocyte-rich classical Hodgkin lymphoma

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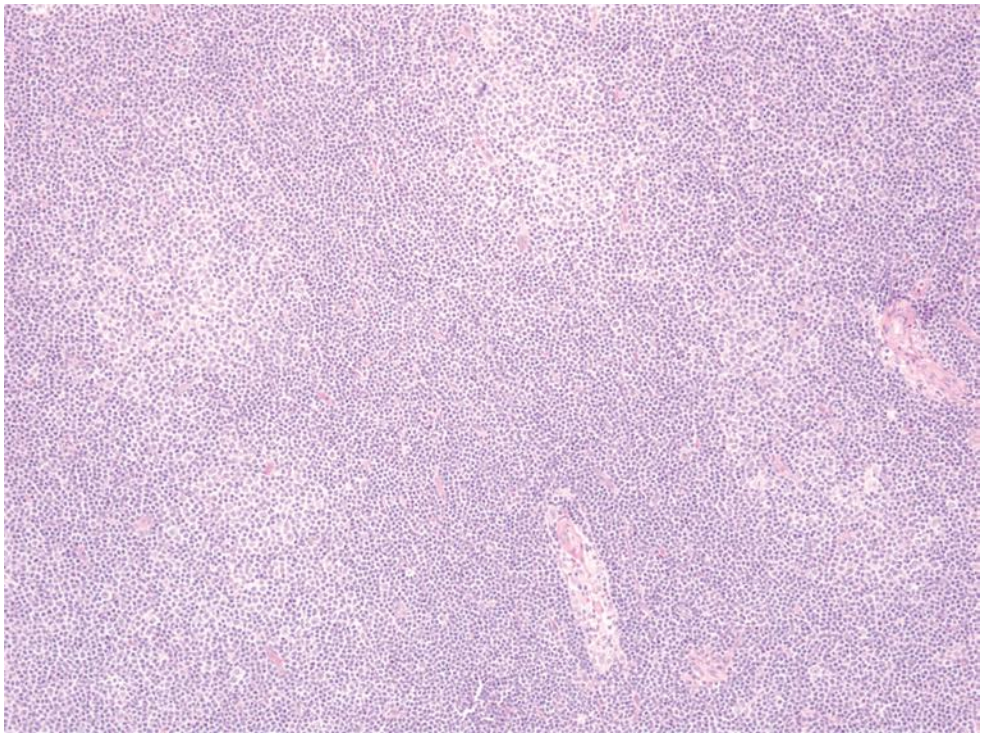


FIG. 7.1

B-cell small lymphocytic lymphoma diffusely effaces the lymph node architecture. The pale areas are proliferation centers.

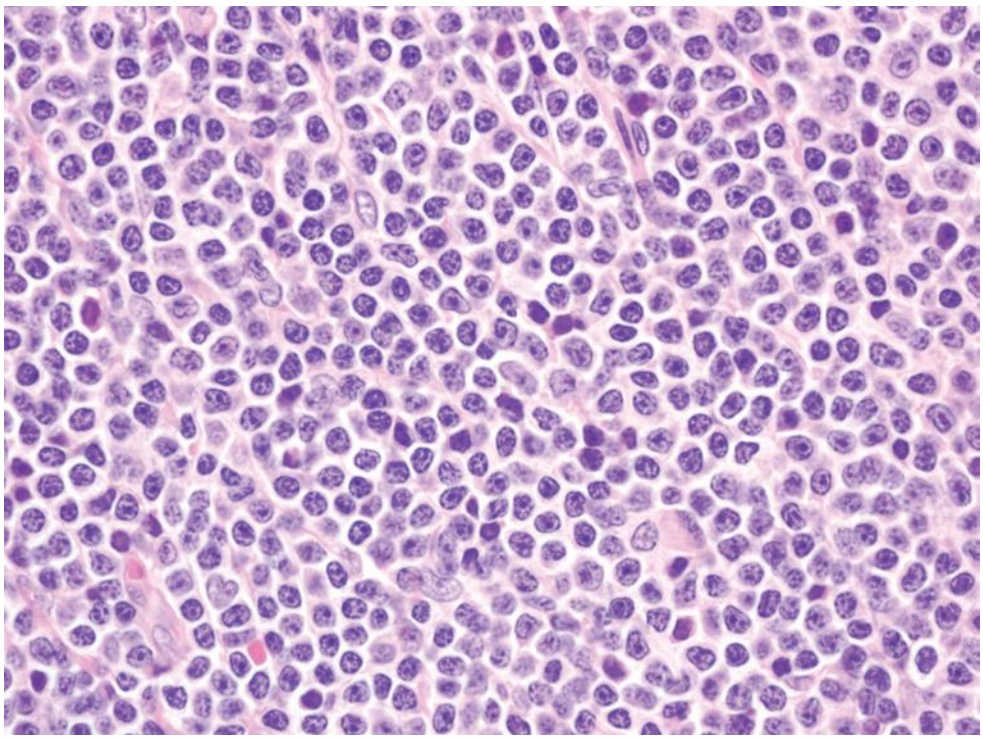


FIG. 7.2

B-cell small lymphocytic lymphoma composed of small lymphocytes with round nuclei, distinctly clumped chromatin, inconspicuous nucleoli, and sparse cytoplasm.

mistaken for large cell lymphoma transformation (see below). However, studies do show an association of prominent, coalescing proliferation centers with adverse prognosis, unfavorable cytogenetic features (17p deletion), and high Ki-67 proliferation index.

In those instances, when the lymph node architecture is partially preserved, proliferation centers can be located in the interfollicular areas (Fig. 7.5), or they can encircle the residual lymphoid follicles. These phenomena are thought to represent early stages of lymph node involvement and are morphologically quite subtle. The interfollicular pattern can be easily overlooked, whereas the perifollicular pattern can be mistaken for marginal zone lymphoma (MZL). One study has suggested that absence of proliferation centers in subtle

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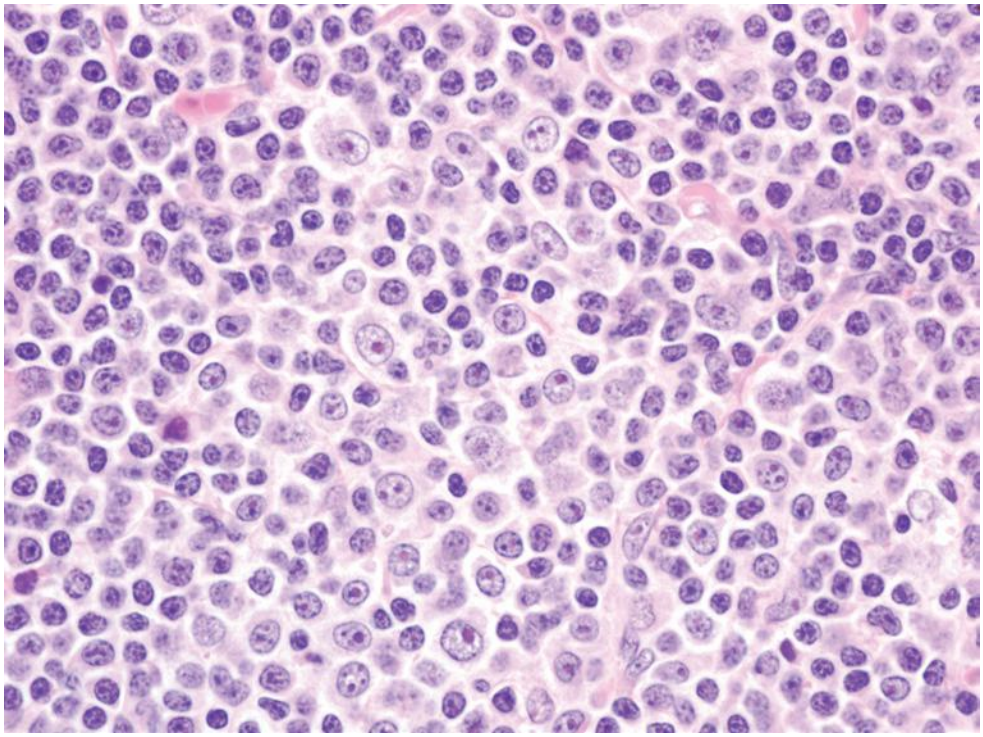


FIG. 7.3
Cellular composition of a proliferation center in B-cell small lymphocytic lymphoma.

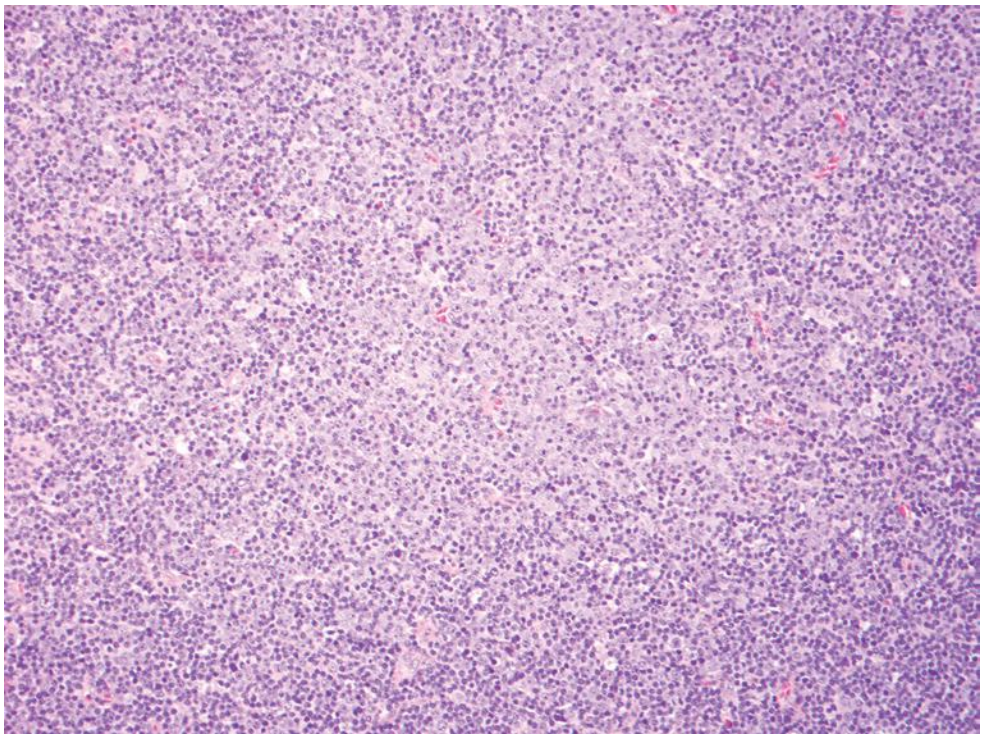


FIG. 7.4
Although prominent, this proliferation center is surrounded by the typical small lymphocytes of B-cell small lymphocytic lymphoma *(right)*.

infiltrates may be an indicator of a so-called tissue-based MBL in patients with minimal nodal disease who have a low rate of progression.

In the involved spleen, a uniform expansion of the white pulp creating grossly visible nodules in a miliary fashion is seen early in the course of the disease. However, at later stages the red pulp becomes involved (Fig. 7.6), and the white pulp is obliterated, producing a diffuse pattern of involvement encompassing the entire splenic parenchyma. The microscopic features are similar to the lymph node with infiltration by small lymphocytes and admixed paraimmunoblasts. When the disease becomes disseminated, essentially any extranodal site can be involved and the same morphologic features are seen, but proliferation centers are not nearly as frequent in

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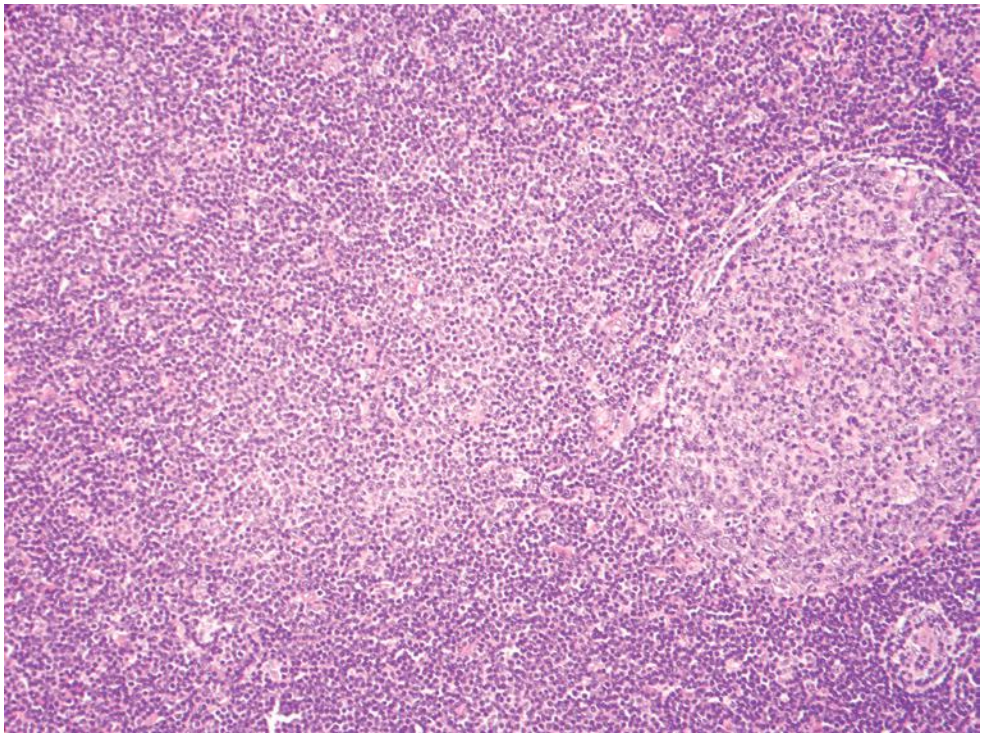


FIG. 7.5
Interfollicular B-cell small lymphocytic lymphoma (SLL). On the right a hyperplastic, atrophic germinal center is present. Note the proliferation center in the left side of the photograph, a low-power diagnostic clue to the presence of B-cell SLL.

extranodal sites as in lymph nodes. Sometimes the infiltrate is sparse and the distinction from lymphoid hyperplasia can be challenging. Special studies to demonstrate monoclonality and to confirm the typical immunophenotype of SLL are prudent in this context.

Immunophenotype

The typical immunophenotype of SLL cells is CD5+, CD10–, CD19+, CD20+ (dim), CD22 (dim), CD23+, CD43+, CD79b–/dim+, CD200+, LEF1+, SOX11–, FMC7–/dim+, and surface immunoglobulin (sIg) light chain dim+. Some cases may not have detectable sIg. Cyclin D1 is not expressed, although the large cells within proliferation centers may be positive for cyclin D1 by immunohistochemistry in up to 20% of cases and can also express MYC protein. Notably these cases lack both t(11;14)(q13;q32) involving *CCND1/IGH* and rearrangements involving the *MYC* gene. The most reliable markers in paraffin section immunohistochemistry are CD5, CD10, CD20, and cyclin D1. CD23 is also helpful but can be difficult to demonstrate on small lymphocytes in some cases. Proliferation centers are often nicely highlighted by immunostaining for CD23. Newer markers that have helped aid in the diagnosis of challenging cases include CD200 and LEF1. CD200 is typically assessed by flow cytometry. This marker has a high sensitivity in distinguishing CLL/SLL from MCL and may have prognostic value in CLL. LEF1, lymphoid enhancer-binding factor 1, is normally expressed in pro-B cells and mature T cells but is downregulated in mature B cells. However, it is expressed in neoplastic B cells in many cases of CLL/SLL, and has shown up to 92% specificity and 70% sensitivity for the diagnosis. LEF1 is typically performed by immunohistochemistry, but may also be performed by flow cytometry. This described immunologic profile of SLL is identical to that of CLL (see Chapter 12 for details). A reported difference is the expression of lymphocyte function–associated antigen (LFA-1), an adhesion molecule, on SLL but not CLL cells. By contrast, CLL cells express the chemokine receptors CCR-1 and CCR-3 that are absent from SLL cells.

CD38 and ZAP-70 expression have become accepted as poor prognostic markers in CLL/SLL. It is worth noting here that both ZAP-70 and CD38 expression can be assessed by immunostaining, and at least in the case of ZAP-70 there is good correspondence between its detection by flow cytometry and immunohistochemistry.

Genetics

Clonal immunoglobulin gene rearrangements are detectable in almost all cases. The molecular genetic features are covered in the section on CLL. There are no specific karyotypic abnormalities in SLL. As with CLL, cases of SLL variably show trisomy 12, del(6q), del(11q), del(13q), and del(17p). These genetic abnormalities, though common in CLL/SLL, have been described in many other lymphoma types. In practice they should be considered prognostic markers (see section on CLL) rather than disease-defining markers for CLL/SLL. (See Table 12.4 for common recurrent mutations in CLL/SLL.)

Transformation

Transformation into an aggressive lymphoma (Richter syndrome) occurs in approximately 5% of SLL cases. Affected

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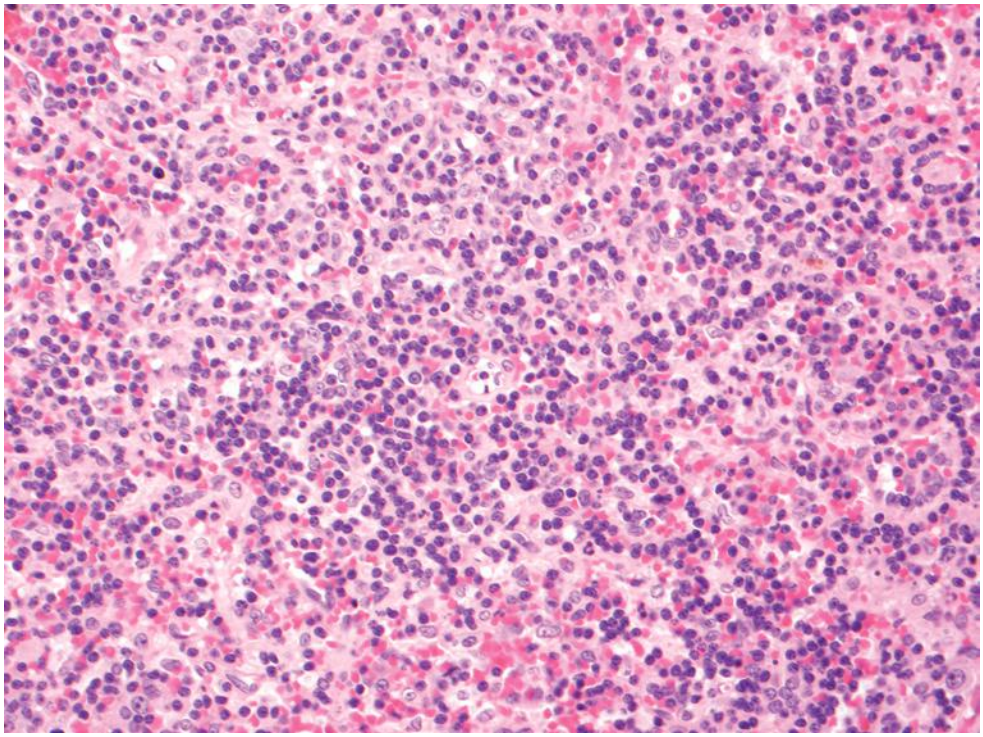


FIG. 7.6
Splenic red pulp infiltrated by B-cell small lymphocytic lymphoma (SLL).

patients present with rapidly growing masses, elevated serum lactate dehydrogenase (LDH), and B symptoms. Most commonly the transformation is in the form of diffuse large B-cell lymphoma, which may or may not be clonally related to the B-cell SLL. The morphologic features are similar to those for diffuse large B-cell lymphoma in general. They are composed of a monomorphous population of large lymphoid cells, resembling centroblasts or immunoblasts that grow in a diffuse pattern, effacing the architecture of the involved tissue (Fig. 7.7), including overrunning any residual SLL/CLL in the sample.

Some SLL cases contain prominent, almost coalescing proliferation centers that give the superficial impression of a large B-cell lymphoma. Recognition of the spectrum of small, medium, and larger lymphocytes present in these cases and appreciation for the manner in which the prominent proliferation centers appear to blend into adjacent small lymphocyte-rich areas helps distinguish this phenomenon from overt transformation. As mentioned above, some studies have found a correlation between prominent proliferation centers and more aggressive disease in CLL. Such cases, however, should not be considered transformation to diffuse large B-cell lymphoma.

Finally, rare cases of SLL are complicated by transformation to classic Hodgkin lymphoma (CHL), usually of nodular sclerosis or mixed cellularity type. Single cell polymerase chain reaction (PCR) studies have shown that the Reed-Sternberg cells in these cases are clonally related to the preexisting CLL clone in some cases but not in others. Epstein-Barr virus (EBV) can usually be detected in the Reed-Sternberg–like cells. The prognosis of patients with CHL complicating SLL/CLL is worse than the typical SLL but still better than that for patients with large B-cell lymphoma transformation. Importantly, as discussed below, transformation to CHL must be distinguished from SLL/CLL with Hodgkin-Reed Sternberg–like cells.

Differential Diagnosis

The differential diagnosis of SLL includes reactive lymphoid hyperplasia, most of the small B-cell lymphomas including MCL, FL, MZL, and LPL; nodular lymphocyte-predominant Hodgkin lymphoma; and lymphocyte-rich CHL.

Tissue architectural effacement by monomorphous small lymphocytes is a finding present in SLL and not lymphoid hyperplasia where the tissue architecture is intact and/or the cellular infiltrates contain other cell types. Because proliferation centers do not occur in reactive lymphoid hyperplasia, recognizing these structures plays a key role in distinguishing SLL from lymphoid hyperplasia. Finally, phenotypic analysis will demonstrate a monotypic B-cell population in SLL compared to a polytypic B-cell population in hyperplasia. Although normal lymph nodes can contain small populations of non-neoplastic CD5+ B cells, they are usually confined to the follicular mantle zones and represent only a small subset of the total B-cell population. If one encounters a substantial population of CD5+ and CD23+ B cells and if they form aggregates in the interfollicular zones, then one should consider SLL rather than a reactive condition.

Distinguishing SLL from the other B-cell malignancies discussed in this chapter is summarized in Tables 7.1, 7.2, and 7.3. A combination of careful morphologic observations

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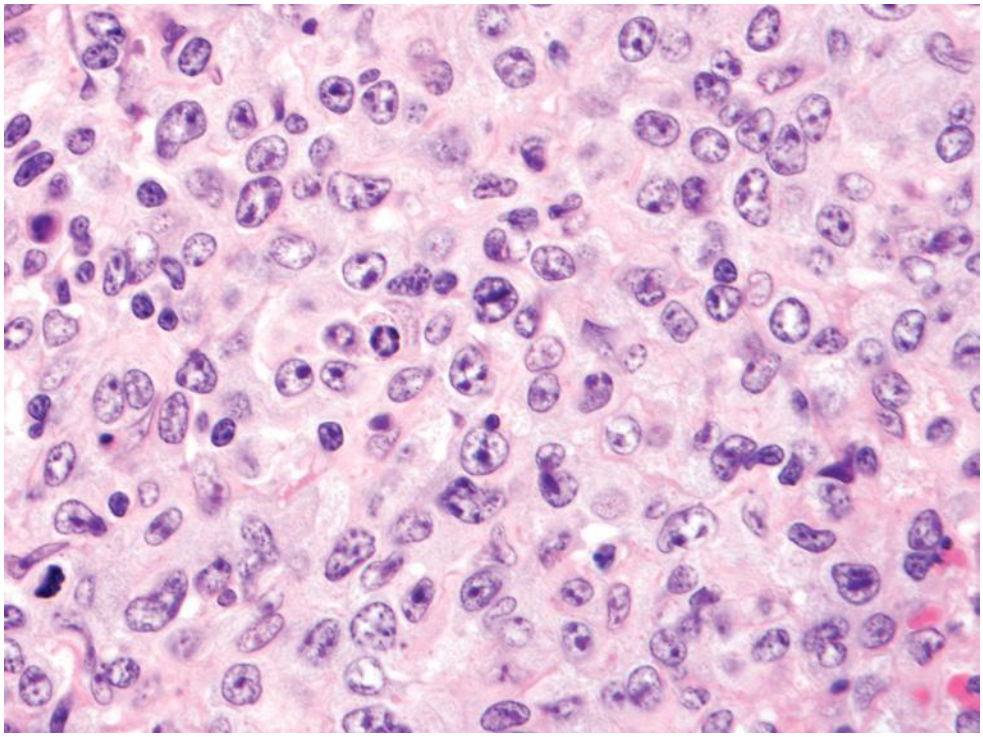


FIG. 7.7
Diffuse large B-cell (Richter's) transformation of B-cell small lymphocytic lymphoma (SLL) occurred in the same spleen specimen as that illustrated in Fig. 7.6.

focusing on the features summarized in Table 7.1 together with judicious use of immunophenotyping (Table 7.2) will readily resolve the differential diagnosis.

A controversial area in the SLL literature is the rare variant of SLL seen in about 5% of cases in which cells show plasmacytoid features accompanied by a serum or urine M component. The line separating this variant of SLL from LPL is blurred. Cases that are CD5+ and CD23+ and have proliferation centers with limited paraproteinemia should be classified as CLL/SLL, whereas cases with *MYD88* L265P mutations are likely to be LPL. Paraprotein levels exceeding 30 g/L with hyperviscosity syndrome are highly unlikely in CLL/SLL. There is no consensus about the prognostic significance of the plasmacytoid variant of SLL. However, a study of 26 such patients showed no difference in the overall survival (OS) from an age-, sex-, and stage-matched group of 52 patients with standard CLL/SLL, albeit with only relatively short follow-up (median of 24 months).

In most cases, nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) grows in a macronodular pattern. The nodules contain small lymphocytes but also include the typical lymphocyte-predominant (LP) variants of Hodgkin cells characteristic of this disorder together with variable numbers of epithelioid histiocytes. The nodularity in SLL is due to the proliferation centers. They are usually smaller than the nodules of NLPHL and contain prolymphocytes and paraimmunoblasts but not LP cells. Phenotypically, the small lymphocytes in the macronodules of NLPHL include CD279+ T cells immediately surrounding the LP cells, polyclonal small B cells, and follicular dendritic cells, whereas the small B cells of SLL have a uniform CD5, CD23, immunoglobulin light chain–restricted phenotype.

In contrast to SLL, lymphocyte-rich CHL contains diagnostic Reed-Sternberg cells expressing CD15 and CD30 with absent CD45 expression. The small lymphocytes represent polyclonal CD5– B cells and T cells. It should be noted that individual, widely scattered CD30+ Hodgkin-like cells can be seen on occasion in the background of otherwise typical SLL. The background inflammatory infiltrate of CHL is absent. The Hodgkin-like cells in SLL are usually EBV+ and represent an EBV-driven phenomenon due to the generally immunosuppressed state of the patient. This should not be considered Hodgkin transformation.

Prognosis and Therapy

Many studies have investigated the role of different clinical, laboratory, and pathologic parameters in predicting prognosis in SLL. Most concede that there is an uncertain correlation between histology of SLL and disease outcome. In the international non-Hodgkin Lymphoma Classification Project the 5-year overall actuarial survival (OAS) of patients with an international prognostic index (IPI) of 0/1, 2/3, or 4/5 were 76%, 51%, and 38%, respectively. Similar to CLL, ZAP-70 positivity, trisomy 12, and deletion of 17p predict a poor prognosis in SLL patients.

SLL is not curable by current conventional therapies. Asymptomatic patients with advanced disease are often observed without treatment, whereas those with symptomatic early stage disease (stage I and II) may be treated with regional or extended field radiation therapy. Those symptomatic patients with advanced stage disease (stage III and IV) are usually

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treated similar to patients with CLL (see Chapter 12 for details)

MANTLE CELL LYMPHOMA

Clinical Features

As they are currently defined, MCLs represent 2% to 8% of non-Hodgkin lymphomas in the United States. They occur in older individuals (median age 63 years) with a decided male predominance (75% of patients). Most patients present with progressive adenopathy involving multiple sites, and there is a relatively high frequency of Waldeyer ring involvement. Although historically considered to be a uniformly aggressive disease, a more indolent form characterized by non-nodal, leukemic disease, SOX11 negativity, and *IGHV*-mutated status is now recognized. Splenic involvement, both with and without lymphadenopathy, occurs frequently, and a small subset of patients present with multiple intestinal lymphomatous polyps. The staging bone marrows from MCL patients are frequently positive (70%), and 20% to 30% have morphologically recognizable abnormal circulating lymphocytes. This disease is almost always widespread (Ann Arbor stage III or IV); approximately one third of patients have B symptoms at the time of diagnosis.

Formerly known as *mantle cell lymphoma in situ*, the WHO now recognizes *in situ mantle cell neoplasia* (ISMCN) as a subset of cases in which there is localized tissue involvement by MCL, without architectural distortion, and which have a low rate of progression to overt lymphoma. These cases likely do not require therapeutic intervention.

Morphology

The gross pathology of MCL in lymph nodes is not distinctive. This disorder produces nodal enlargement characterized by homogeneous, tan cut surfaces with or without small nodules. When MCL involves the spleen, it produces splenic enlargement, usually greater than 1000 g with small, 1- to 3-mm, miliary, white nodules scattered throughout the splenic parenchyma. In a peculiar pattern of involvement in the gastrointestinal (GI) tract, MCL can also produce numerous sessile and pedunculated polyps throughout the entire length of the small and large intestine in a pattern termed *multiple intestinal lymphomatous polyposis*.

MCLs are characterized by three growth patterns: mantle zone, nodular, and diffuse, in increasing order of frequency. In the mantle zone pattern, prominent mantle zones composed of the neoplastic cells surround reactive germinal centers that can be either normal sized or small and atrophic (Fig. 7.8A and 7.8B). When the germinal centers are normal size, distinguishing MCL from follicular hyperplasia can be a challenge unless one recognizes that the architecture of the

MANTLE CELL LYMPHOMA—FACT SHEET

Clinical Features
- 2% to 8% of non-Hodgkin lymphomas
- Median age 7th decade, male predominance
- Progressive adenopathy
- Splenic enlargement without adenopathy
- Multiple intestinal lymphomatous polyposis
- Two subtypes: one more aggressive with SOX11+, nodal presentation; one more indolent with SOX11- disease, leukemic presentation, and non-nodal disease
- High stages at presentation

Morphology
- Mantle zone, nodular or diffuse architecture
- Monomorphous small lymphocytes with irregular nuclei clumped chromatin, inconspicuous nucleoli, and sparse cytoplasm
- Blastoid variants: lymphoblast-like, centroblast-like, pleomorphic

Immunophenotype
- CD19+, CD20+, sIg+, IgM with or without IgD
- CD5+, SOX11+, LEF1 usually negative
- CD23 negative or weakly positive in subset
- Cyclin D1+

Genetics
- Clonally rearranged immunoglobulin genes
- t(11;14)(q13;q32)
- *CCND1* gene translocated into *IGH* locus
- Point mutations in Ig genes absent in most, present in some

Prognosis and Therapy
- Multi-agent chemotherapy with rituximab
- Incurable
- Prognosis suggested by mantle cell lymphoma international prognostic index (MIPI), pattern of involvement of lymph node, Ki-67 proliferative rate (>30% portends poor prognosis), and blastoid cytology
- In patients younger than 65 whose tumors do not have adverse prognostic features treated with aggressive therapy and autotransplant median survival approaches 10 years. Others median survival variable: 4–5 years not uncommon

Differential Diagnosis
- Reactive lymphoid hyperplasia
- Chronic lymphocytic leukemia/small lymphocytic lymphoma
- Follicular lymphoma, grade 1–2
- Nodal marginal zone B-cell lymphoma
- Splenic marginal zone B-cell lymphoma
- Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue
- Lymphoplasmacytic lymphoma
- Lymphocyte-predominant Hodgkin lymphoma
- Lymphocyte-rich classical Hodgkin lymphoma

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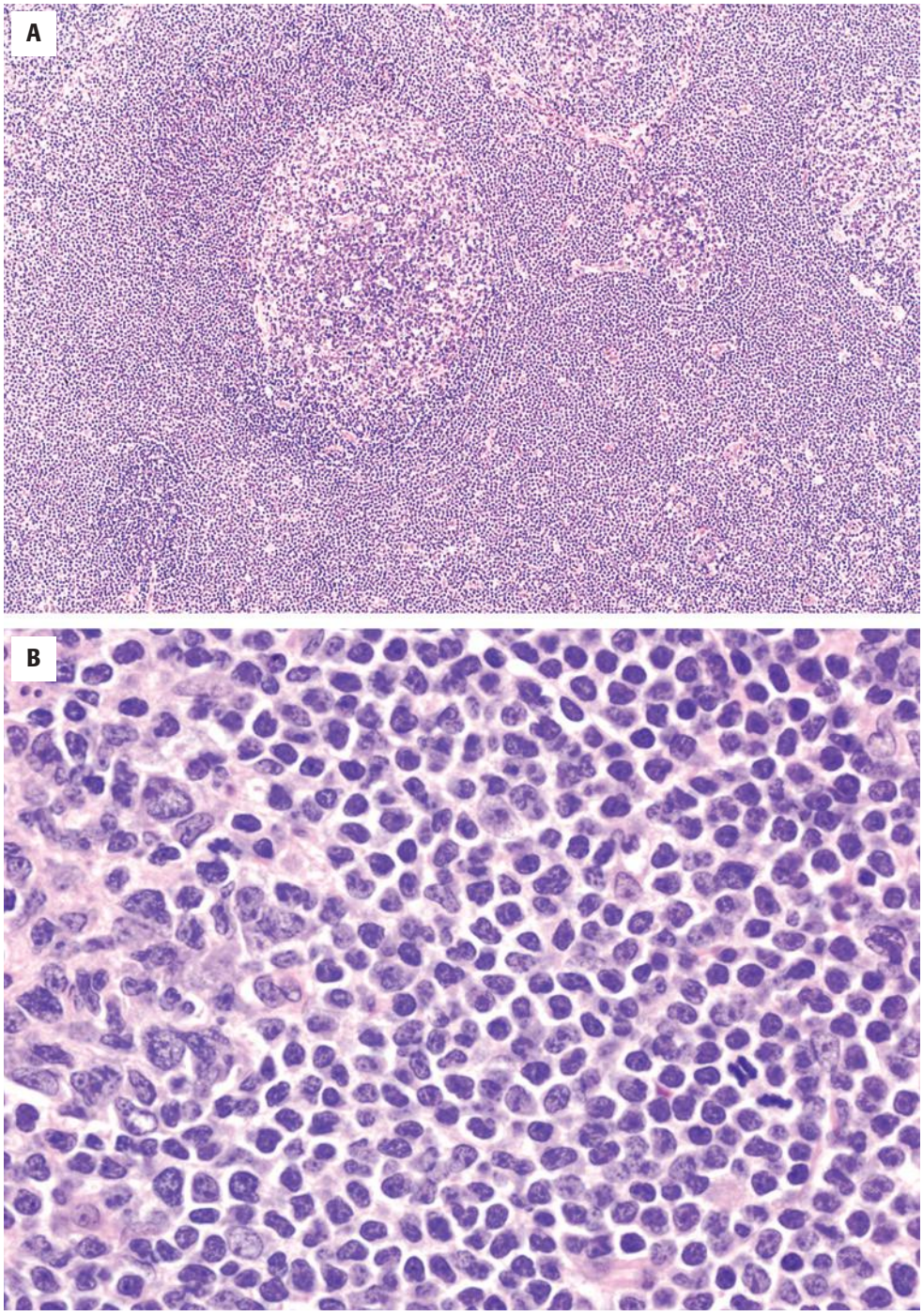


FIG. 7.8

Mantle cell lymphoma, mantle zone pattern. (A) The neoplastic cells surround hyperplastic germinal centers and infiltrate the interfollicular areas. (B) Non-neoplastic germinal center cells are illustrated on the left. The mantle cell lymphoma cells occupy the rest of the image.

internodular areas is effaced by the expanded "cloud" of neoplastic mantle zone cells. Numerous coalescing nodules of tumor cells devoid of germinal centers are characteristics of nodular pattern MCL (Fig. 7.9), whereas a diffusely growing lymphoma cell population that effaces the underlying architecture of the involved tissue defines the diffuse pattern (Fig. 7.10). Hyalinized small blood vessels course through the lymphoma infiltrates in a substantial fraction of diffuse pattern MCLs and can be a useful low-power morphologic clue to the diagnosis of this tumor type.

In the prototypic case, MCL is composed of a highly monomorphous population of small lymphocytes with spheroidal nuclei containing irregularities, cleaves, and grooves. The chromatin is clumped, nucleoli are inconspicuous, and the cytoplasm is sparse (Fig. 7.11). Proliferation centers and intermixed centroblasts or immunoblasts are absent. In addition, MCLs often contain intermixed, singly distributed epithelioid macrophages. Because this cell population is unusual in other lymphoma of small B-lymphocytes, its presence is a useful clue to the diagnosis of MCL. Plasma cell differentiation

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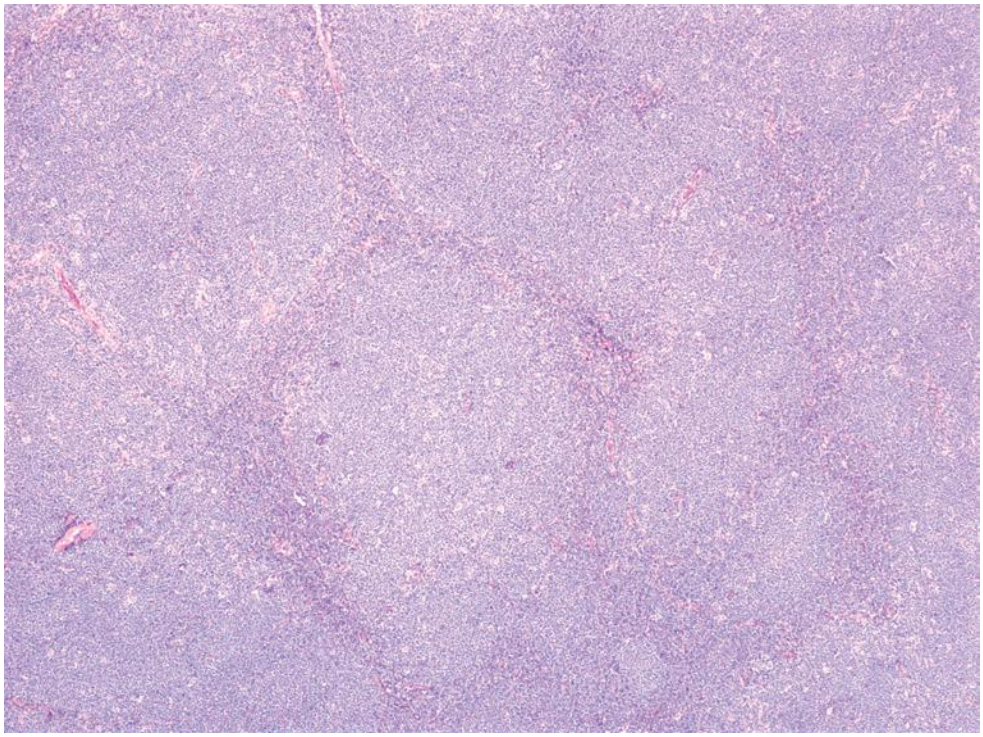


FIG. 7.9
Mantle cell lymphoma, nodular pattern. Near-confluent nodules efface the lymph node architecture.

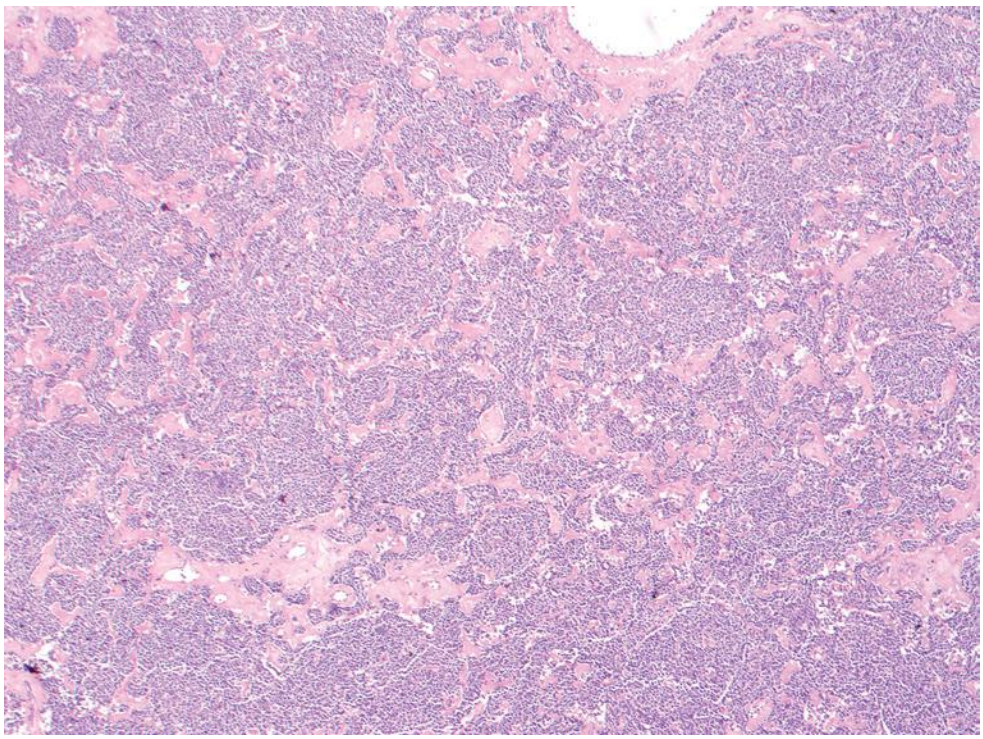


FIG. 7.10
Mantle cell lymphoma, diffuse pattern. Note the blood vessels surrounded by hyalinized collagen.

has also been described in MCL. These cases contain small lymphocytes morphologically and phenotypically identical to those of standard MCL cases that are mixed with varying numbers of plasma cells that are monotypic for the same light chain as the MCL lymphocytes.

In a small subset of cases, the neoplastic mantle cells have "monocytoid" characteristics. These include somewhat larger nuclei and less clumped chromatin than standard MCL cases together with voluminous clear or lightly eosinophilic cytoplasm (Fig. 7.12). This cytology can mimic exactly the cytology of nodal and splenic MZLs, highlighting the importance of ancillary studies in supporting the diagnosis of MCL.

One can recognize three other cytologic variants of MCL. In the first, the neoplastic cells resemble lymphoblasts. They

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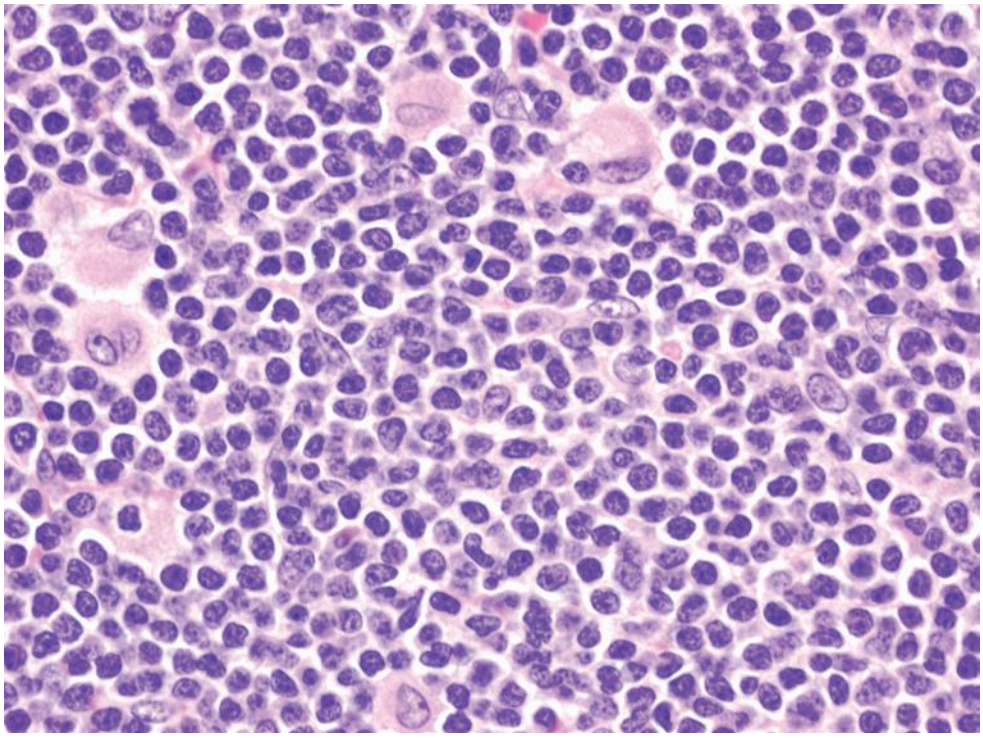


FIG. 7.11
Mantle cell lymphoma, standard cytology. Monomorphous small lymphocytes with irregular nuclei, condensed chromatin, inconspicuous nucleoli, and sparse cytoplasm. Intermixed singly distributed macrophages are also present.

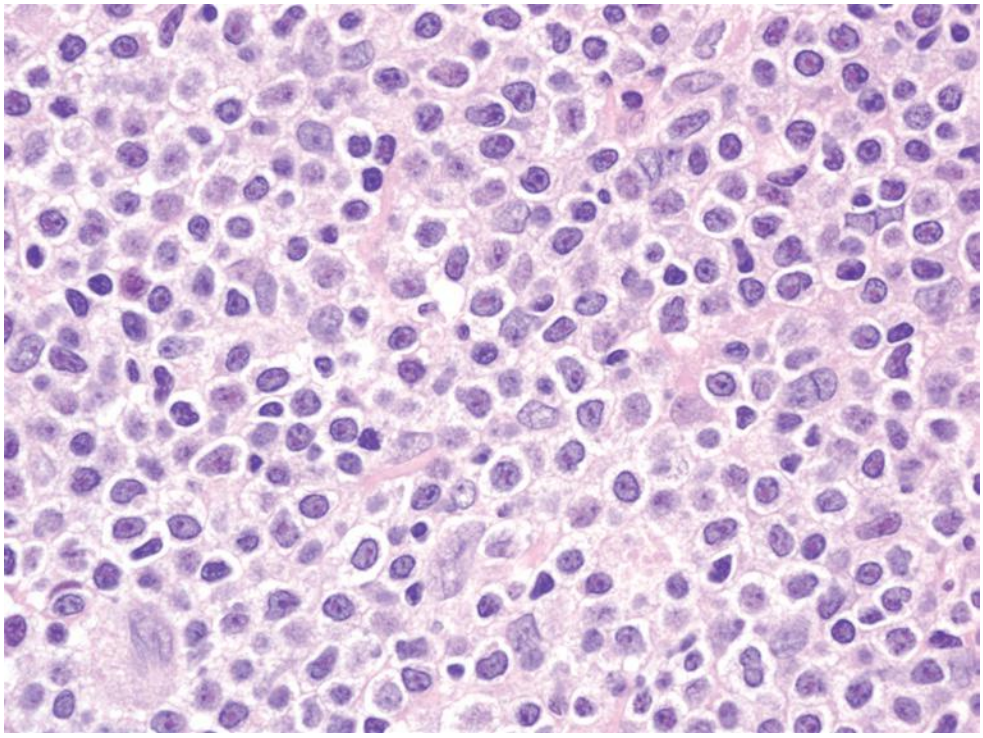


FIG. 7.12
Mantle cell lymphoma, monocytoid cytology.

are medium sized with irregular nuclei containing stippled chromatin, small nucleoli, and imperceptible cytoplasm (Fig. 7.13A). They are often associated with numerous mitotic figures. In the second, the neoplastic cells resemble centroblasts with round nuclei, multiple nucleoli often attached to nuclear membranes, and more voluminous amphophilic cytoplasm. In the third, the nuclei of the neoplastic cells are pleomorphic, vary in size and shape, are often hyperchromatic, and have variably prominent nucleoli (Fig. 7.13B). The WHO lymphoma classification groups these last three cytologic variants into the category blastoid/pleomorphic variant of MCL. Blastoid/pleomorphic MCLs can occur de novo, in which case they

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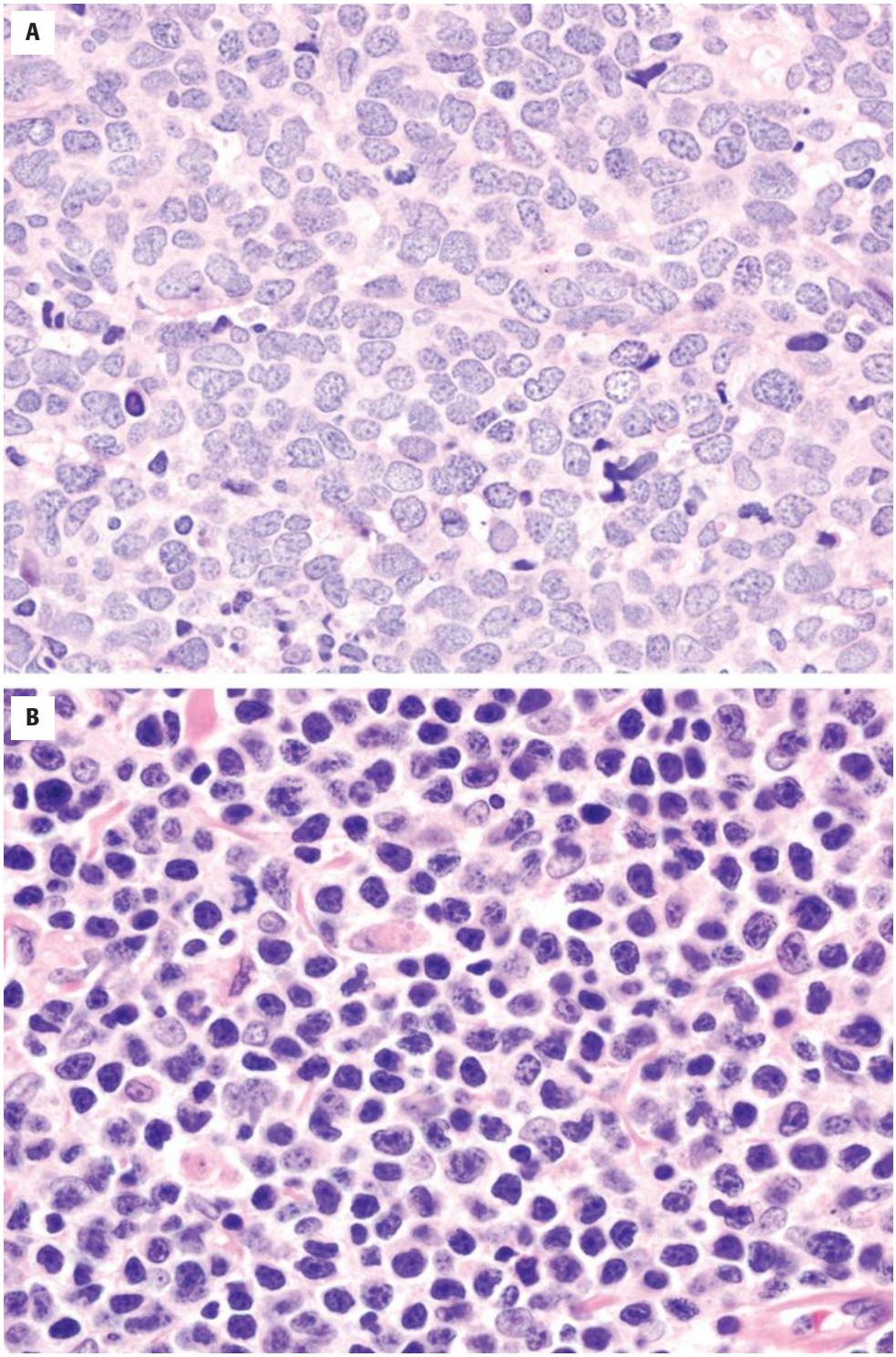


FIG. 7.13

Blastoid variants of mantle cell lymphoma. A, Lymphoblastoid cytology. B, Pleomorphic cytology.

are associated with increased risk for adverse outcome. Alternatively, standard MCLs with progression can acquire blastoid cytologic characteristics.

In ISMCN, the architecture of the lymph node or tissue remains intact and undistorted, and it is usually only by the serendipitous recognition of small clusters of cyclin D1+ cells in nonexpanded mantle zones that this phenomenon is recognized (Fig. 7.14).

Involvement of Extranodal Sites

Mantle cell lymphomas frequently involve extranodal sites. The bone marrow is positive in 70% of patients. Paratrabecular, nodular, and/or interstitial infiltrates are found in involved bone marrow biopsy specimens (Fig. 7.15). In contrast to the marked monomorphism of the tumor cells of typical MCLs in fixed tissue specimens, the neoplastic cells in

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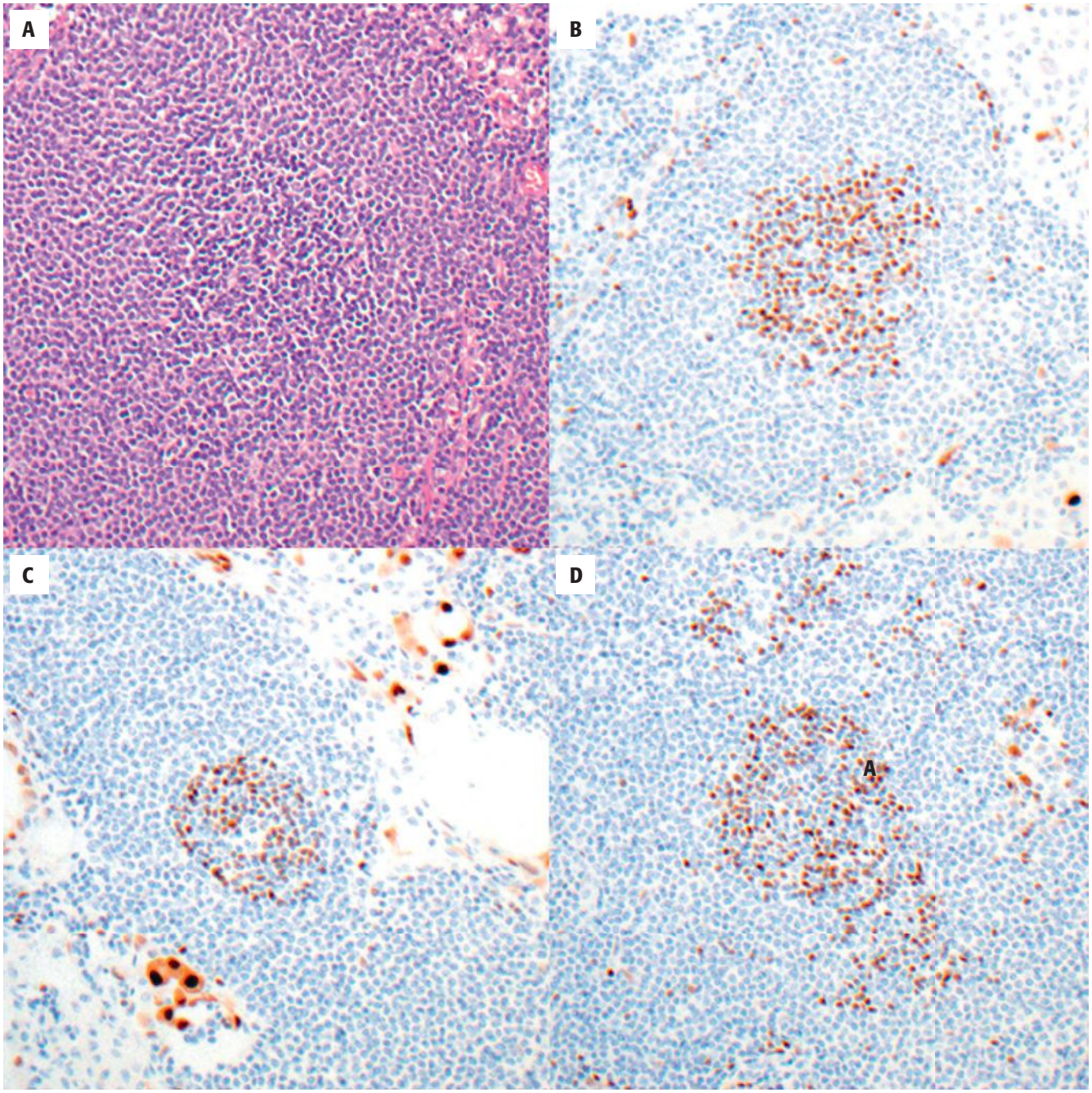


FIG. 7.14

In situ mantle cell neoplasia. A, The center of this follicle contains a small aggregate of darkly staining lymphocytes that represent ISMCL. B–D, Immunoperoxidase staining of paraffin sections for cyclin D1 demonstrating cyclin D1-positive small lymphocytes confined to the centers of the follicles.

air-dried Wright-Giemsa stained bone marrow aspirate and blood smears are quite polymorphous (Fig. 7.16). Nuclear size and shape vary, the chromatin has a reticulated pattern, and nucleoli can be quite prominent. Peripheral blood involvement by MCL can mimic B-cell chronic lymphocytic leukemia both by absolute lymphocyte count and cytology. The cases previously thought to be B-cell prolymphocytic leukemia with the t(11;14)(q21;q32) are now considered to represent leukemic phase MCLs. In the spleen, MCL involves the white pulp, and a subset of cases will demonstrate marginal zone differentiation toward the periphery of the white pulp nodules. As mentioned above, MCL is one of the lymphoma types that can cause multiple intestinal lymphomatous polyposis (Fig. 7.17).

Phenotype

Mantle cell lymphoma cells express pan B-lymphocyte antigens such as CD19, CD20 (Fig. 7.18A), CD79a, CD79b, and PAX-5. They are positive for IgM or IgM plus IgD and

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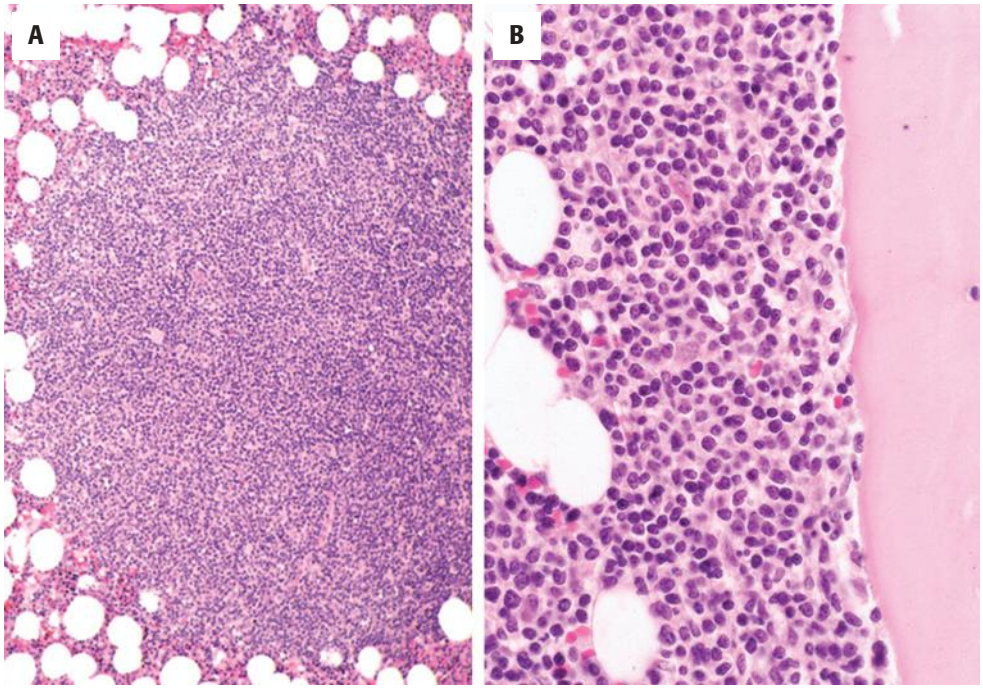


FIG. 7.15

Bone marrow involvement by mantle cell lymphoma. A, Intertrabecular nodule. B, Paratrabecular aggregate.

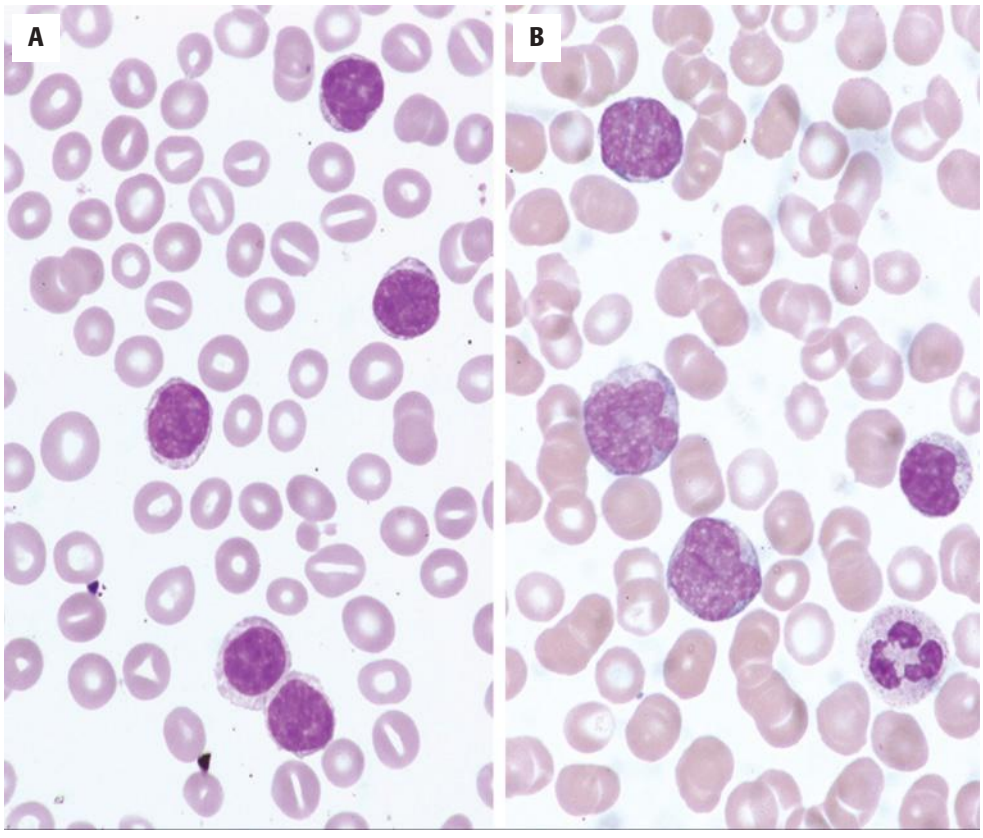


FIG. 7.16

Blood involved by mantle cell lymphoma. A, Monomorphous small lymphocytes. B, Nuclear size and shape variability and small nucleoli characterize this case.

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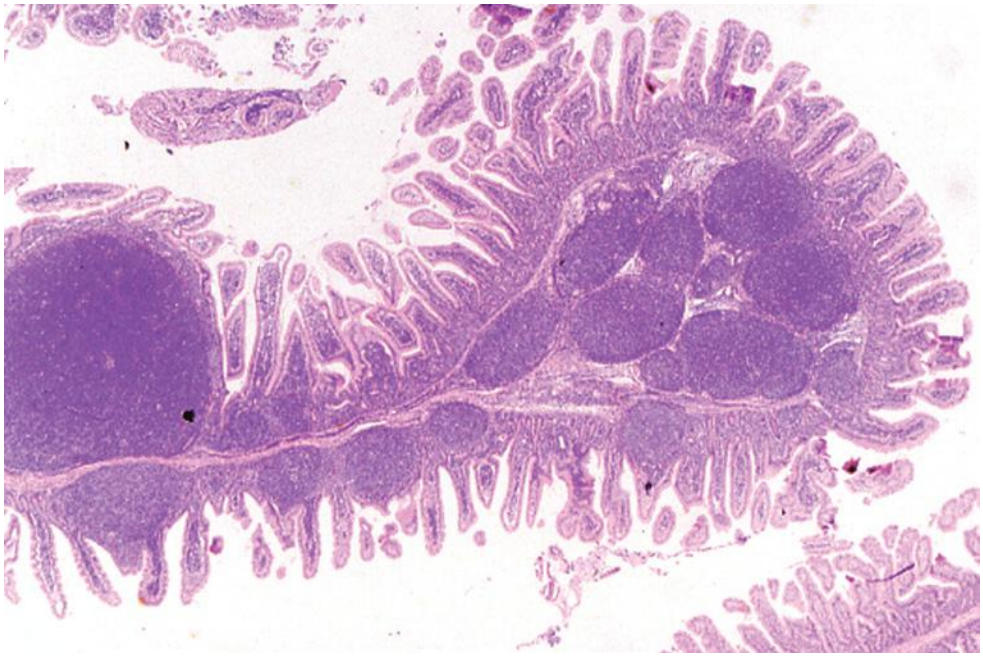


FIG. 7.17
Multiple intestinal lymphomatous polyposis due to mantle cell lymphoma.

exhibit immunoglobulin light chain restriction. More MCL cases express lambda immunoglobulin light chain than kappa immunoglobulin light chain. Greater than 95% are positive for CD5 (Fig. 7.18B) but negative for other T-cell antigens, such as CD3 (Fig. 7.18C), and most either completely lack CD23 expression or show weak CD23 marking in a subset of the tumor cells (Fig. 7.18D). They typically lack or have low-intensity expression of CD200 and are most often LEF1-negative, both of which may aid in the distinction from CLL/SLL. They are usually also negative for CD10 and BCL6. Exceptions to this typical phenotype exist. CD5− MCL cases have been described. They can be recognized on the basis of typical morphologic features and expression of cyclin D1 and will have the classic t(11;14)(q13;q32) *CCND1/IGH* as described below. A very small number of MCLs is positive for the follicle center cell marker BCL6, and MCL is one of the types of B-cell lymphomas that can co-express both CD5 and CD10. As a consequence of the t(11;14)(q13;q32) (see below), the nuclei of the majority of MCLs are positive for cyclin D1 (Fig. 7.18E), a marker demonstrated best by paraffin section immunohistochemistry. More recently, cyclinD1-negative MCLs have been described, which can pose a diagnostic challenge to the pathologist. Expression of SOX11 by immunohistochemistry is seen in a high proportion (>90%) of MCL and appears useful in recognition of cyclin D1 negative MCL. Interestingly, SOX11-negative cases seem to represent a subtype of MCL, which has a more indolent course, leukemic/non-nodal involvement, and mutated *IGHV*. SOX11 is not expressed in other small B-cell lymphomas or DLBCL (including CD5+ DLBCL). It can be expressed in some cases of Burkitt lymphoma and lymphoblastic lymphoma, but these entities are not typically in the differential diagnosis of MCL.

Genetics

Almost all cases of MCL contain a balanced translocation involving the cyclin D1 gene *(CCND1)* on chromosome 11q13. The breakpoints occur in a region of *CCND1* 5′ to the coding region of the gene so that intact cyclin D1 protein can be produced as a result of the translocation. In most cases, the immunoglobulin heavy chain gene *(IGH)* on chromosome 14q32 is the partner in the translocation, but in a very small subset of cases the kappa immunoglobulin light chain gene (2p11) or the lambda immunoglobulin light chain gene (22q11) are the partner loci. At *IGH*, the translocation involves the VDJ joining regions of the gene, and *CCND1* comes under the regulatory control of the *IGH* enhancer sequences, causing *CCND1* overexpression. Because the morphology and phenotype of MCL overlap with other small B-cell lymphomas, demonstrating cyclin D1 positivity in the neoplastic cells by paraffin section immunohistochemistry or detecting *CCND1/IGH* fusion by fluorescence in situ hybridization (Fig. 7.19) is typically considered an essential confirmatory finding for this diagnosis.

Cyclin D1–negative cases of MCL have been identified as those cases that have typical morphologic and phenotypic attributes (CD5+, CD23− B-cell lymphoma) of MCL often combined with positivity for cyclin D2 or cyclin D3 and absence of staining of the tumor for p27. These criteria are based on gene expression profiling (GEP) experiments that demonstrated identical GEP signatures of cyclin D1+ and cyclin D1− cases defined in this way. More recently, SOX11 immunohistochemistry has aided in further recognition of this subset of cases. A subset of cyclin D1− MCL cases harbor translocations involving cyclin D2. Cyclin D3 rearrangements have also been evaluated, although they are rare and appear less

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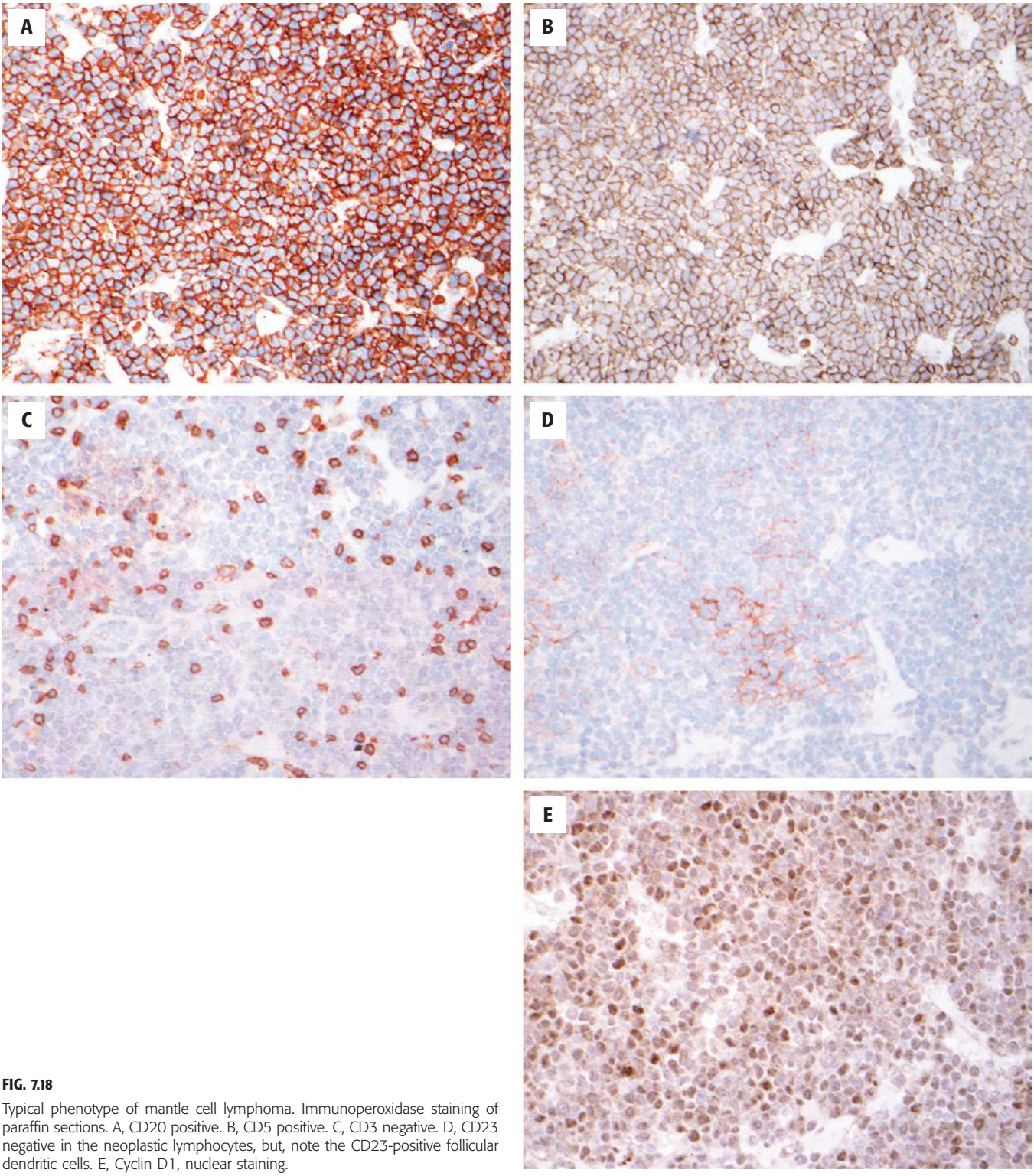


FIG. 7.18

Typical phenotype of mantle cell lymphoma. Immunoperoxidase staining of paraffin sections. A, CD20 positive. B, CD5 positive. C, CD3 negative. D, CD23 negative in the neoplastic lymphocytes, but, note the CD23-positive follicular dendritic cells. E, Cyclin D1, nuclear staining.

specific for the diagnosis of MCL. This is an area that requires additional study and clinical analysis due to the rarity of these cases.

In addition to *CCND1* translocations, MCLs typically contain a high number of other non-random chromosome abnormalities. These include gains of 3q26, 7p21, and 8q24 and losses of 1p13-p31, 6q23-q27, 9p21, 11q22-q23, 13q11-q13, 13q14-q34 and 17p13-pter. *ATM* gene mutations occur in a substantial subset of cases, and trisomy 12 is a relatively frequent abnormality in this disease. Blastoid variants are also characteristically tetraploid, and progression from standard variants to aggressive variants can be associated with acquisition of *MYC* amplification or translocation, *P16* deletion or hypermethylation, and/or *TP53* mutation/deletion.

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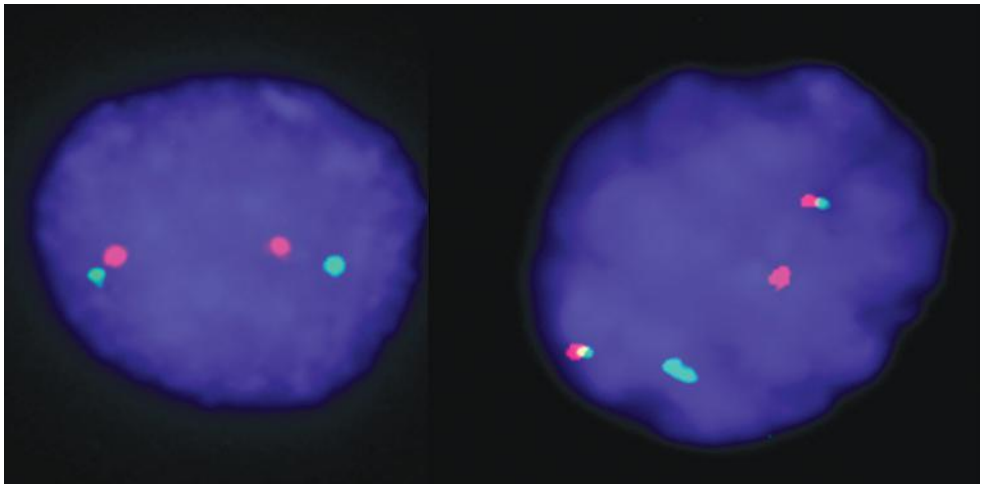


FIG. 7.19

Fluorescence in situ hybridization with probes that recognize *CCND1 (red)* and *IGH (green)*. The nucleus on the left is normal; two copies of *CCND1* and two of *IGH* on normal chromosomes 11q13 and 14q32, respectively. On the right is a mantle cell lymphoma nucleus. It contains one normal red *CCND1* and one normal green *IGH* signal representing the non-translocated chromosomes 11q13 and 14q32. The two yellow fusion signals represent the derivative chromosomes 11 and 14 involved in the reciprocal t(11;14)(q13;q32).

On a molecular level, clonal immunoglobulin heavy and light chain gene rearrangements can be demonstrated in virtually all cases of MCL. As alluded to earlier, most cases of MCL have unmutated or minimally mutated *IGHV*. However, it is now accepted that MCL arises along two different pathways, with the more common type having the characteristic nodal, aggressive presentation and unmutated *IGHV*. A distinct group, however, shows mutated *IGHV* and follows a more indolent course. This subgroup tends to be SOX11– and have non-nodal presentations.

Next-generation sequencing (NGS) studies suggest that in addition to translocations involving *CCND1*, there are recurrent point mutations in the *CCND1* gene in 35% of MCL cases. Additional driver mutations are commonly identified in the *ATM* and *TP53* genes. *NOTCH1* and *NOTCH2* mutations are associated with poor prognosis. These along with other recurrently mutated genes may provide prognostic markers as well as targets for therapy. As with most other lymphomas, NGS testing is not routinely performed clinically, and the best way to integrate the results of these studies with other clinicopathologic features is still being evaluated.

Differential Diagnosis

The differential diagnosis of MCL includes all of the lymphoma types addressed in this chapter. Tables 7.1, 7.2, and 7.3 highlight the features that distinguish among them. In addition, blastoid variant MCL morphologically resembles B- and T-lymphoblastic lymphoma/leukemia and acute myeloid leukemia. These diseases are primarily distinguished from MCL on the basis of phenotype. T-lymphoblastic lymphoma/leukemia cells are positive for terminal deoxynucleotidyl transferase (TdT) and have an immature T-cell phenotype. B-lymphoblastic lymphoma/leukemia is also TdT positive, the blasts express CD34 in a high percentage of the cases, and they have a CD19, CD20 variable, CD5–, surface immunoglobulin, and cyclin D1– phenotype. Myeloid lineage blasts contain azurophilic granules and/or Auer rods in Wright-Giemsa–stained smears. By immunohistochemistry applied to paraffin sections they express myeloperoxidase and lysozyme and lack staining for cyclin D1, and they are variably positive for CD13, CD33, and CD117 (CKIT).

Prognosis and Therapy

The median survival for MCL is only 3 to 4 years with no plateaus in the survival curves. Several groups have attempted to refine predicting prognosis using a proposed Mantle Cell Lymphoma International Prognostic index (MIPI). In this scheme, age, performance status, white blood cell count, and lactate dehydrogenase levels are combined into an index that predicts low-, intermediate-, and high-risk groups. Addition of proliferative rate as assessed by Ki-67 staining has been suggested as an independent prognostic marker (higher risk associated with proliferation >30%) in MCL.

Treatment of MCL is challenging. There is no uniformly agreed upon standard initial therapy. Therapy ranges from watch-and-wait to aggressive chemotherapy, depending on patient risk factors. Common regimens have included R-maxi-CHOP or R-HyperCVAD with or without methotrexate and cytarabine. Other regimens, including bendamustine and rutuxumab, are being evaluated, and targeted therapies, such as the BTK inhibitor ibrutinib, have proven effective in MCL. Depending on the patient's age and comorbid conditions, these may be followed by autologous stem cell transplant.

FOLLICULAR LYMPHOMA

Clinical Features

Accounting for approximately 20% of all non-Hodgkin lymphomas, FL is the second most common lymphoma type in

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FOLLICULAR LYMPHOMA—FACT SHEET

Clinical
- 20% of non-Hodgkin lymphomas
- Median age 7th decade, slight female predominance
- Progressive adenopathy, waxing and waning in some
- Multiple intestinal lymphomatous polyposis; duodenal cases a unique indolent subset
- Primary cutaneous follicular lymphoma
- Pediatric follicular lymphoma with different biology
- Leukemic phase rare
- High stages at presentation in majority of patients; limited stage characteristic of pediatric type, primary cutaneous, duodenal

Morphology
- Nodular pattern with varying proportions of diffuse growth
- Spectrum of neoplastic centrocytes and centroblasts
- Diffuse variant associated with different genetics
- Other morphologic variants: floral variant, with plasmacytic differentiation, with marginal zone B-cell differentiation
- Grading based on number of centroblasts/400× field:
  - Grade 1, less than 5
  - Grade 2, between 5 and 15
  - Grade 3, greater than 15
  - Grade 1–2 of 3 an acceptable alternative to distinguishing grade 1 from grade 2

Immunophenotype
- CD19+, CD20+, sIg+, IgM with or without IgD, occasional cases IgG or IgA+
- CD10+, BCL6+, BCL-2+ (in most nodal grade 1–2 cases)
- Associated with CD21- or CD23-positive follicular dendritic cells

Genetics
- t(14;18)(q32;q21): *IGH/BCL2*
- Clonally rearranged immunoglobulin genes
- Point mutations in immunoglobulin genes and in 5′ noncoding region of *bcl-6* gene

Prognosis and Therapy
- Grades 1 and 2 follicular lymphoma:
  - Watch and wait approach for asymptomatic stage III and IV patients
  - Low-intensity single or multiagent chemotherapy with rituximab for symptomatic patients
  - 8- to 10-year median survival
  - Outcome predicted by Follicular Lymphoma International Prognostic Index (FLIPI)
  - Transformation to diffuse large B-cell lymphoma heralds aggressive disease
- Grade 3 follicular lymphoma:
  - Anthracycline-containing multiagent chemotherapy with rituximab
  - Long-term clinical remissions in 40% of patients
  - Outcome predicted by International Prognostic Index

Differential Diagnosis
- Follicular lymphoid hyperplasia
- Chronic lymphocytic leukemia/small lymphocytic lymphoma
- Mantle cell lymphoma
- Nodal marginal zone B-cell lymphoma
- Splenic marginal zone B-cell lymphoma
- Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue
- Lymphoplasmacytic lymphoma
- Lymphocyte-predominant Hodgkin lymphoma
- Lymphocyte-rich classic Hodgkin lymphoma

the United States and Western Europe. The disease occurs at a median age of 60 years and with a slight female predominance; pediatric cases are rare and, as discussed below, represent a distinct clinicopathologic entity. Adult patients most often present with gradually progressive or waxing and waning painless lymph node enlargement involving cervical, supraclavicular, axillary, and/or inguinal regions. Less common are abdominal presentations, characterized by abdominal or back pain due to either mesenteric or retroperitoneal adenopathy. Isolated splenomegaly and a primary peripheral blood leukemic phase, superficially resembling CLL are rare initial manifestations of FL. Gastrointestinal involvement is not uncommon, and specifically FL involving the duodenum is now recognized to have distinct clinical features. Following staging evaluations, FL patients are usually found to have widespread disease; 40% of patients have spleen involvement, 50% have liver involvement, and 55% to 70% have bone marrow involvement. Thus, most patients present in Ann Arbor stage III or IV. Only 20% have B symptoms (fever, weight loss, night sweats) or elevated lactate dehydrogenase levels.

The WHO now recognizes two subtypes of FL that appear to represent distinct clinicopathologic entities: pediatric-type FL and duodenal-type FL. In addition, FLs with *IRF4* rearrangements, also most common in young patients, will be grouped under the heading of "large B-cell lymphoma with *IRF4* rearrangements." These subtypes will be discussed below.

Finally, as with MCL, FL appears to have an in situ counterpart, *in situ follicular neoplasia* (ISFN). ISFN itself has a low rate of progression but may be associated with concurrent or prior overt FL.

Pathologic Features

Morphology

Follicular lymphomas recapitulate the architecture, cytology, and phenotype of normal germinal centers. Most grow in a nodular pattern (Figs. 7.20 and 7.21). The nodules efface the lymph node architecture, extending from the cortex to the hilum, overrunning the sinuses and invading beyond the capsule of the lymph node into the perinodal soft tissue (Fig. 7.22). The neoplastic follicles are crowded together,

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may coalesce (Fig. 7.23), and tend to be smaller and have less prominent mantle zones than normal, reactive follicles. In many cases the neoplastic nodules are accompanied by a diffusely growing component, and, in rare examples, FLs grow in a purely diffuse pattern (Fig. 7.24). These predominantly diffuse cases have been shown in recent years to represent a subgroup with recurrent molecular and cytogenetic abnormalities and reproducible phenotypic and morphologic features. Although currently the WHO does not recognize this variant as a distinct subgroup of FL, evidence is increasing that may support this in the future. When both nodular and diffuse patterns are present in the same tissue sample, the WHO classification recommends that the percentage of the diffuse component be specified in the report.



FIG. 7.20
Follicular lymphoma in a lymph node. The multinodular architecture is readily apparent.

Branching, tapering blood vessels associated with varying amounts of collagen fibrosis commonly accompany FLs (Fig. 7.25). The fibrotic areas can be intranodular, perinodular, associated with diffuse areas, or be so extensive that they obscure the underlying neoplastic lymphoid infiltrates. In the latter cases, wide sampling of the tissue often uncovers more typical features of FL. The fibrovascular stroma can also surround clusters of neoplastic follicular center cells, packeting them in a manner that suggests an epithelial or a melanocytic malignancy.

Just as a spectrum of cell types is present in normal germinal centers, FLs vary in their cytologic composition from case to case or from area to area in an individual case. Centrocytes predominate in most FLs (Fig. 7.26A). They have nuclei that are basically ovoid with irregular, angulated contours and superimposed notches, grooves, and cleaves. In hematoxylin and eosin (H&E)-stained sections, centrocytes lack nucleoli, and the chromatin distribution varies from delicate to coarsely clumped and hyperchromatic. Small centrocytes have nuclei that are smaller than the nuclei of intermixed histiocytes whereas large centrocytes have nuclei that are larger than those of the macrophages. The second cell population that can be identified in most examples of FL is the centroblasts (Fig. 7.26C). They have round nuclei, dispersed chromatin, nucleoli that are usually multiple and adjacent to the nuclear membranes, and basophilic cytoplasm.

The relative proportions of centrocytes and centroblasts in an FL case form the basis for grading the neoplasm. Centroblasts are counted in ten consecutive 400× microscopic fields in ten consecutive follicles. The mean number of centroblasts per 400× field is then determined. Grade 1 is assigned to those cases in which there are less than five large centroblasts per 400× field (Fig. 7.26A). Grade 2 is assigned to those cases with between 5 and 15 centroblasts per 400×

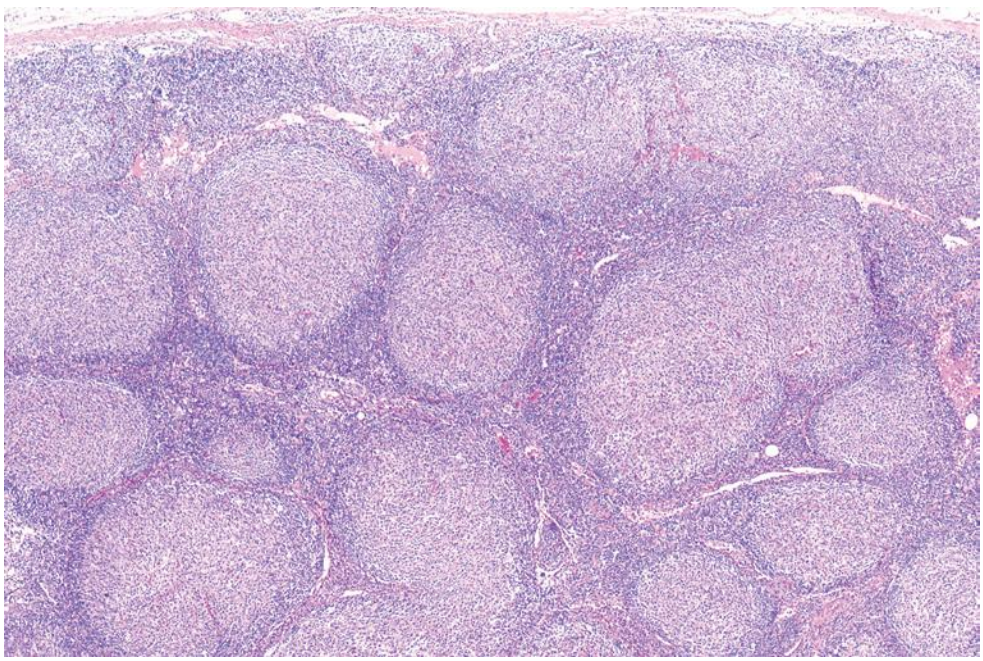


FIG. 7.21
Follicular lymphoma. Multiple neoplastic follicles are crowded into the lymph node.

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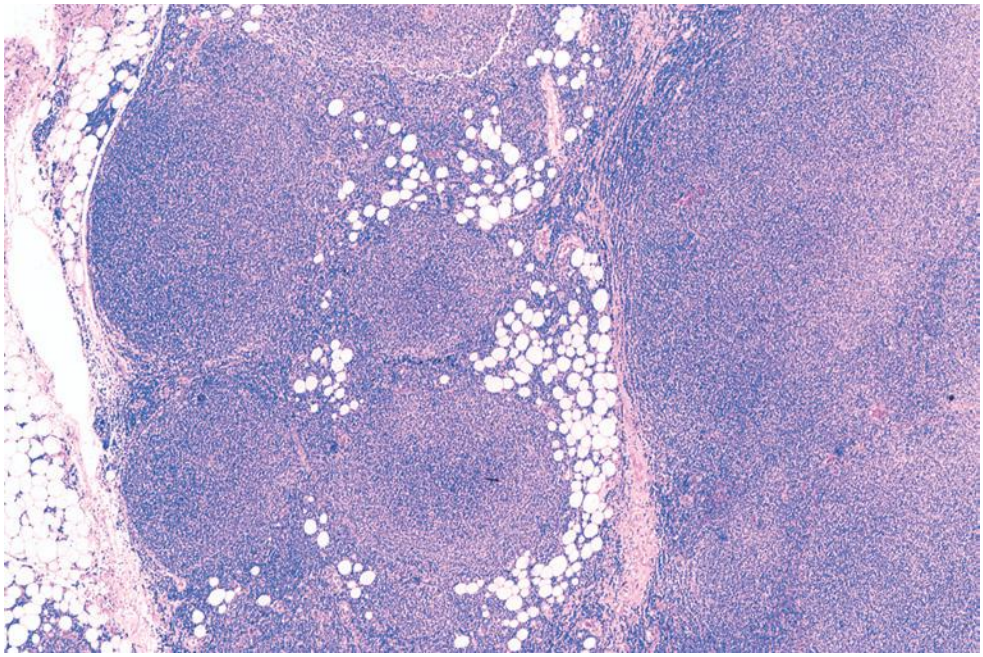


FIG. 7.22

Extensive extracapsular infiltration by follicular lymphoma is illustrated on the left side of the photograph. On the right, the lymph node architecture is effaced by lymphoma growing in a more vaguely nodular pattern.

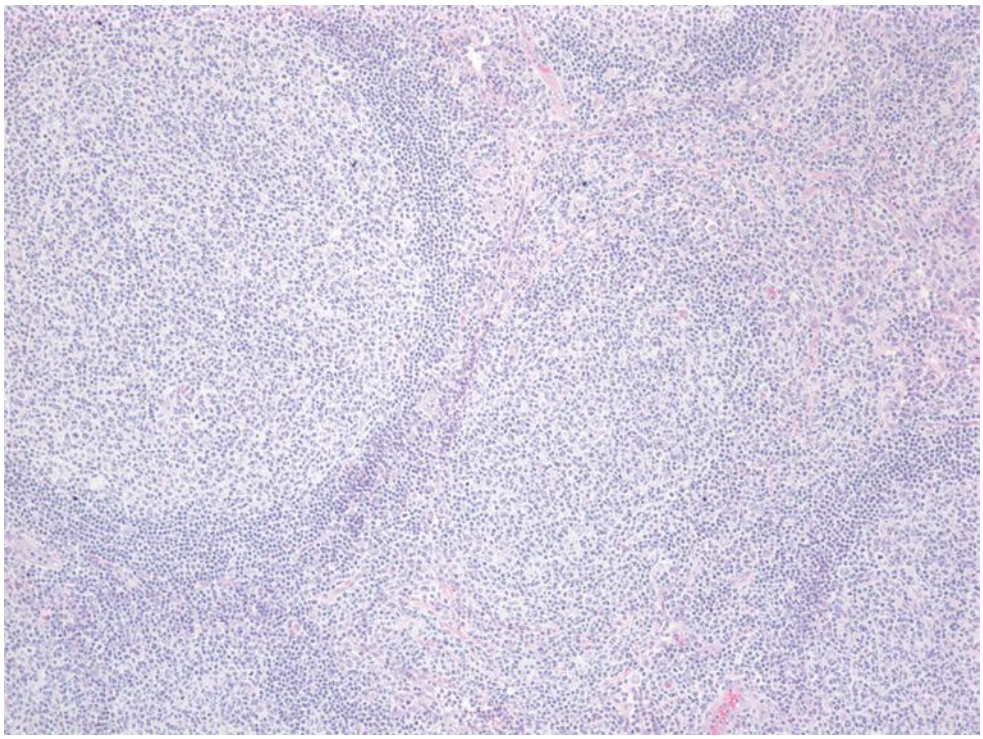


FIG. 7.23

Follicular lymphoma; near confluent neoplastic follicles and interfollicular infiltration by the neoplastic cells.

field (Fig. 7.26B), and grade 3 FLs have more than 15 centroblasts per 400× field. Grade 3 is further subdivided into grades 3a and 3b. If virtually all of the cells in the neoplastic follicles are centroblasts the tumor is assigned grade 3b (Fig. 7.26C). If there is a mixture of centrocytes together with the centroblasts, the lymphoma is graded 3a. Because there is variability between microscopes in the area defined by a 400× field, conversion factors have been published to aid in reproducible grading. Finally, because grade 1 and grade 2 FLs comprise a morphologic continuum and because they are both clinically indolent, the WHO classification now deems it acceptable (preferable) to use the designation grade 1 to 2 of 3 for cases that meet grading criteria for either grade 1 or grade 2 FL. This approach is particularly useful for small biopsy specimens where sampling limitations make grading difficult.

Several unusual morphologic patterns of FL collectively account for no more than 10% of all FL cases. Although

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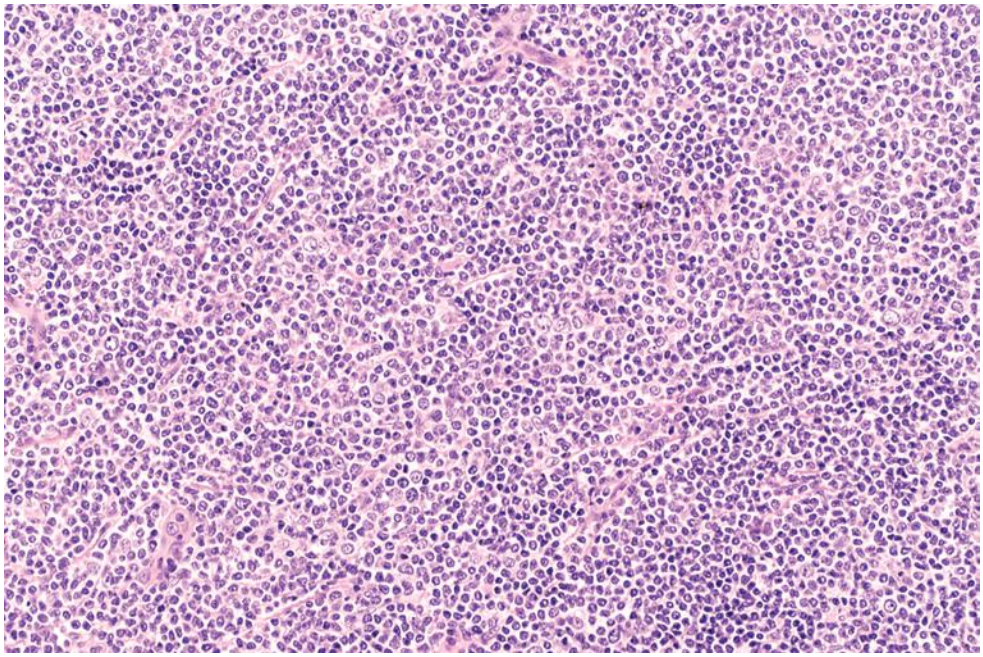


FIG. 7.24
Diffuse follicule center lymphoma.

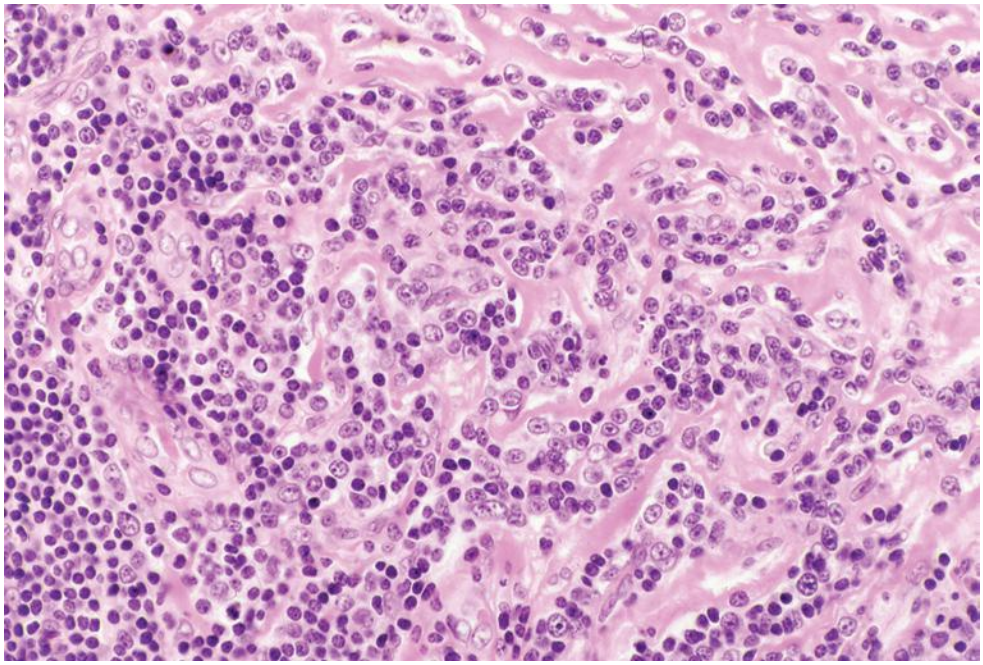


FIG. 7.25
Collagen sclerosis in a follicular lymphoma.

these are not separated out by the WHO as distinct clinicopathologic entities, recognizing their features is important for proper diagnosis. These variants are morphologic reflections of biologic functions of normal follicular center cells: immunoglobulin production, plasma cell differentiation, and marginal zone B-cell differentiation. When the neoplastic follicular center cells, usually centrocytes, contain intracytoplasmic immunoglobulin that distorts the nucleus and forms intracytoplasmic vacuoles, the term *signet ring cell lymphoma* has been applied. The vacuoles represent dilated rough endoplasmic reticulum containing flocculent material—the immunoglobulin. In cases in which the immunoglobulin isotype is IgM, the vacuoles are periodic acid-Schiff positive. This unusual morphology probably results from an imbalance between immunoglobulin synthesis and the ability of the neoplastic follicular center cells to transport the immunoglobulin normally to the surface of the cell. There is no independent prognostic significance to signet ring cell cytology in FL.

Cytologically normal, polyclonal plasma cells, usually distributed between the neoplastic follicles, occur relatively frequently in FLs. However, in a small proportion of cases, FLs contain intrafollicular or extrafollicular plasma cells that are clonally related to the underlying FL (Fig. 7.27). The

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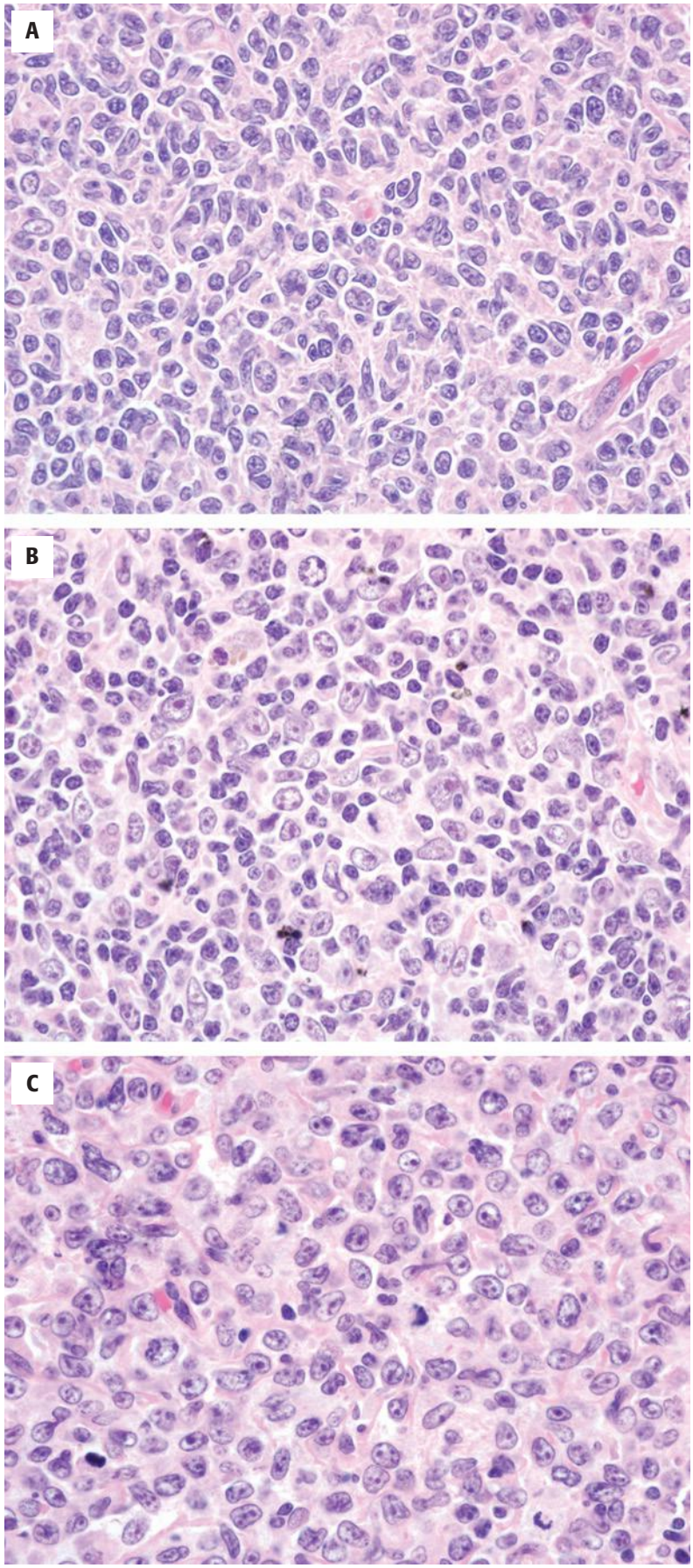


FIG. 7.26

Cytologic composition of follicular lymphoma. A, Grade 1. B, Grade 2. C, Grade 3.

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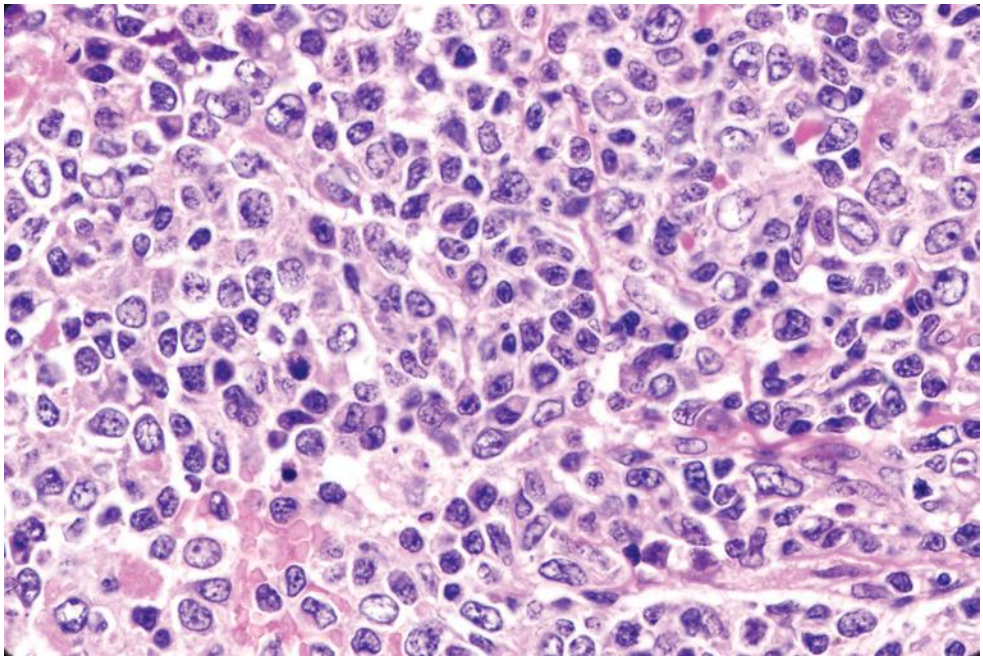


FIG. 7.27
Follicular lymphoma with plasma cell differentiation. The neoplastic follicle is present in the upper right side portion of the photograph. The neoplastic plasma cells are clustered in the center of the photograph.

plasma cells are often cytologically abnormal with nuclear irregularities and intranuclear (Dutcher bodies) or intracytoplasmic (Russell bodies) immunoglobulin inclusions. Using paraffin section immunohistochemistry, the abnormal plasma cells can be shown to express the same light chain type that is expressed by the neoplastic follicular center cells (Fig. 7.28A and 7.28B). This morphology and immunoarchitecture represent the neoplastic recapitulation of the normal process of plasma cell differentiation of normal germinal center B cells.

A subset of FL exhibits marginal zone B-cell differentiation (Fig. 7.29A and 7.29B). In these cases, the neoplastic follicles, composed of centrocytes and centroblasts, are surrounded by coronas of monocytoid-appearing cells with distinctive, voluminous pale eosinophilic or clear cytoplasm. The immunoarchitecture in these cases is complex. The morphologically more usual FL cells distributed in the centers of the nodules are immunoglobulin light chain restricted B cells that express BCL2 together with CD10 and/or BCL6. By contrast, the neoplastic monocytoid-appearing cells, though expressing BCL2 and the same immunoglobulin light chain type as the neoplastic follicular center cells, often lack staining for CD10 and BCL6. At one time these cases were considered to be composite follicular and *monocytoid B-cell lymphomas*. However, studies using microdissection and PCR have demonstrated that the marginal zone cells in these cases contain the same immunoglobulin gene rearrangement and identical *BCL2/IGH* gene sequences as the phenotypically and cytologically typical neoplastic follicular center cells. Thus, this process is now thought to represent marginal zone B-cell differentiation in FL.

Finally, an architectural variant of FL, termed *floral variant*, has also been described (Fig. 7.30). The neoplastic follicles are large; they contain clustered neoplastic germinal center cells surrounded by large "clouds" of non-neoplastic mantle zone lymphocytes.

Phenotype

Follicular lymphoma cells express the pan B lymphocyte antigens CD19, CD20 (Fig. 7.31A), CD22, CD79a, CD79b, and PAX-5. A substantial subset of cases is positive for CD23. IgM with or without IgD is the heavy chain type most frequently expressed by FLs, but IgG+ and IgA+ cases also occur. In almost all cases, immunoglobulin light chain restriction can be demonstrated by flow cytometry or immunohistochemistry. Rarely, the cells of FLs do not express immunoglobulin. In our experience, surface immunoglobulin-negative FLs are more likely to be grade 3 or to occur in children and young adults or to be primary in the skin. Similar to normal germinal center B cells, the cells in FLs in lymph nodes and anatomic sites other than bone marrow, blood, and skin are positive for CD10 (Fig. 7.31C) and/or BCL6 in a very high proportion of cases (90%). Compared to flow cytometry, paraffin section immunohistochemistry may be a more sensitive modality by which to detect CD10 expression in these cases. In addition, in approximately half of FLs, CD10 is expressed only by the lymphocytes within neoplastic nodules and not by the lymphoma cells present in the interfollicular areas of the lymph node. Furthermore, compared to those involving lymph nodes, a greater fraction of FLs in leukemic phase or involving the bone marrow fail to stain for CD10. Other germinal B-cell markers for which FLs are positive include LMO2, GCET, and HGAL. With only rare exceptions, FLs are negative for CD5 (Fig. 7.31F). The morphologically recognizable nodular pattern of FLs has an immunoarchitecture that recapitulates that of normal germinal centers. In addition to the lymphomatous B cells, neoplastic

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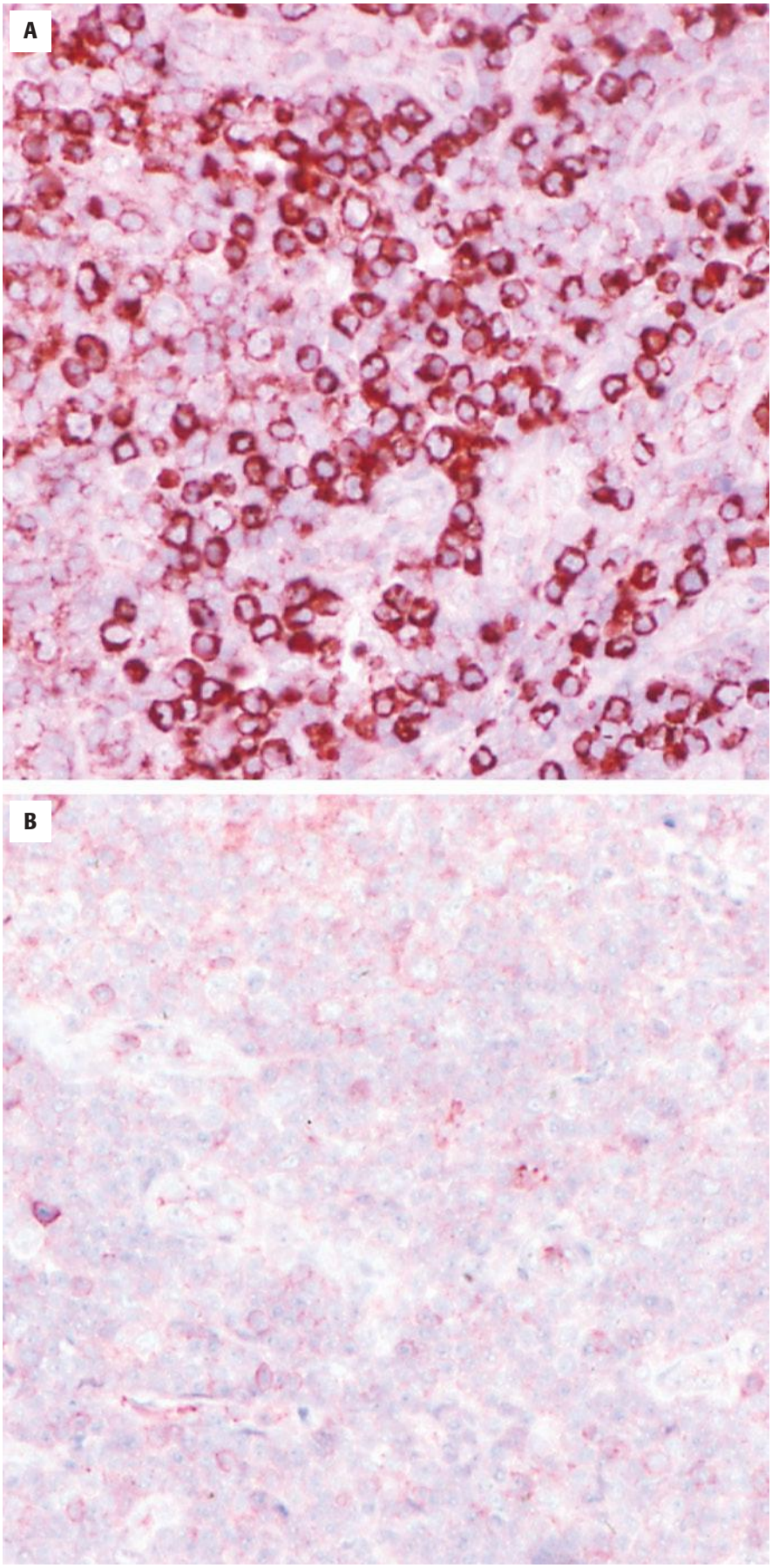


FIG. 7.28

Follicular lymphoma with plasma cell differentiation. Immunoperoxidase stain for kappa immunoglobulin light chains (A) and lambda immunoglobulin light chains (B). The plasma cells have a monotypic staining pattern for kappa light chains.

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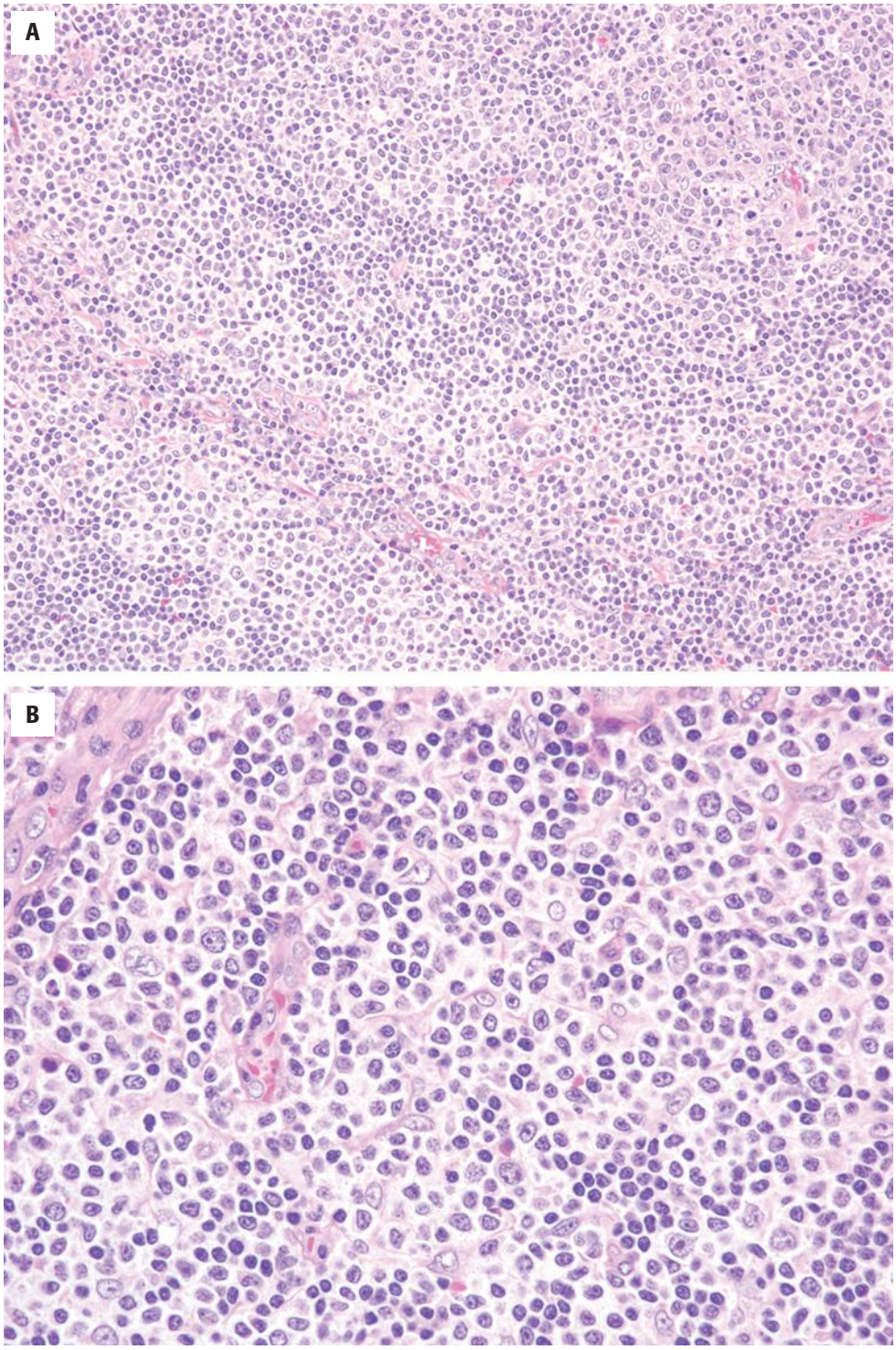


FIG. 7.29

Marginal zone B-cell differentiation in a follicular lymphoma. A, The neoplastic germinal center is at the upper right of the photograph. Marginal zone differentiation is at the lower left with the cytology illustrated in B.

follicles contain well-defined meshworks of normal CD21 and/or CD23+ (Fig. 7.31B) follicular dendritic cells, relatively few CD8+ cytotoxic T lymphocytes, and varying numbers of CD3+ (Fig. 7.31E) interfollicular T cells and CD57/CD279+ intrafollicular T cells. In most cases the neoplastic follicles are also surrounded by, often attenuated, polyclonal, IgD+ mantle zone lymphocytes.

As a consequence of the t(14;18)(q21;q32) (see below), aberrant expression of cytoplasmic BCL2 protein expression is a hallmark of most FL cases (Fig. 7.31D). This marker is positive in nearly 85% of grade 1–2 FLs, 75% of grade 3 FLs, and a varying percentage of primary cutaneous FLs. As discussed below, pediatric-type FL cases lack the *IGH-BCL2* translocation, by definition. Because normal follicular center cells lack BCL2 protein expression, BCL2 is a useful marker to distinguish FL from follicular lymphoid hyperplasia. In this application BCL2 positivity in follicular center cells supports a diagnosis of FL, but absence of BCL2

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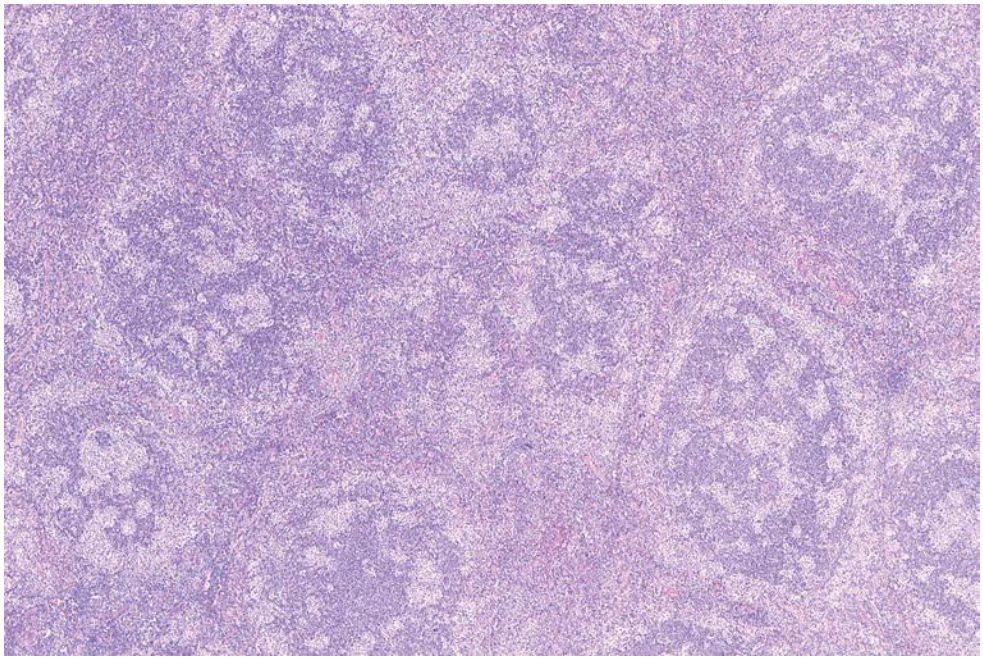


FIG. 7.30
Floral variant follicular lymphoma.

expression by follicular center cells, though characteristic of follicular hyperplasia, does not exclude FL. Because almost all other low-grade B-cell lymphomas physiologically express BCL2, this marker is not useful to discriminate between FL and other low-grade B-cell lymphoma types. It should be noted that some cases of BCL2– FL may actually express BCL2 that is mutated or otherwise altered such that the epitope recognized by the commonly used mouse monoclonal antibody is absent. Such cases may be detected with other BCL2 antibodies, such as a rabbit monoclonal antibody, and for this reasons some laboratories maintain both options.

A small subset of otherwise typical low-grade FLs may show a high proliferative fraction by Ki67 staining (>30%). Reports are somewhat variable, but some studies have found these cases to have a more aggressive clinical course than low-grade FL with Ki-67 less than 30%.

Genetics

The genetic hallmark of FL, present in 85% of cases, is the t(14;18)(q32;q21). This abnormality translocates *BCL2* into the VDJ region of the immunoglobulin heavy chain gene (*IGH),* placing *BCL2* under the regulatory control of the *IGH* promotor and causing overexpression of BCL2 protein. Because BCL2 is an inhibitor of apoptosis, the translocation rescues the neoplastic cells from apoptotic cell death, causing their accumulation in the tissues. The t(14;18) can be demonstrated by routine cytogenetic analysis, PCR techniques with primers that recognize *BCL2* and *IGH*, and by fluorescence in situ hybridization (FISH). The latter is most practical for diagnosis, as it can be applied to paraffin-embedded tumor tissues. In most cases, it is unnecessary to demonstrate the t(14;18) in suspected FLs because the morphology and immunoarchitecture are highly characteristic of the entity. However, in those low-grade B-cell lymphoma cases in which morphology and phenotype are unusual and the differential diagnosis includes FL, demonstrating the t(14;18)(q32;q21) by FISH or PCR helps to confirm the diagnosis. Although the t(14;18)(q32;q21) is the cytogenetic abnormality that is most characteristic of FL, it is usually not the sole karyotypic abnormality in the neoplastic cells. Additional trisomies, monosomies, gains, losses, and other translocations, none of which is specific for FL, usually accompany the t(14;18)(q32;q21), indicating that from a genetic perspective FLs are pathophysiologically complex.

In a small subset of FLs, there are translocations involving the *BCL6* gene at chromosome 3q27. The partner chromosome is usually 14q32 at the *IGH* locus. The tumor cells in these cases do not contain *BCL2* rearrangements, and these FLs are more likely to be examples of grade 3b FL, suggesting that they are pathogenetically distinct from FLs with the t(14;18)(q32;q21).

FLs with a predominantly diffuse growth pattern are usually negative for the t(14;18)(q32;q21) and commonly show deletions at chromosome 1p36. This abnormality is a common secondary abnormality in FL in general but appears enriched in this group. The significance of this abnormality is not certain, although the *TNFRSF14* gene is implicated. More recently mutations in *STAT6* were also found to be enriched in diffuse FLs.

FLs have clonally rearranged immunoglobulin genes. A hallmark of normal follicular center B cells and postfollicular B cells is point mutations in the VDJ regions of the immunoglobulin genes and in the 5′ noncoding sequences of *BCL6* (the latter an off-target effect of AID activity). Although the biological significance of the *BCL6* point mutations is unknown, point mutations in the immunoglobulin genes result

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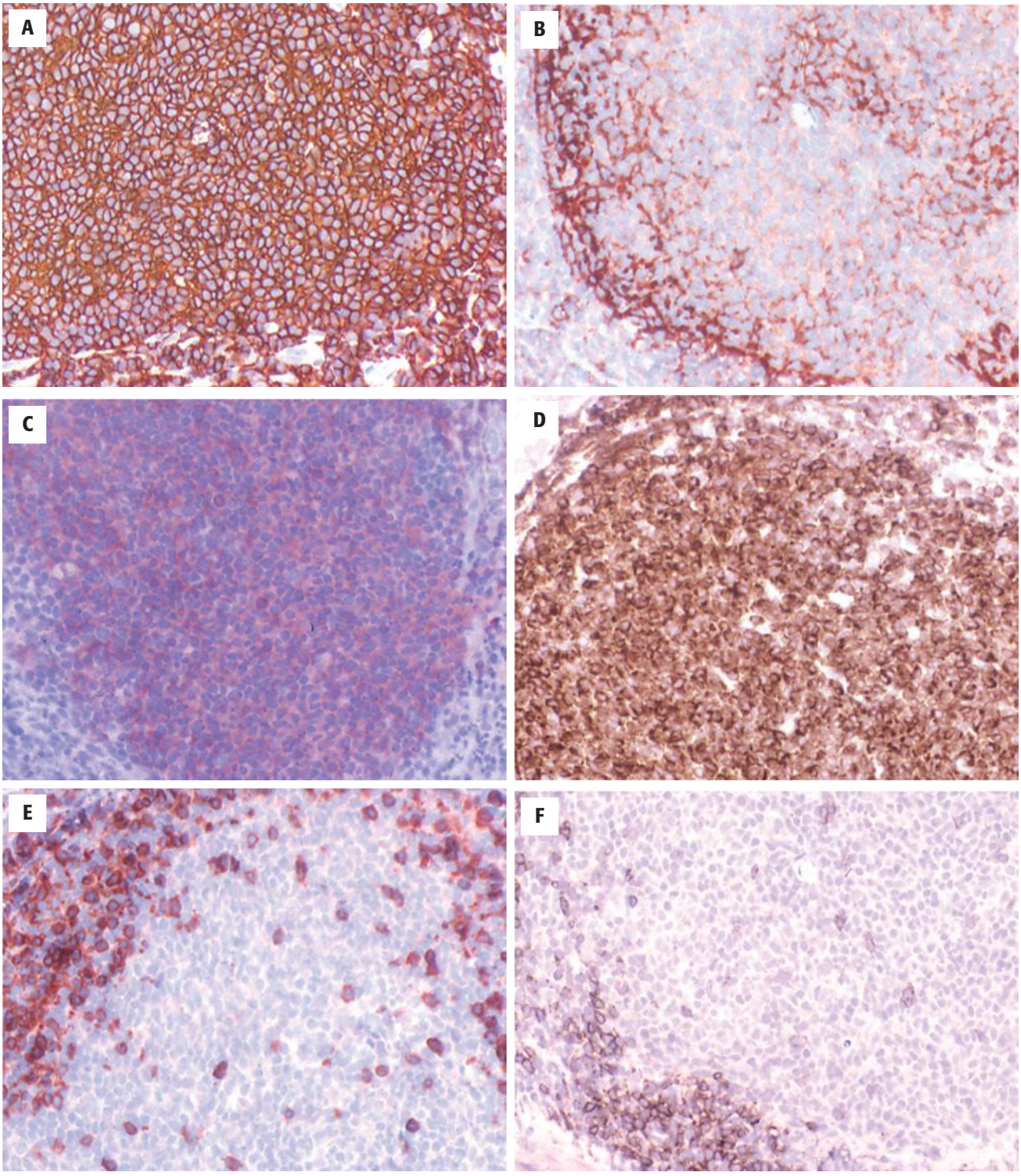


FIG. 7.31

Typical phenotype of follicular lymphoma. Immunoperoxidase staining of paraffin sections of serial sections of a single neoplastic follicle. A, CD20 positive. B, CD23 negative; note the associated CD23-positive follicular dendritic cell meshwork. C, CD10 positive. D, bcl-2 positive. E, CD3 negative. F, CD5 negative.

in immunoglobulin molecules that have enhanced affinity for the antigen that has initiated the B-cell immune response. In keeping with their presumed origin from normal follicular center cells, FL cells also contain immunoglobulin and *BCL6* gene point mutations.

Recent NGS studies have revealed a number of recurrent mutations in FL. However, their clinical significance is still uncertain in most cases. Genes involved in epigenetic regulation are commonly mutated genes. *EZH2* mutations, for example, are common (20% to 25%) in FL and appear to

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occur as an early event. Table 7.4 lists some of the more commonly mutated genes. A panel of seven genes including *EZH2*, along with *ARID1A, MEF2B, EP300, FOXO1, CREBBP*, and *CARD11,* has been proposed as part of a clinicogenetic risk model to improve upon the Follicular Lymphoma International Prognostic Index (FLIPI) currently used by clinicians in FL. This has been termed the *m7-FLIPI*.

Perhaps further emphasizing the potential importance of molecular testing, a subset of FLs (≈5%) have been identified that harbor *NOTCH1* mutations. These cases are characterized by marked female predominance, lack of t(14;18), and propensity for splenic involvement. Although the numbers of cases are small, these may also show a propensity to transform to DLBCL.

Follicular Lymphoma in Unusual Circumstances and in Extranodal Sites

Pediatric-Type Follicular Lymphoma

The WHO now recognizes pediatric-type FL as a distinct entity. Although first described in children, cases have also been reported in adults. Overall this is a rare entity, representing only 1% to 2% of B-cell Non-Hodgkin lymphoma (NHL) in the pediatric age group. Patients with pediatric-type FL present with low-stage disease, typically involving lymph nodes in the head and neck region and less frequently involving the GI tract.

The pediatric-type FL cases have characteristic histopathologic features that distinguish them from typical FL. These include large, expansile follicles (Fig. 7.32) with high proliferation rate, reminiscent of follicles seen in florid follicular hyperplasia. The neoplastic follicles in pediatric-type FLs are usually composed of medium-sized, blastoid cells that do not exactly resemble either centroblasts or centrocytes (Fig. 7.33). Caution must be taken in these cases first to recognize this as a lymphomatous process and second to distinguish this cytologically from a high-grade process.

The neoplastic follicles are composed of B cells that express CD10 (Fig. 7.34A) and BCL6, are typically negative for MUM 1, and most, but not all, are BCL2− (Fig. 7.34B). By definition, these cases must lack the t(14;18) *IGH-BCL2* translocation. They typically also lack rearrangements of *BCL6* and *IRF4*. Demonstrating a high Ki-67 proliferation index, defined in most studies as greater than 30%, is also a diagnostic criterion of pediatric-type FL.

By next-generation sequencing (NGS), pediatric-type FLs have relatively few mutations compared with other FLs, probably explaining their indolent behavior. Mutations in the *TNFRSF14* gene, located at 1p36, and found in 54% of cases seem to dominate the landscape. Although mutations in this gene are common in standard FL as well, their relative predominance in the pediatric-type FL group may suggest a role in pathogenesis. However, additional studies are ongoing, and likely much remains to be understood about the mutational landscape in this unique cohort of patients.

Rendering a pediatric-type FL diagnosis can be challenging, due to the frequent young age of the patients and the histopathologic features that overlap with follicular hyperplasia. Therefore, demonstrating kappa or lambda immunoglobulin light chain restriction by immunophenotyping (Fig. 7.34C and D) or clonal immunoglobulin gene rearrangements is

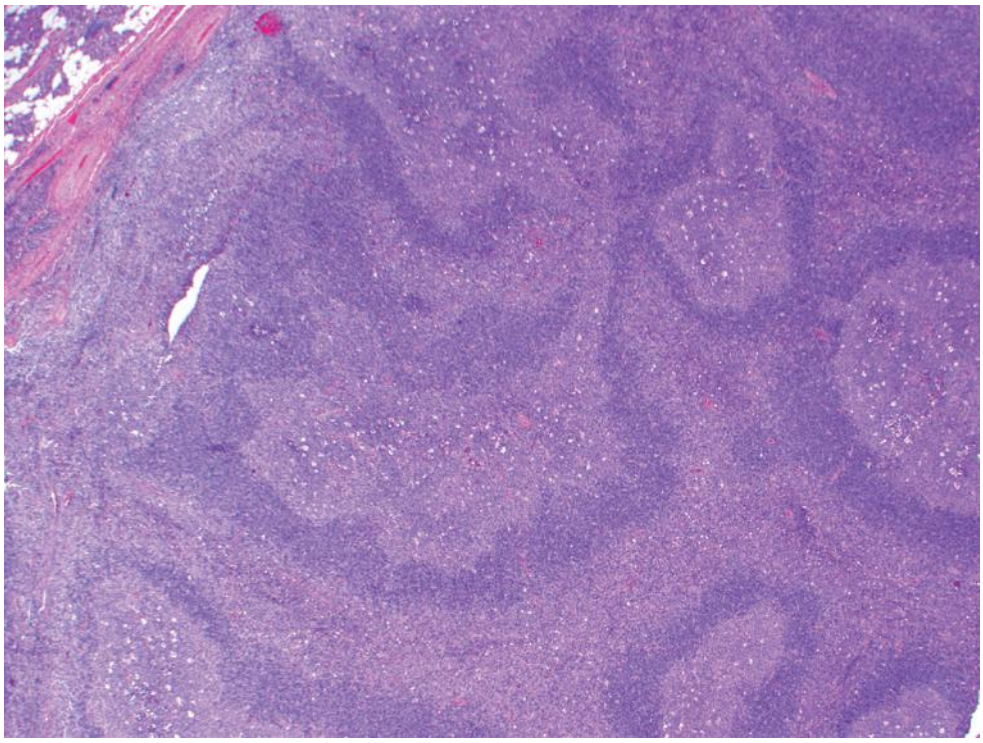


FIG. 7.32
Pediatric follicular lymphoma. Large expansile follicles.

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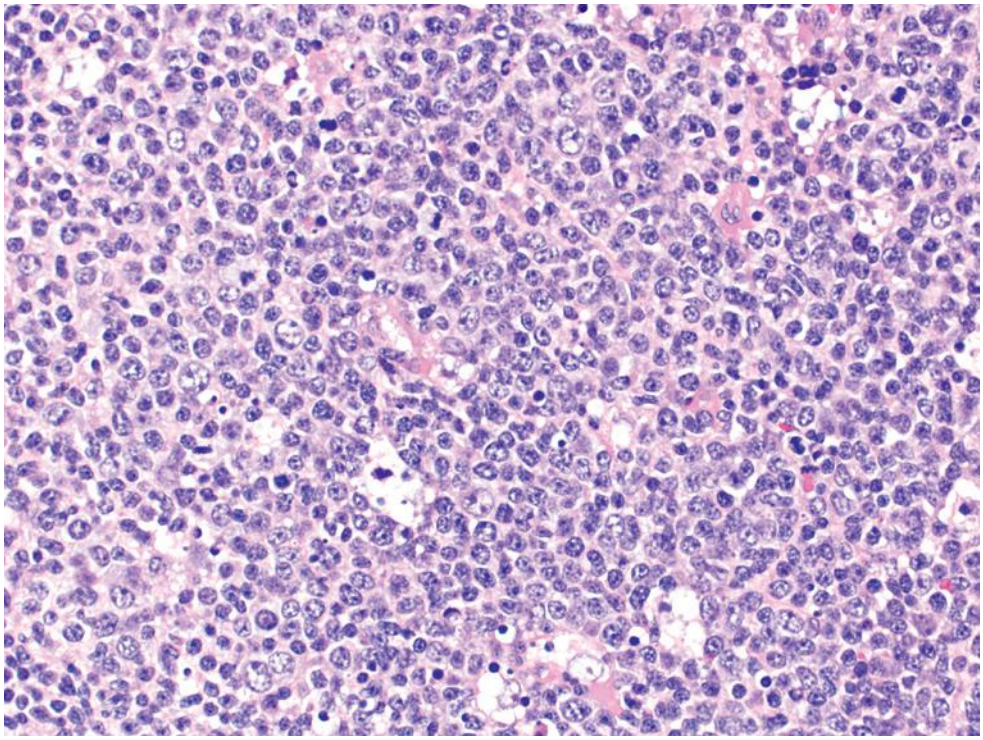


FIG. 7.33
Pediatric follicular lymphoma. Cytologic composition featuring blastlike abnormal lymphocytes.

often required to support the diagnosis. FISH studies must be performed to demonstrate lack of a *BCL2* rearrangement, and Ki-67 staining must show a high proliferation rate. Strict adherence to the diagnostic criteria is essential, because pediatric-type FL, whether in children or adults, has a very indolent course and may not require systemic therapy.

Of note, rare FL cases present in the testis, most often in children. These cases may be pathophysiologically distinct from the "pediatric-type" FL cases because they have occasional *BCL6* rearrangements. However, they are phenotypically similar in that they often lack *BCL2* expression and lack *IGH-BCL2* rearrangements. These cases also have an indolent course.

Large B-Cell Lymphoma and Follicular Lymphoma With *IRF4* Rearrangement

Although technically defined as *large B-cell lymphoma,* this group encompasses cases with the histology of either DLBCL or high-grade FL that have *MUM1/IRF4* gene rearrangements as demonstrated by FISH. In 2016, the WHO recognized this group of patients as a distinct provisional entity. As with pediatric-type FL, these cases occur most often in children. Waldeyer ring is the most common site of presentation. Most patients present with low-stage disease, in spite of the often high-grade–appearing histopathologic appearance. They have a favorable prognosis and do not behave aggressively, although the disease course appears less predictably indolent than pediatric-type FL.

By immunohistochemistry, FL with *IRF4* rearrangements express typical markers of FL such as CD10 and BCL6, but only variably express BCL2 and do not have *BCL2* gene rearrangements. In contrast to pediatric-type FL, they are strongly and uniformly positive for MUM1 by immunohistochemistry. However, because MUM1 protein can be expressed by FLs in the absence of *MUM1I/RF4* gene rearrangements, a *MUM1/IRF4* gene rearrangement must be demonstrated (usually by interphase FISH) to fulfill the diagnostic criteria for FL with *IRF4* gene rearrangement. The *MUM1/IRF4* rearrangement in these cases frequently involves an immunoglobulin gene partner.

Primary Cutaneous Follicle Center Cell Lymphoma (FCL)

Skin involvement can occur in systemic FL, and, in this situation they are morphologically, phenotypically, and genetically similar to their nodal counterparts. However, when follicle center lymphomas arise primarily in and only involve the skin, they exhibit important differences from those that arise in lymph nodes. Clinically, they produce single or grouped erythematous papules and nodules often in the head and neck, shoulder girdle, upper chest, and back regions. After careful staging excludes extracutaneous disease, they are readily treated by conservative local therapies and rarely disseminate or cause the death of the patient.

In primary cutaneous FCL, a Grenz zone separates the nodular infiltrates in the dermis from the epidermis. In prototypic cases the centers of the nodules are composed of a mixture of centrocytes and centroblasts without prominent tingible body macrophages or mitotic figures. Distinct mantle cell populations circumscribe the follicular center cells in most cases, and the internodular areas are either sparsely cellular or contain infiltrates of bland T lymphocytes. Cutaneous FCLs are composed of B cells that express BCL6 and often CD10. However, the neoplastic lymphoid cells in primary

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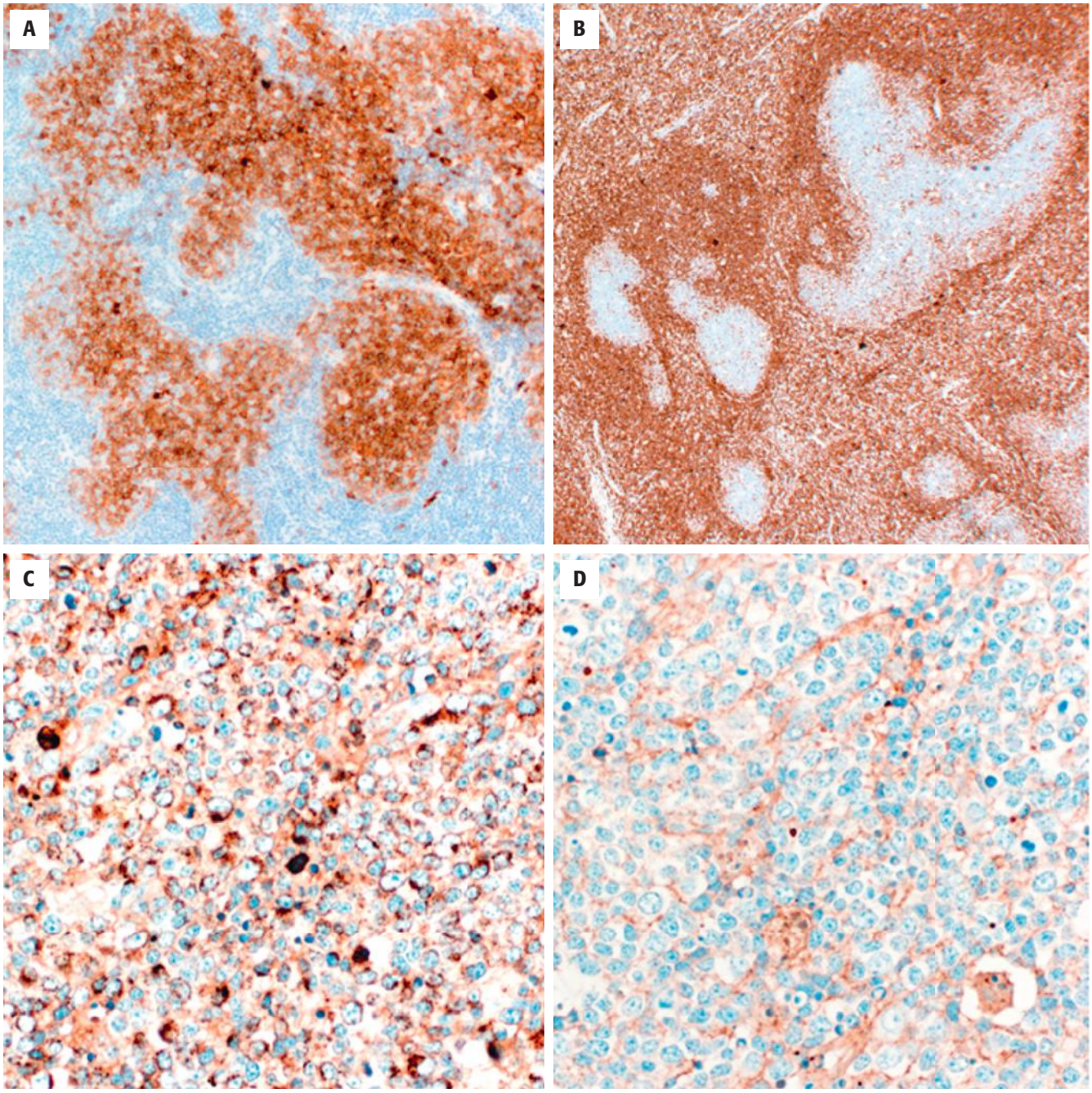


FIG. 7.34

Immunophenotype of pediatric follicular lymphoma. The neoplastic cells express CD10 (A) and kappa light chains (C). They are negative for BCL-2 (B) and lambda light chains (D).

cutaneous FLs are more frequently BCL2 protein and immunoglobulin light chain negative than nodal FL. By both morphology and phenotype they can be very difficult to distinguish from cutaneous follicular lymphoid hyperplasia. In difficult cases, establishing that there is an abnormal follicular center cell population in the skin using morphology and immunohistochemistry and showing clonal immunoglobulin gene rearrangements by molecular genetic methods are necessary to make the diagnosis of cutaneous FL. The European Organisation for Research and Therapy of Cancer and the World Health Organization representatives issued a joint classification for primary cutaneous lymphomas. Those thought to be the cutaneous counterparts of the nodal FL are termed *primary cutaneous follicle center lymphoma*, and regardless of the cytologic composition of the neoplastic follicles, they are NOT graded. Notably, cases showing a completely diffuse pattern, but composed of centrocytic and centroblastic cells with a germinal center phenotype, are also appropriately diagnosed as primary cutaneous follicle center lymphoma. Indeed, a diffuse pattern is, in our experience, a

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common presentation. Presence of a follicular pattern with BCL2 expression should prompt a careful search for evidence of systemic FL before settling on a diagnosis of primary cutaneous FCL.

Follicular Lymphoma in the Gastrointestinal Tract and Duodenal-Type Follicular Lymphoma

Follicular lymphoma can present in the intestine as multiple sessile and pedunculated polyps—multiple intestinal lymphomatous polyposis. In these cases, numerous nodules of monomorphous follicular center cells extensively infiltrate the lamina propria of the intestine. When small centrocytes predominate they can be morphologically quite similar to intestinal lymphomatous polyposis due to MCL. Immunophenotyping will readily distinguish between these two lymphoma types. Follicular lymphomatous polyposis in the intestine is almost always associated with mesenteric lymph node involvement.

On the other hand, duodenal involvement by FL is often confined to that location (approximately half of duodenal FLs are confined to the duodenum). Because of their unique features, the WHO now recognizes duodenal-type FL as a distinct entity. These lesions almost always involve the second portion of the duodenum, forming periampullary sessile polyps with lamina propria and submucosal nodular lymphoid infiltrates. The lymphomas are grade 1 to 2 and are composed of CD10 and/or BCL6+, BCL2+ tumor cells. However, there is a high frequency (71%) of association with *Helicobacter pylori*, and these tumors show restricted usage of *IgVH4* genes, attributes that they share with extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue. Thus, primary duodenal FLs are hypothesized to be pathophysiologically different from their systemic counterparts.

Bone Marrow and Spleen Involvement

At diagnosis, the bone marrow is involved by FL, grades 1 and 2 in approximately 55% to 70% of cases and in a lower percentage of FL grade 3 cases. A paratrabecular distribution is most characteristic, but intertrabecular nodules and interstitial infiltrates also occur. In the bone marrow, FLs are usually composed of a monomorphous population of small centrocytes with few centroblasts; in a small fraction of cases, the whole spectrum of follicle center cell types or monomorphous large centroblasts are present. The cell composition of the lymphoma in the bone marrow may not reflect that of the diagnostic lymph node specimen. Optimally the bone marrow report should include a statement comparing the morphology of the lymphoma in the staging bone marrow sample to that in the lymph node. Follicular lymphomas in the marrow are usually associated with reticulin fibrosis or very sparsely and focally involve the bone marrow so that they are not often sampled by bone marrow aspiration. However, when aspirate smears are involved, FL cells are usually small to medium sized with irregular, grooved nuclei, condensed chromatin, inconspicuous nucleoli, and sparse agranular cytoplasm. An overt leukemic phase of FL occurs in less than 10% of FL patients, but careful examination of the blood will reveal abnormal lymphocytes in the blood smears of many patients with FL. The cytologic features of circulating FL cells are identical to those in aspirate smears of involved bone marrow specimens.

In the spleen, FLs preferentially involve the white pulp. Neoplastic centrocytes and/or centroblasts are present in the centers of white pulp nodules, and from there they can irregularly infiltrate the splenic red pulp. Marginal zone differentiation is common in FLs involving the spleen. Haloes of medium size lymphocytes with abundant pale cytoplasm and less irregular nuclei than the neoplastic centrocytes surround the neoplastic follicles, making difficult the distinction between FL with marginal zone differentiation and SMZL. Immunoperoxidase stains demonstrating the BCL2+ CD10 and/or BCL6+ neoplastic follicular center cells (see below) help to define this lymphoma type in difficult cases. A small subset of splenic FL is BCL2−. These cases typically show less frequent CD10 expression, higher proportion of grade 3 histology, higher Ki-67 proliferation rate, less frequent *BCL2* rearrangements, and more commonly have disease restricted to the spleen.

In Situ Follicular Neoplasia

Unusual cases are seen in which lymph nodes have normal architecture but in which the germinal center cells in a subset of the follicles strongly express BCL2. This phenomenon was formerly termed *FL in situ* but is now recognized by the WHO classification as in situ follicular neoplasia (ISFN) to acknowledge the uncertain malignant potential of these lesions (Fig. 7.35). Before using this designation one must be certain the finding is limited to only a few follicles in the lymph node, that these follicles do not distort the architecture of the lymph node, that the BCL2+ lymphocytes are strongly CD10 and/or BCL6+ follicular center cells, and that there is no infiltration of CD10 or BCL6+ lymphocytes into the interfollicular areas. When diagnosed in this way, ISFN can be seen in several clinical settings. In some patients, contemporaneous clinical staging reveals overt disease in other lymph node groups or other extranodal sites. In this situation, it is likely that ISFN actually represented very focal lymph node involvement by disseminated FL. However, in other patients, staging will not uncover any other sites of disease. In this situation, only a small number of patients subsequently develop overt FL, but most never do. When defined in this way, ISFN is of little clinical significance, and patients may be reassured. Therefore, a diagnosis of ISFN is of uncertain clinical significance independent of rigorous staging and subsequent follow-up.

Transformation of Follicular Lymphomas

Depending on how it is defined, FL transforms to a more aggressive B-cell lymphoma in 20% to 60% of cases. Diffuse large B-cell lymphoma in which the neoplastic cells resemble centroblasts or immunoblasts is the most common tumor to which FLs transform (Fig. 7.36A). The large B-cell lymphoma cells express B-cell lineage antigens and usually retain a

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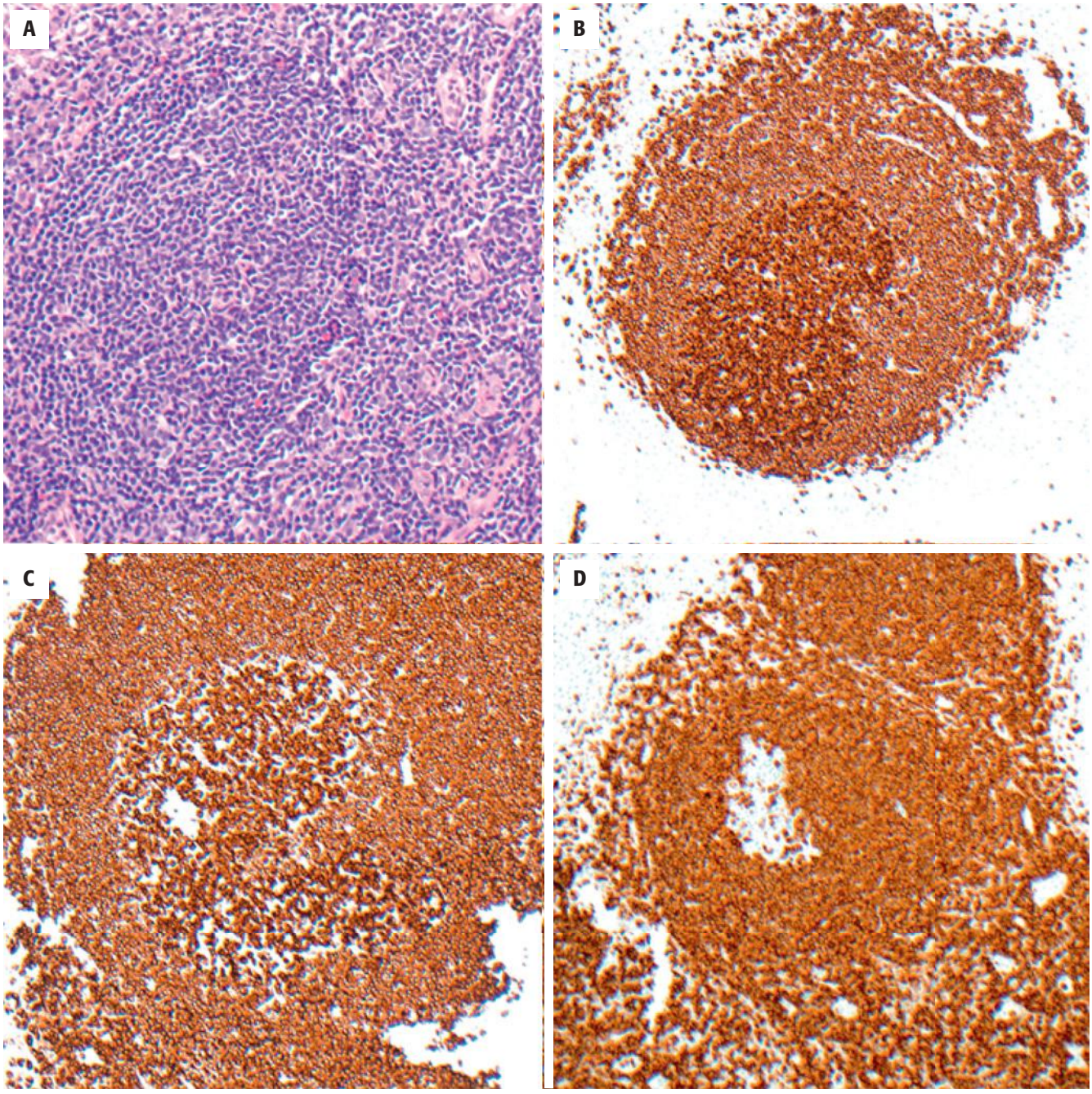


FIG. 7.35

In situ follicular neoplasia. A, The neoplastic cells occupy the center of this nodule. B and C, Immunoperoxidase stains for BCL-2 demonstrating strong BCL-2 positivity within the germinal center cells. D, Normal follicle from the same case with BCL-2 negative germinal center cells.

germinal center B-cell phenotype (CD10 and BCL6+). They typically are clonally related to the FLs from which they arise (e.g., contain the same immunoglobulin heavy chain rearrangements and identical *BCL2/IGH* fusion sequences) but contain novel genetic alterations that have been reproducibly observed to be characteristic of transformation. Occasionally, the transformed lymphoma has cytologic characteristics of a higher grade lymphoma. Accompanying this change, the tumor cells can acquire the t(8;14)(q24;q32) involving *MYC* (8q24) and *IGH* (14q32), and along with the t(14;18) would be classified as a high-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements ("double-hit" lymphoma). Finally, rare cases of FL transform to a neoplasm that morphologically is indistinguishable from lymphoblastic lymphoma (Fig. 7.36B). In these cases the lymphoblastoid neoplastic cells retain the general phenotypic characteristics of FLs, including surface immunoglobulin expression and absence of TdT. Whatever the form that transformation takes, the disease becomes more clinically aggressive and from that point on prognosis is guarded.

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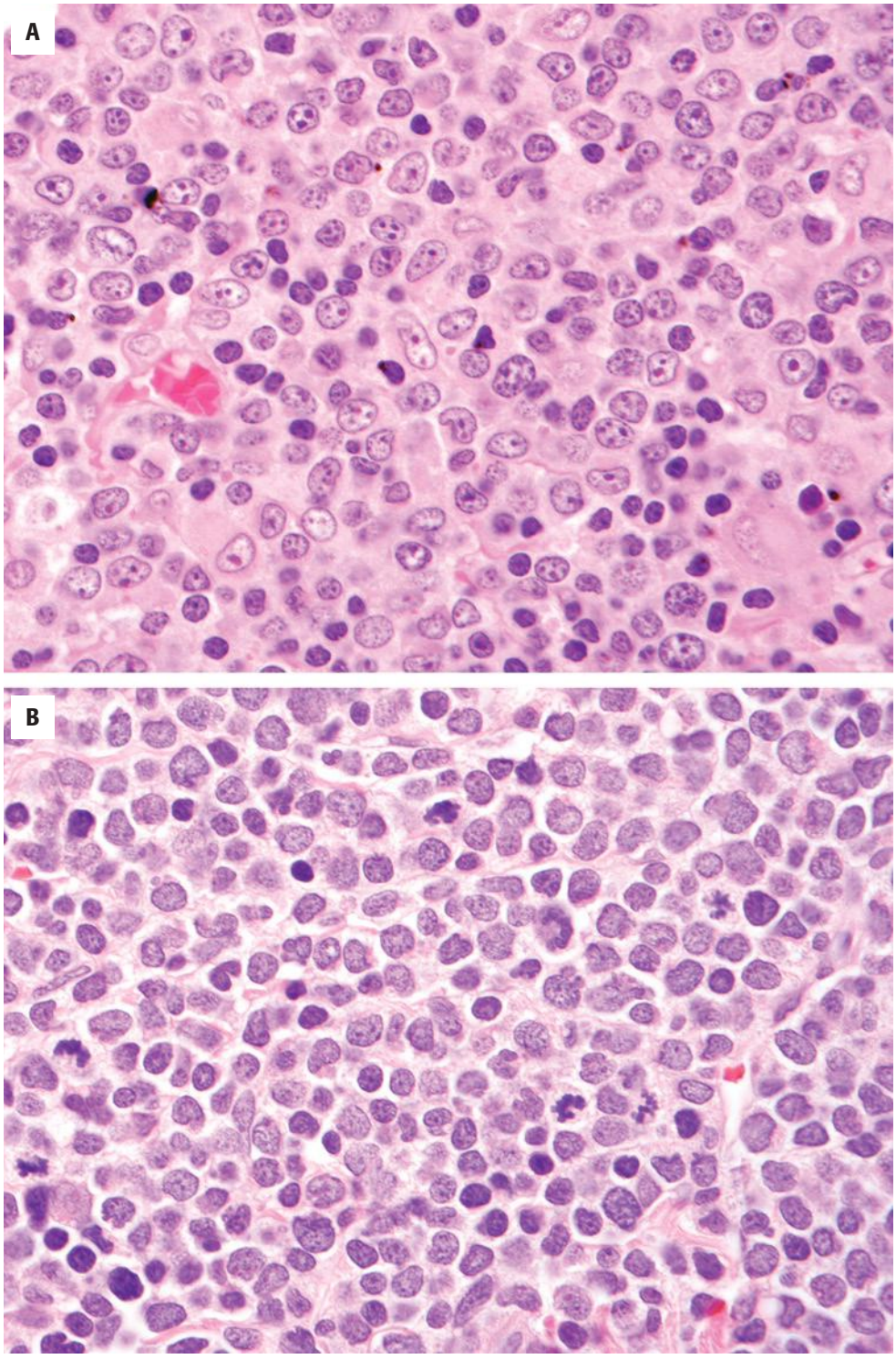


FIG. 7.36

Transformation of follicular lymphoma. A, Diffuse large B-cell lymphoma; the cells have cytologic features of centroblasts. Note the large cell size, dispersed chromatin, multiple nucleoli, and eosinophilic cytoplasm. B, Blastic transformation. The cells are intermediate size with irregular nuclear contours, stippled chromatin, small or inconspicuous nucleoli, and sparse cytoplasm. Note the multiple mitotic figures.

Differential Diagnosis

Follicular lymphoma must be distinguished from reactive follicular hyperplasia. Lymph node architectural effacement, uniform size follicles, coalescence of follicles with loss of interfollicular tissue, and monomorphism of the intrafollicular lymphocyte population are all features that favor a diagnosis of FL over follicular hyperplasia. Demonstrating an immunoglobulin light chain restricted CD10+ B-cell population by flow cytometry or immunohistochemistry strongly favors a diagnosis of FL over follicular hyperplasia. Demonstrating that nodules of follicular center cells (CD10 and/or BCL6+ and associated with CD21 or CD23+ follicular dendritic cell meshworks) express BCL2 also strongly favors FL over follicular hyperplasia.

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Any lymphoma that grows in a nodular pattern is in the differential diagnosis of FL: MCL, MZL, and SLL/CLL with proliferation centers. These disorders are morphologically, phenotypically, and genetically distinct from one another and can usually be readily separated from one another (see Tables 7.1, 7.2, and 7.3).

Blastic transformation of FL is separated from B and T precursor lymphoblastic lymphoma/leukemia and acute myeloid leukemia in a manner totally analogous to that used to distinguish these conditions from blastoid MCL (see above). Phenotypically, the cells in blastic transformation of FL resemble B precursor lymphoblastic lymphoma/leukemia in that they express CD19 and CD10, but they are usually TdT and CD34–. Rare case reports have documented clonally related transformations that express TdT.

Prognosis and Therapy

The treatment approach to, and prognosis of, FLs varies depending on histologic grade and Ann Arbor stage. For grade 1 and 2 FLs, choice of therapy is driven by several considerations. It has long been appreciated that FL is an indolent disease and that aggressive initial therapies do not materially alter the survival. Thus, for asymptomatic stage III and IV patients with no vital organ compromise due to the lymphoma, a watch-and-wait approach is commonly employed. When treatment is necessary, rituximab, cyclophosphamide, vincristine, and prednisone (R-CVP) or bendamustine and rituximab (BR) followed by rituximab maintenance is often the first line of therapy. Following initial therapy the lymphoma almost invariably recurs, and the patients can be retreated with the expectation of a subsequent response. Eventually, responsiveness diminishes and the patients succumb to progressive disease or transformation to large B-cell lymphoma. The median survival for adult FL patients is approximately 9 years. For grade 3 FL, standard treatment is rituximab-cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) chemotherapy, which has been shown to produce long-term remissions in at least a subset of the treated patients.

Because there is heterogeneity in the clinical outcomes for FL patients, attempts have been made to devise a prognostic index similar to that which has been successfully applied to diffuse large B-cell lymphomas. The Follicular Lymphoma International Prognostic Index (FLIPI) has been recently proposed to meet this need. Five adverse prognostic factors were identified: age greater than 60 years, Ann Arbor stage III or IV, elevated serum LDH, hemoglobin level less than 12 g/dL, and more than four nodal sites of involvement by lymphoma. Low-risk patients have no or only one adverse factor, intermediate-risk patients have two adverse factors, and high-risk patients have three or more adverse factors. Ten-year survivals of 71%, 51%, and 27% of patients are predicted by the low-, intermediate-, and high-risk groups, respectively. Incorporation of molecular features to improve on prognostic models (such as the m7-FLIPI) is the subject of ongoing investigation.

As mentioned above, the duodenal-type FLs, pediatric-type FLs, and primary cutaneous follicle center lymphomas deserve special mention. These typically have a very indolent, nonprogressive course. The cutaneous cases may require local treatment only, whereas duodenal FL may not require therapy at all. Similarly, ISFN does not require treatment once staging procedures have ruled out other clinical sites of disease.

EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA OF MUCOSA-ASSOCIATED LYMPHOID TISSUE

Clinical Features

Mucosa-associated lymphoid tissue lymphoma comprises 7% to 8% of all B-cell lymphomas and is a disease of older adults with a median age of 60 years. There is a slight female predominance. As the name implies, MALT lymphomas preferentially arise in non–lymph node sites. They are most common in the stomach, salivary glands, lungs, orbit and ocular adnexae, thyroid, skin, and breast. Despite their morphologic resemblance to Peyer patches, MALT lymphomas are actually quite rare in the intestines, except for a peculiar type of MALT lymphoma, immunoproliferative small intestinal disease. The epidemiology of MALT lymphomas varies by anatomic site. In general, MALT lymphomas arise in organs devoid of, or sparsely populated by, lymphoid tissue. An inflammatory stimulus, either autoimmune or infectious, recruits lymphocytes and immune accessory cells to these sites, and it is within the inflammatory milieu that MALT lymphomas arise. The inflammatory disorders linked to MALT lymphomas in various anatomic sites are listed in Table 7.5. Because of the association of gastric MALT lymphomas with *Helicobacter pylori* infections (90% of gastric MALT lymphomas are associated with *H. pylori*), there is a higher

TABLE 7.5

Site-Specific Inflammatory Disorders Related to the Pathogenesis of MALT Lymphomas

Anatomic Site	Associated Condition
Stomach	*Helicobacter pylori* gastritis
Salivary gland	Sjögren syndrome
Thyroid	Hashimoto thyroiditis
Lacrimal gland	*Chlamydia psittaci* infection
Skin	*Borrelia burgdorferi* infection
Immunoproliferative small intestinal disease	*Campylobacter jejuni* infection

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EXTRANODAL MARGINAL ZONE B CELL LYMPHOMA OF MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT)—FACT SHEET

Clinical
- 5% to 6% of non-Hodgkin lymphomas
- Median age 7th decade, slight female predominance
- Involves extranodal sites: stomach, lung, salivary glands, orbit, skin, etc.
- Symptoms depend on presenting anatomic site
- Associated conditions
  - *H. pylori* gastritis
  - Sjögren syndrome
  - Hashimoto thyroiditis
- Low Ann Arbor stage at presentation

Morphology
- Five elements assembled into structures resembling Peyer patch:
  - Neoplastic small lymphocytes, centrocyte-like cells
  - Transformed lymphocytes resembling centroblasts and immunoblasts
  - Plasma cells, clonally related to the small lymphocytes in 30%
  - Reactive germinal centers
  - Lymphoepithelial lesions
- Follicular colonization in subset

Immunophenotype
- CD19+, CD20+, sIg+, IgM without IgD, rarely IgG or IgA
- Monotypic plasma cells in 30%
- CD10 and CD5 negative
- CD43 variably positive

Genetics
- Clonally rearranged immunoglobulin genes
- t(11;18)(q21;q21): *BIRC3/MALT1*
- t(14:18)(q32;q21): *IGH/MALT1*
- t(1:14)(p22:q32): *BCL-10/IGH*
- t(3;14)(p13;q32): *FOXP1/IGH*
- Trisomies 3, 8, 18 present in cases without translocations
- *TNFAIP3* deletion/hypermethylation
- Point mutations in Ig genes

Prognosis and Therapy
- For gastric MALT lymphoma associated with *H. pylori*:
  - Antibiotic therapy to eradicate *H. pylori*—probability of success decreases with increasing Ann Arbor stage, depth of infiltration of the gastric wall by lymphoma, and presence of *API2/MALT1* translocation
- For other anatomic sites:
  - Local radiation
  - Low-intensity single or multi-agent chemotherapy with rituximab
- Excellent prognosis for low-stage gastric MALT lymphoma
- Median survival 5 to 6 years for Ann Arbor stage III and IV patients

Differential Diagnosis
- Reactive lymphoid hyperplasia
- Chronic lymphocytic leukemia/small lymphocytic lymphoma
- Mantle cell lymphoma
- Follicular lymphoma, particularly those with plasmacytic and/or marginal zone B-cell differentiation
- Nodal marginal zone B-cell lymphoma
- Splenic marginal zone B-cell lymphoma
- Lymphoplasmacytic lymphoma

incidence of this lymphoma type in geographic regions (Veneto region of Italy, for example) where there is a high incidence of *H. pylori* infection. Despite the ubiquitous distribution of *Campylobacter jejuni,* immunoproliferative small intestinal disease occurs almost exclusively in the Middle East and in the Cape region of South Africa.

The initial manifestations of MALT-type lymphomas vary by the organ primarily involved by the tumor but in general symptoms are quite nonspecific. For the stomach, dyspepsia and epigastric pain are common symptoms. For the lung, cough and chest pain herald the symptomatic onset of the disease. For the salivary glands, thyroid, orbit, and skin, tumors involving those sites in association with symptoms of the salient associated autoimmune disease are present. A subset of MALT lymphoma patients have serum monoclonal gammopathies, usually of IgM isotype. Symptoms of the underlying autoimmune diseases in patients whose lymphomas arise in salivary gland and thyroid can also be present concurrent with the onset of MALT lymphoma.

At the time of diagnosis, MALT lymphomas are usually localized to the organ in which they arise. The distribution of MALT lymphomas across the different Ann Arbor stages is likely to vary by anatomic site, but few systematically analyzed data allow for definite conclusions about this point except for the stomach. For all primary anatomic sites, aggressive staging will uncover bone marrow involvement in 20% of patients, spread to another non-nodal site in 12% of patients, and involvement of lymph nodes in 7.5% of patients. Primary gastric MALT lymphomas have a very indolent evolution, remaining localized to the stomach for long periods of time prior to dissemination to other sites. This is likely to be the behavior of MALT lymphomas originating in other organs as well.

Pathologic Features

Morphology

Regardless of the extranodal organ involved by the MALT lymphoma, five key elements define the morphology of these tumors: the neoplastic marginal zone B cells, varying numbers of transformed lymphocytes that resemble immunoblasts or centroblasts, plasma cells that can be polyclonal or clonally related to the neoplastic B cells, lymphoepithelial lesions, and reactive germinal centers. These elements are arranged architecturally in a manner that recapitulates the Peyer patch

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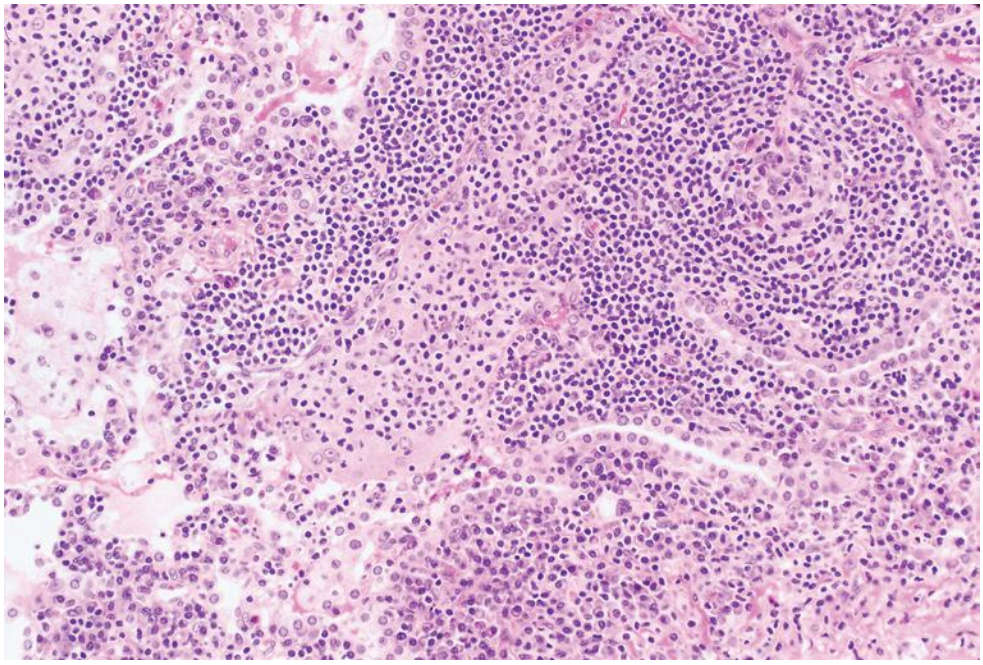


FIG. 7.37

Mucosa-associated lymphoid tissue (MALT) lymphoma involving lung. The resemblance to a Peyer's patch is noted here. The germinal center is surrounded by neoplastic marginal zone cells that infiltrate into altered pulmonary epithelium forming lymphoepithelial lesions.

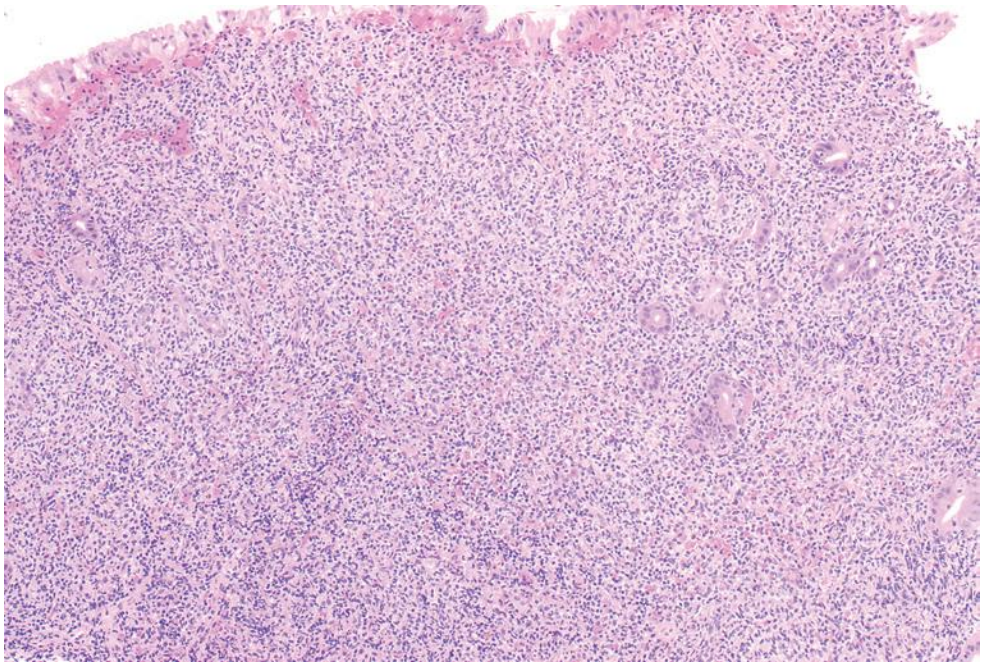


FIG. 7.38

Gastric mucosa-associated lymphoid tissue (MALT) lymphoma. The tumor cells extensively infiltrate the lamina propria.

(Fig. 7.37). A prototypic gastric MALT lymphoma is illustrated in Figs. 7.38, 7.39, and 7.40.

There is case-to-case variability in the cytologic features of the neoplastic marginal zone B cells. In most cases, the neoplastic marginal zone cells are small with irregular nuclear contours, condensed chromatin, and abundant pale staining cytoplasm. Because these cells resemble centrocytes, they have been termed *centrocyte-like cells* (Fig. 7.40). The tumor cells may have more voluminous clear or pale eosinophilic cytoplasm and resemble monocytoid B cells or have the cytologic characteristics of small lymphocytes with round nuclei and sparse cytoplasm. In addition to the neoplastic small lymphocytes, most MALT lymphomas also contain variable numbers of singly distributed, large transformed lymphocytes with the cytologic features of either centroblasts or immunoblasts. If the large cells begin to form clusters or especially if they aggregate in confluent sheets, then a diagnosis of diffuse large B-cell lymphoma arising in a MALT lymphoma should be entertained. The term *MALT lymphoma* should not be used to refer to an aggressive large B-cell lymphoma arising in an extranodal site; the WHO term *diffuse large B-cell lymphoma* is appropriate in this circumstance.

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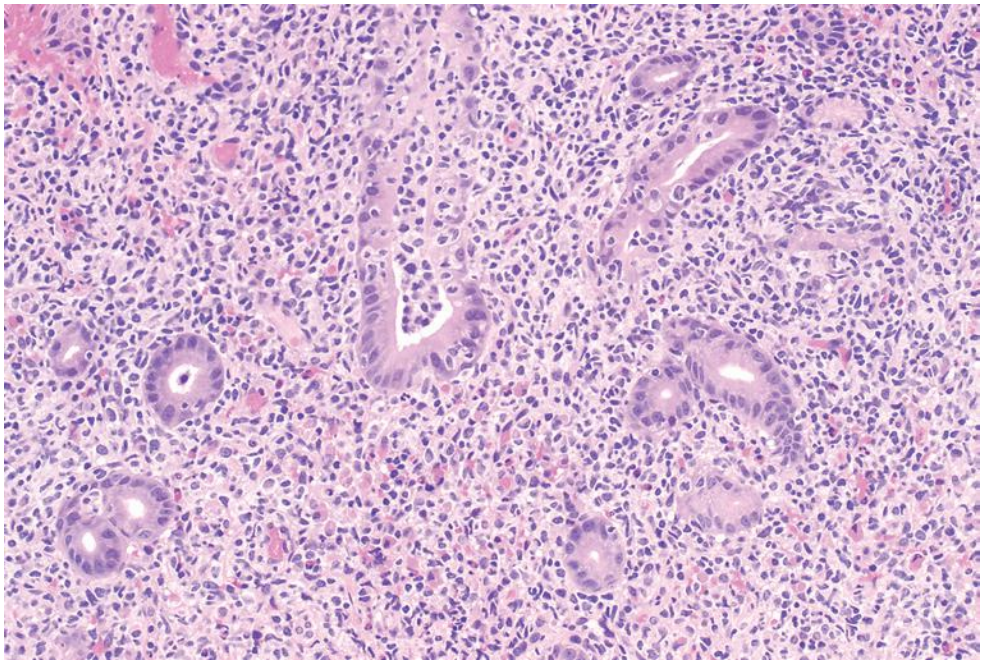


FIG. 7.39

Gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Residual gastric glands are separated by the neoplastic lymphocytes (centrocyte-like cells). Note the lymphoepithelial lesion in the center of the field.

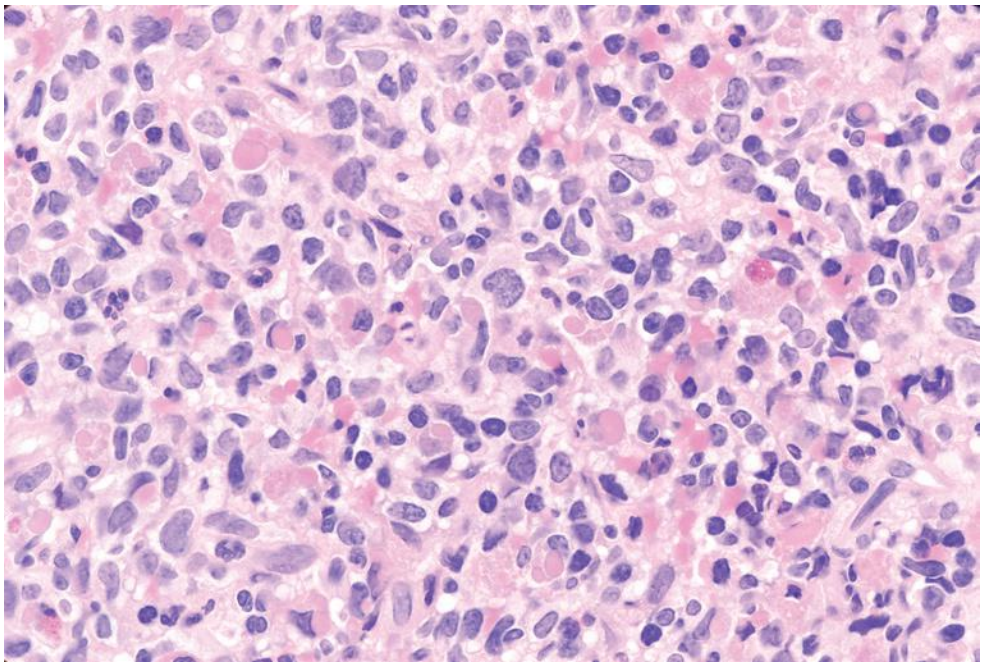


FIG. 7.40

Gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Varied cytologic composition including centrocyte-like cells, larger transformed lymphocytes, and eosinophilic immunoglobulin inclusions.

In approximately 30% of cases immunoglobulin light chain restricted plasma cells are part of the tumor (Fig. 7.41) and, in rare cases, they predominate the cellular infiltrates almost to the exclusion of neoplastic marginal zone B lymphocytes. Sometimes the plasma cells contain intranuclear (Dutcher bodies) or intracytoplasmic (Russell bodies) immunoglobulin inclusions or exhibit nuclear pleomorphism. Characteristically, the plasma cells are distributed adjacent to the mucosal surface involved by the lymphoma, or if the MALT lymphoma involves the skin, they are most frequently found immediately under the epidermis and at the border between the dermal collagen and the nodular infiltrates of the tumor.

Most obvious in gastric, pulmonary, salivary gland, and thyroid gland MALT lymphomas is infiltration of altered epithelium by the neoplastic cells (lymphoepithelial lesions [LELs]) (see Fig. 7.39). LELs are unusual in cutaneous, bladder, and conjunctival MALT lymphomas. LELs are composed of three or more neoplastic lymphocytes associated with alteration or destruction of the epithelium. Although LELs help to identify a B-cell lymphoma as a MALT type

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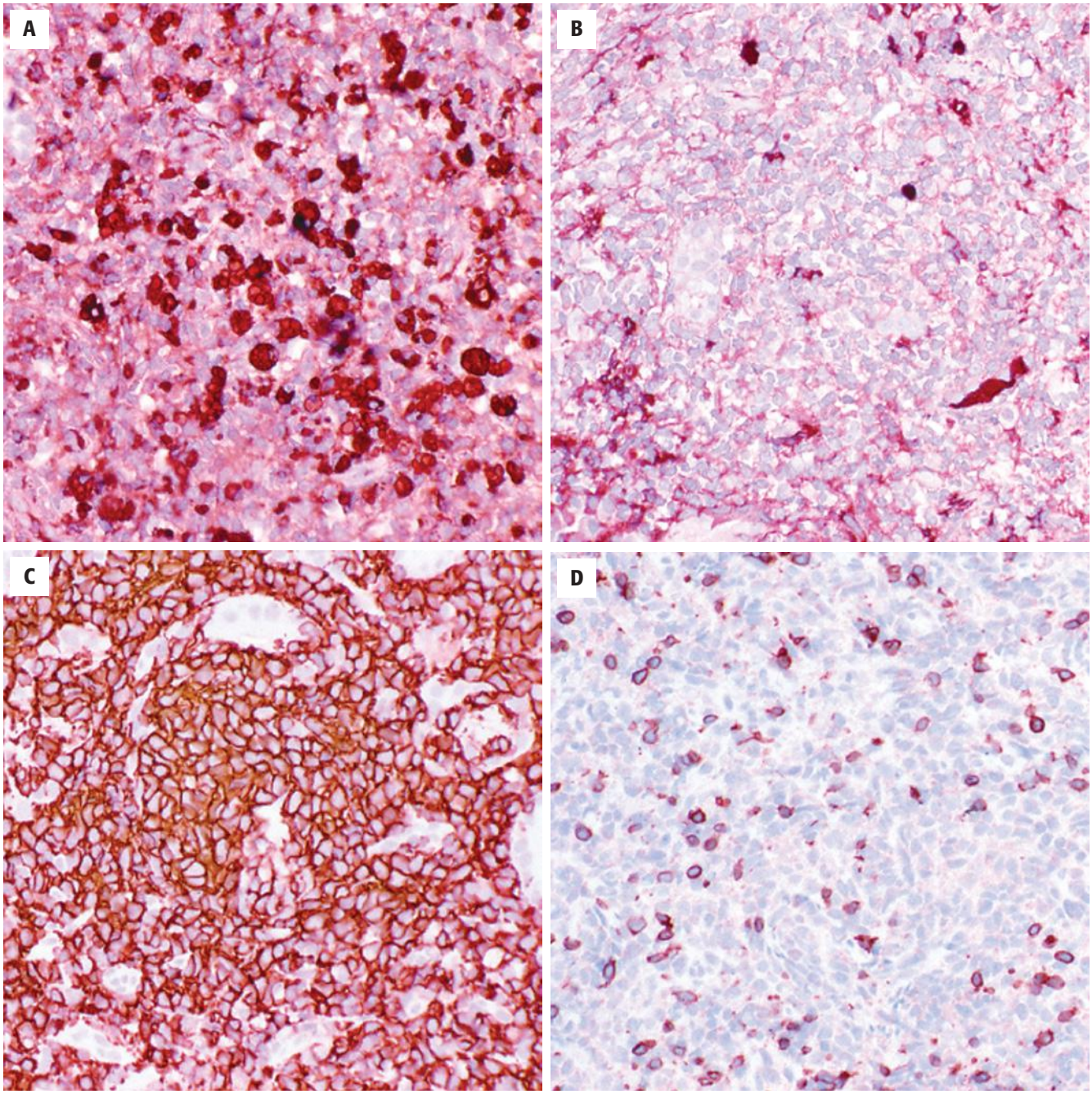


FIG. 7.41

Gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Immunoperoxidase staining of paraffin sections for kappa (A) and lambda (B) immunoglobulin light chains, CD20 (C) and CD5 (D). The plasma cells show kappa light chain restriction. Extracellular immunoglobulin globules also selectively stain for kappa light chains. The neoplastic cells express CD20 and are negative for CD5.

lymphoma, they can also be seen in a variety of reactive conditions in most mucosa-associated anatomic sites. The notable exception is the stomach in which prominent LELs are a diagnostic clue to the presence of lymphoma.

Non-neoplastic germinal centers associated with follicular dendritic cells are a common feature in MALT lymphomas. Uncommonly, MALT lymphomas are associated with localized amyloid or free immunoglobulin light chain deposition that can be so pronounced that the underlying MALT lymphoma is obscured. In these instances, wide sampling of the lesion is necessary to uncover the characteristic cellular infiltrates of the lymphoma.

In some cases of MALT lymphoma, the neoplastic cells infiltrate the germinal centers (follicular colonization). This phenomenon takes three forms. First, the neoplastic marginal zone cells retain their native cytologic characteristics and replace the follicular center cells. Second, the neoplastic cells enter the germinal center and differentiate into plasma cells. In this situation the germinal center contains a mixture of non-neoplastic follicular center cells and monotypic plasma

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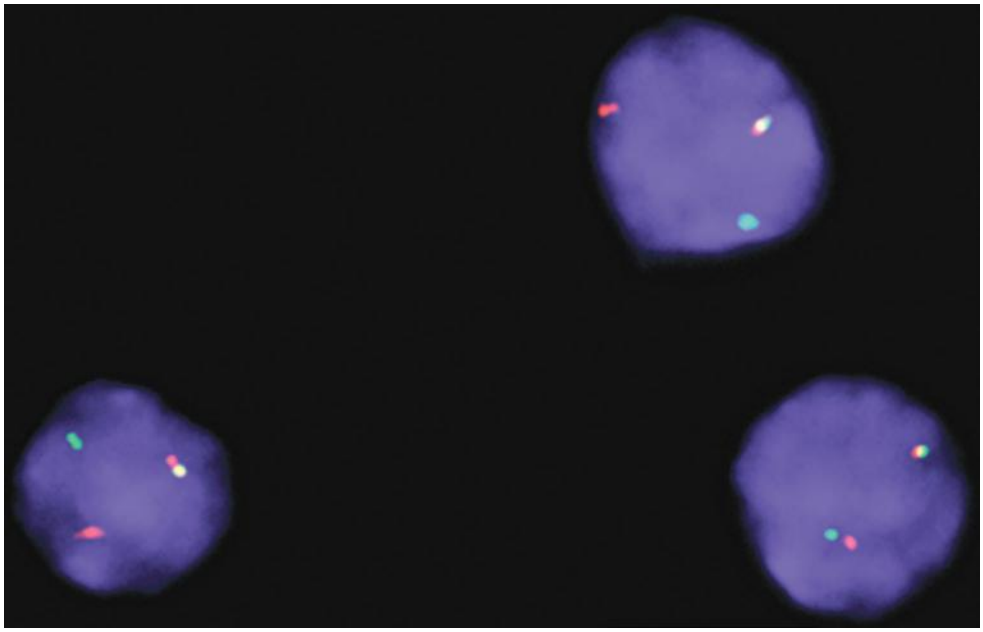


FIG. 7.42

Fluorescence in situ hybridization using probes that recognize the 5′ and 3′ ends of *MALT1* on chromosome 18q21. The cell in the lower right shows the two normal fused 5′ *(red fluorochrome tagged)* and 3′ *(green fluorochrome tagged*) signals representing the intact *MALT1* genes on the two normal chromosomes 18q21. The fused signals appear yellow. The upper and left nuclei are mucosa-associated lymphoid tissue (MALT) lymphoma cells. In each the 5′ and 3′ *MALT1* signals have split into separate 5′ red and 3′ green signals, indicating that a translocation has occurred with breakpoints in *MALT1*. Each of these nuclei also contains one normal yellow *MALT1* fusion signal representing the non-translocated chromosome 18q21.

cells derived from the MALT lymphoma. Finally, under the influence of the follicular microenvironment, the neoplastic cells can enter the germinal center and transform into large centroblasts. This latter manifestation of follicular colonization is the most difficult to distinguish from florid follicular hyperplasia or grade 3 FL and occurs most often in MALT lymphomas arising in the thyroid.

Expansion of the paracortex by a mixed population of small marginal zone B cells with or without plasma cells is the most common pattern of lymph node involvement by MALT lymphoma. In occasional cases, the lymph nodes contain a prominent concentric halo of centrocyte-like cells surrounding germinal centers. This pattern resembles that seen when lymph nodes are involved by SMZL. Thus, in a lymph node, it can be very difficult to distinguish splenic, nodal, and extranodal marginal zone lymphomas from one another. Considering the anatomic distribution of the lymphoma prior to classifying it is prudent in this context.

Immunophenotype

The cells of MALT lymphoma express the pan B lymphocyte antigens CD19, CD20 (see Fig. 7.41), CD22, CD79a, CD79b, and PAX-5 and show either kappa or lambda light chain restriction. When the intermixed plasma cells are part of the lymphoma, they too express the same immunoglobulin light chain as the neoplastic small lymphocytes.

MALT lymphoma cells can be positive for CD21 and CD35, antibodies to which also highlight the intermixed follicular dendritic cells and help in the recognition of follicular colonization. Typically, the cells of MALT lymphoma are negative for CD5 (see Fig. 7.36), CD10, and BCL6, but exceptionally, these tumors can express one or more of these markers. CD43 is expressed by a subset of MALT lymphomas. This useful finding helps to distinguish reactive B-cell infiltrates from MALT lymphomas.

Recently, immunoglobulin superfamily receptor translocation-associated 1, *IRTA1*, has been published as a potential specific immunohistochemical marker for MZLs of both nodal and extranodal types. This protein is expressed also in normal marginal zone B cells in extranodal sites, as well as in monocytoid B cells. Interestingly, both SMZL and other small B-cell lymphomas overwhelmingly lack expression of this marker.

Genetics

Four translocations are highly specific for extranodal marginal zone B-cell lymphoma of MALT and have different frequencies in MALT lymphomas from different anatomic sites. Most common is t(11;18)(q21;q21), which occurs in MALT lymphomas of stomach, lung, and occasionally orbit (Fig. 7.42). As a result of this translocation, two genes, *BIRC3 (API2)* from 11q21 and *MALT1* from 18q21, are fused, producing a chimeric protein coded for by the 5′ end of *BIRC3* and the 3′ end of *MALT1*. In the t(14;18)(q32;q21) a full-length *MALT1* gene is brought under the control of the immunoglobulin heavy chain gene enhancer at 14q32, resulting in overproduction of the MALT1 protein. This translocation is most common in non-gastrointestinal MALT lymphomas, particularly the liver, orbit, skin, and lung. In the t(1;14)(p22;q32) *BCL10* from 1p22 is brought under the control of the immunoglobulin heavy chain enhancer (14q32). Thus, BCL10 protein is overexpressed and localizes to the nucleus of the neoplastic cells. The t(1;14)(p22;q32) is uncommon, and its frequency distribution by involved organ is uncertain. In all of these translocations, elements of the signaling pathway from the immunoglobulin receptor to activation of nuclear

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factor kappa B (NF-κB) are altered, resulting in non-physiologic activation of NF-κB. Finally, rare MALT lymphoma cases have the t(3;14)(p12.1;q32) that involves *FOXP1* and *IGH*. The pathophysiologic effects of this translocation remain to be determined.

A further genetic abnormality that occurs in MALT lymphomas is deletion or hypermethylation of *TNFAIP3,* a gene found at chromosome 6p23.3 that codes for a protein called A20. A20 is a negative regulator of NF-κB signaling, both by directly inhibiting elements of the NF-κB canonical pathway and by effects on ubiquitinylation of NF-κB pathway members. The net result is that *TNFAIP3* acts as a tumor suppressor, which when deleted or inactivated by hypermethylation, results in unrestrained NF-κB pathway activation, achieving an effect similar to that caused by three of the other translocations involved in MALT lymphomas.

In addition to the common translocations, trisomies of chromosomes 3, 8, and 18 are also common genetic abnormalities in MALT lymphomas. Trisomies and the translocations discussed above are usually mutually exclusive genetic events in MALT lymphomas, suggesting that these tumors arise by multiple pathogenetic pathways, but the pathogenetic and prognostic significance of the trisomies is unknown.

Prognosis and Therapy

The therapeutic approach to gastric MALT lymphomas differs from the general approach to MALT lymphomas that arise in other organs. For gastric MALT lymphomas, clinical stage, presence of *H. pylori*, and presence of the t(11;18)(q21;q21) determine the therapeutic approach and the probability of complete remission of the lymphoma. It has been shown that 73% of gastric MALT lymphoma patients who have Ann Arbor stage IE (lymphoma confined to the stomach), *H. pylori*–positive tumors without the t(11;18)(q21;q21) will achieve a complete remission of the lymphoma treated only with antibiotics to eradicate the *H. pylori* infection. In addition, within the stage IE patient group, increasing depth of invasion of the gastric wall by the lymphoma is associated with increasing risk for treatment failure with antibiotics. Those patients with stage IIE (lymphoma in stomach and regional lymph nodes), *H. pylori,* and t(11;18)(q21;q21)-positive tumors have only a 5% chance of complete remission following antibiotic therapy. The lymphomas only gradually respond to antibiotic therapy (up to 18 months), and response can be associated with morphologically occult molecular genetic evidence of persistence of the clonal neoplastic B-cell population. Persistence of the clone as determined by molecular analysis does not preclude long-term clinical remission. Thus, long-term monitoring with endoscopy and biopsies is required to determine if histologic regression has occurred and/or to document the need for cytotoxic therapy in non-responding symptomatic patients, but the role for molecular monitoring is limited.

Patients with MALT lymphomas confined to a single anatomic site are conventionally treated with either radiation or low-intensity single or multiple agent chemotherapy with rituximab. For all MALT lymphoma patients the prognosis is very favorable.

Differential Diagnosis

Lymphoid hyperplasia in extranodal sites is common as part of a variety of organ-specific inflammatory conditions. MALT lymphomas, with their heterogeneous architecture and cell composition, can closely resemble these reactive processes. In general, monomorphous cell populations, particularly sheets of monocytoid cells, cytologically abnormal plasma cells with nuclear irregularities and Dutcher bodies, and in the stomach prominent lymphoepithelial lesions all point to a diagnosis of MALT lymphoma. Showing light chain restriction in the B cell or plasma cell population in the right morphologic context also supports a MALT lymphoma diagnosis.

Distinguishing MALT lymphomas from other types of indolent B-cell lymphomas is accomplished using the morphologic, phenotypic, and genetic attributes listed in Tables 7.1, 7.2, and 7.3. Because MALT lymphomas can have plasmacytic differentiation, distinction from tissue involvement by lymphoplasmacytic lymphoma (LPL) is especially important. The monocytoid cytology common in MALT lymphomas contrasts with its rarity in LPL. Demonstrating the *MYD88* L265P mutation in a lymphoma favors LPL but should be used with some caution in this setting, because this mutation has been found in up to 10% of MZLs.

It should be stressed that not all neoplastic lymphoid infiltrates in extranodal sits are MALT lymphomas. All of the lymphoma types discussed in this chapter, with the possible exception of nodal marginal zone lymphoma (because of its operational definition), can involve extranodal sites. Therefore, when encountering a neoplastic small lymphocytic infiltrate in an extranodal site, one must be certain that it meets the morphologic and phenotypic criteria for MALT lymphoma and that other indolent lymphoma types, particularly SLL, FL, and MCL, are excluded before rendering a MALT lymphoma diagnosis.

SPLENIC MARGINAL ZONE LYMPHOMA

The current definition of the clinical and pathologic features of SMZL represents the convergence of two separately described disorders: splenic lymphoma with villous lymphocytes (SLVL) and primary SMZL. As initially described, the typical patient with SLVL presented with absolute lymphocytosis and an enlarged spleen. The lymphocytes were medium sized with voluminous cytoplasm and polar cytoplasmic projections. The spleen contained white pulp expansion by

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SPLENIC MARGINAL ZONE LYMPHOMA—FACT SHEET

Clinical Features
- 1% of non-Hodgkin lymphomas
- Median age 7th decade, no gender predominance
- Symptomatic splenomegaly
- Frequent peripheral blood leukemic phase
- Paraprotein in 25%
- High Ann Arbor stage at diagnosis

Morphology
- Splenic white pulp nodules with infiltration of the red pulp
- Dimorphic cytology: small lymphocytes resembling mantle zone lymphocytes in center of nodules, larger cells resembling normal splenic marginal zone lymphocytes at periphery of nodules
- Bone marrow with intertrabecular nodular, paratrabecular, and intravascular infiltrates

Immunophenotype
- CD19+, CD20+, sIg+, IgM with or without IgD, occasional cases IgG or IgA+
- CD5±, CD10–, BCL6–
- May express CD11c, CD22, and CD103 (dim)

Genetics
- Clonally rearranged immunoglobulin genes
- del 7q31-32 in subset
- +3 in subset
- Point mutations in immunoglobulin genes and in 5′ noncoding region of BCL6 gene in subset, germline immunoglobulin and BCL6 genes in subset
- *NOTCH2* mutations
- *KLF2* mutations

Prognosis and Therapy
- Watch and wait approach for asymptomatic patients
- Splenectomy, single agent rituximab, or splenic radiation for patients with symptomatic splenomegaly or cytopenias
- Low-intensity single or multi-agent chemotherapy for symptomatic patients
- 8- to 10-year median survival
- Transformation to diffuse large B-cell lymphoma in 13% heralds aggressive disease

Differential Diagnosis
- Marginal zone B-cell hyperplasia
- Mantle cell lymphoma with marginal zone B-cell differentiation in the spleen
- Follicular lymphoma with marginal zone B-cell differentiation in the spleen
- Hairy cell leukemia

the neoplastic cells. The typical patient with primary SMZL presented with symptomatic splenomegaly. The spleen contained white pulp–based nodules of neoplastic lymphocytes that resembled the normal splenic marginal zone B cells. Subsequent morphologic review of SLVL spleen specimens, phenotypic studies, and genetic analysis determined that most cases of SLVL and primary SMZL represented the same entity that in the WHO classification is termed *SMZL*. There are some exceptions. Some cases of FL, LPL, and even MCL can present with symptomatic splenomegaly and a leukemic phase in which the circulating lymphocytes resemble those of SMZL. Additionally, FLs and MCLs involving splenic white pulp can acquire morphologic features nearly indistinguishable from SMZL (i.e., undergo marginal zone differentiation). Thus, a combined morphologic, phenotypic, and in some cases, genetic approach must be applied to leukemic cases and spleen specimens to ensure accurate and reproducible diagnosis of SMZL.

Clinical Features

Splenic marginal zone lymphoma is rare, accounting for no more than 1% to 2% of all non-Hodgkin lymphomas. It occurs in older individuals (median age is 68 years) with an equal gender distribution. Most patients present with either symptomatic splenomegaly or lymphocytosis associated with an enlarged spleen. Of patients who present with lymphocytosis, 77% have splenomegaly. Of patients who present with symptomatic splenomegaly, 68% have peripheral blood involvement. The bone marrow is positive for lymphoma in at least 80% of patients at diagnosis, and in 30% the liver is involved. Abdominal adenopathy is present in up to 25% of patients, but peripheral and mediastinal adenopathy are rare at diagnosis. Thus, SMZL is characterized by a high frequency (87% of patients) of Ann Arbor stage IV disease at initial presentation. A small number of patients with SMZL have monoclonal gammopathy, usually IgM and far less frequently IgA or IgG isotype.

Pathologic Features

Morphology

This disease has its most characteristic morphology in the spleen. Grossly, the spleen is enlarged with numerous tan nodules on the cut surface (Fig. 7.43). The neoplastic lymphocytes preferentially involve the splenic white pulp, expanding the marginal zones and variably and irregularly infiltrating into the adjacent red pulp (Figs. 7.44 and 7.45). In the most characteristic cases, the white pulp nodules contain a small atrophic germinal center. The neoplastic lymphocytes centrifugally expand out from the margin of the atrophic germinal center and have dimorphic cytologic features (Fig. 7.46). The neoplastic cells immediately adjacent to the

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germinal center resemble mantle zone lymphocytes with round nuclei, condensed chromatin, and sparse cytoplasm. These, in turn, are surrounded by coronas of neoplastic cells that resemble normal splenic marginal zone lymphocytes. They are small to medium sized with more open chromatin small nucleoli and abundant pale staining cytoplasm. In most cases the neoplasm includes large transformed lymphocytes with features similar to immunoblasts situated near the border between the expanded white pulp and the adjacent red pulp. Cases with the cytologic and phenotypic features of SMZLs, but with a diffuse red pulp infiltrative pattern were previously considered to be diffuse variants of SMZLs. They now are considered a provisional entity termed *splenic diffuse red pulp small B-cell lymphoma*. The attributes of this provisional entity are discussed below.

In lymph nodes, SMZLs recapitulate the splenic architecture (Fig. 7.47). The neoplastic cells surround non-neoplastic germinal centers or produce multifocal cortical nodules without germinal centers. In the liver, SMZLs produce multifocal portal nodules and irregularly infiltrate the hepatic sinusoids. Bone marrow involvement by SMZL is highly characteristic (Fig. 7.48). Although intertrabecular nodules, paratrabecular aggregates, and interstitial infiltrates can occur, the neoplastic lymphocytes typically involve the bone marrow sinusoids. Immunohistochemistry for CD20 is particularly useful to highlight the linear arrays of intrasinusoidal neoplastic cells. In a patient who presents with splenomegaly and lymphocytosis, demonstrating by flow cytometry that the circulating abnormal lymphocytes have the proper phenotype for MZL (see below) and that the bone marrow has intravascular involvement, one can usually make a confident diagnosis of SMZL without splenectomy or lymph node biopsy. The typical cytology of splenic SMZL in blood is represented in Fig. 7.49.

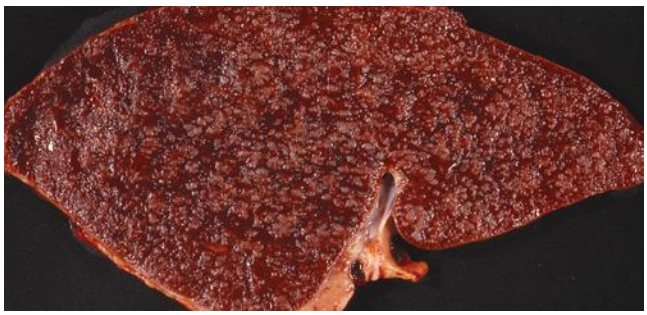


FIG. 7.43

Splenic marginal zone lymphoma. Note the prominence of the white pulp nodules.

Phenotype

The cells of SMZL express the pan B-lymphocyte antigens CD19, CD20, CD22, and PAX-5 and show either kappa or lambda immunoglobulin light chain restriction. Most frequently, they express IgM and are weakly positive for IgD. They are rarely IgG or IgA positive. In occasional cases one can demonstrate that plasma cells within the non-neoplastic germinal centers or at the margins of the neoplastic nodules are part of the lymphoma because they too express the same immunoglobulin light chain as the neoplastic small lymphocytes. SMZL cells are occasionally positive for CD5 (20% of cases), DBA.44, CD11c, and CD103. Care must be taken in these instances to exclude MCL and hairy cell leukemia. However, typically the cells of this lymphoma type are negative

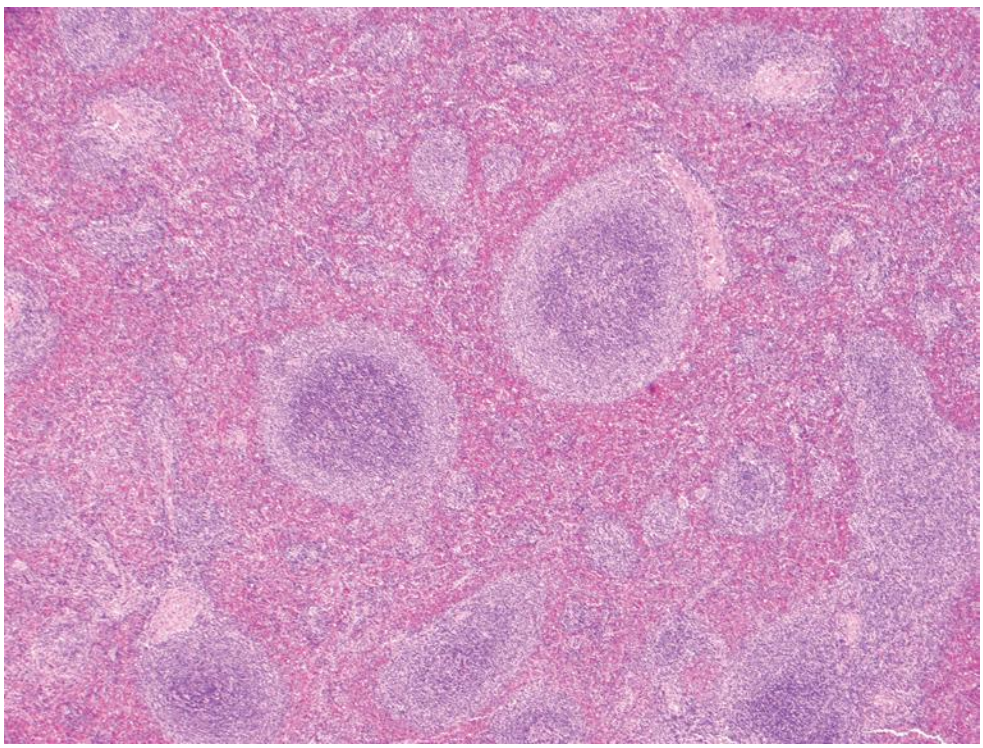


FIG. 7.44

Splenic marginal zone lymphoma. There is expansion of the white pulp by the neoplasm.

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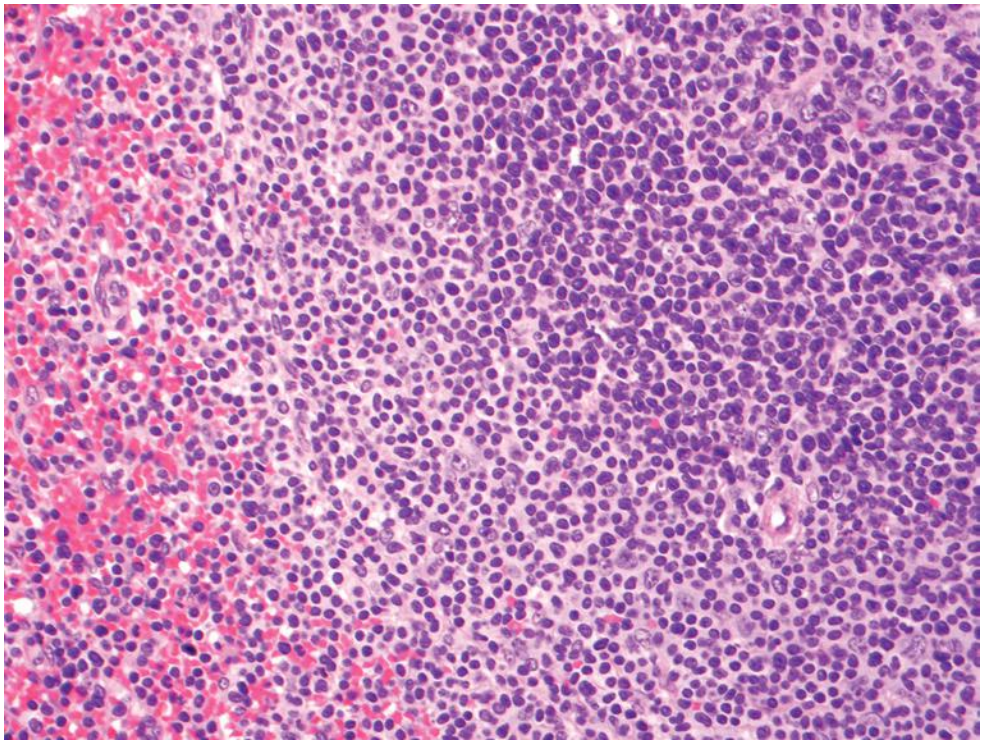


FIG. 7.45

Splenic marginal zone lymphoma. Note the dimorphic cytologic features, neoplastic small mantle zone-like cells in the center of the nodule, and marginal zone-like cytologic features of the neoplastic cells at the periphery of the nodule. Note also the infiltration of the red pulp by the neoplastic cells at the periphery of the white pulp nodule.

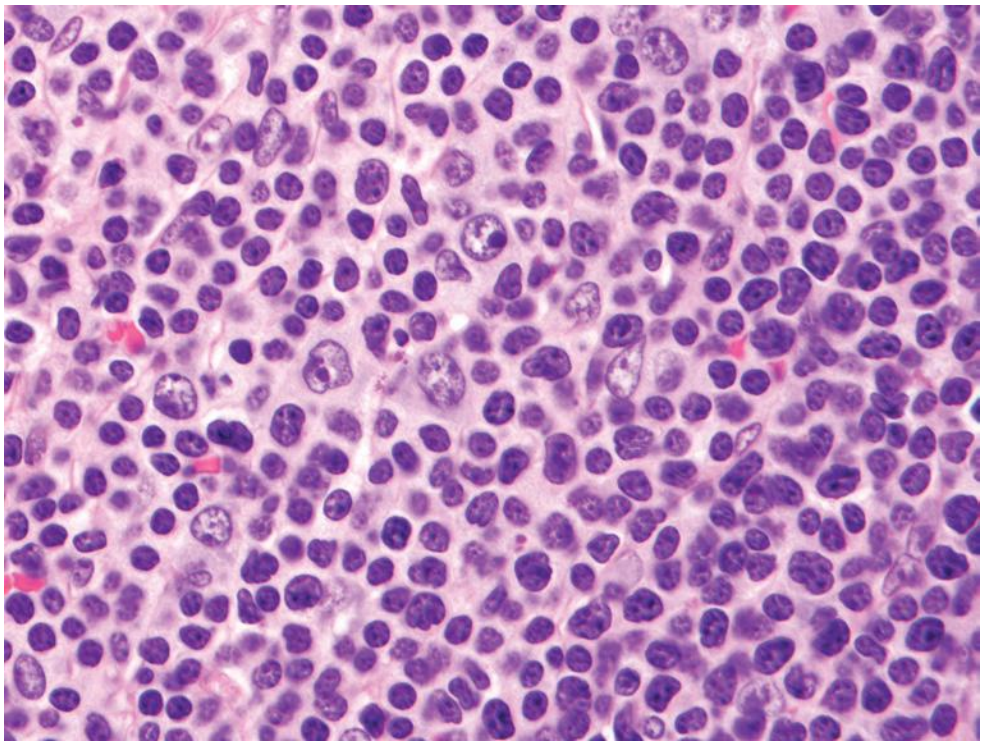


FIG. 7.46

Splenic marginal zone lymphoma. Cytologic features, including scattered large transformed lymphocytes.

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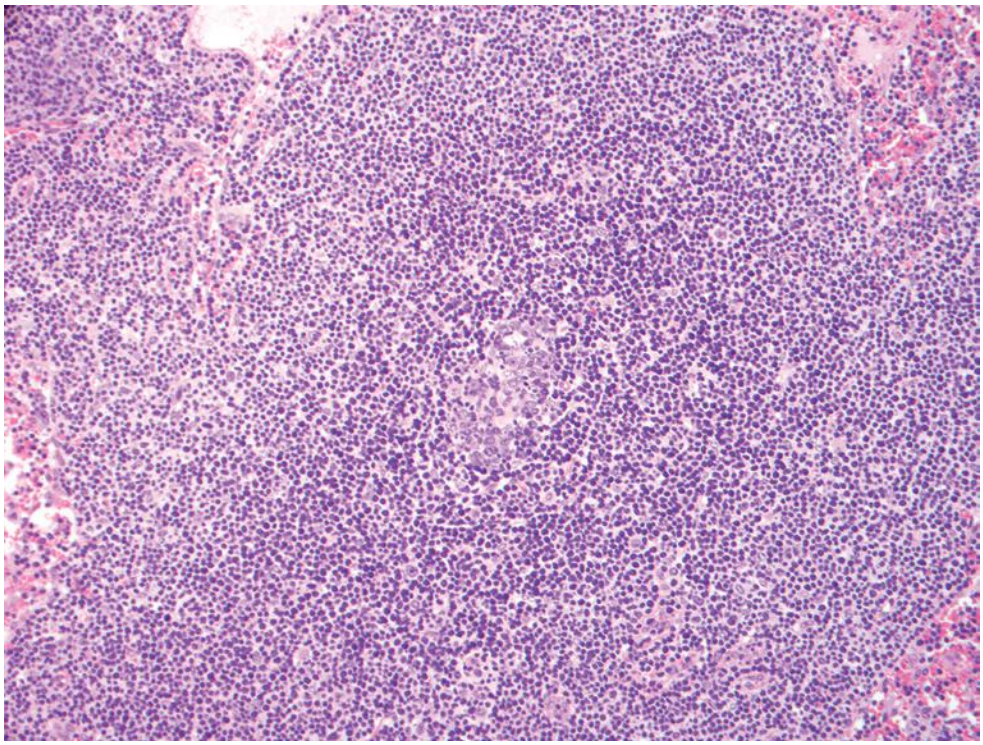


FIG. 7.47

Splenic marginal zone lymphoma involving a lymph node. In the center of the nodule is an atrophic non-neoplastic germinal center surrounded by the same dimorphic cell population as is present in the spleen.

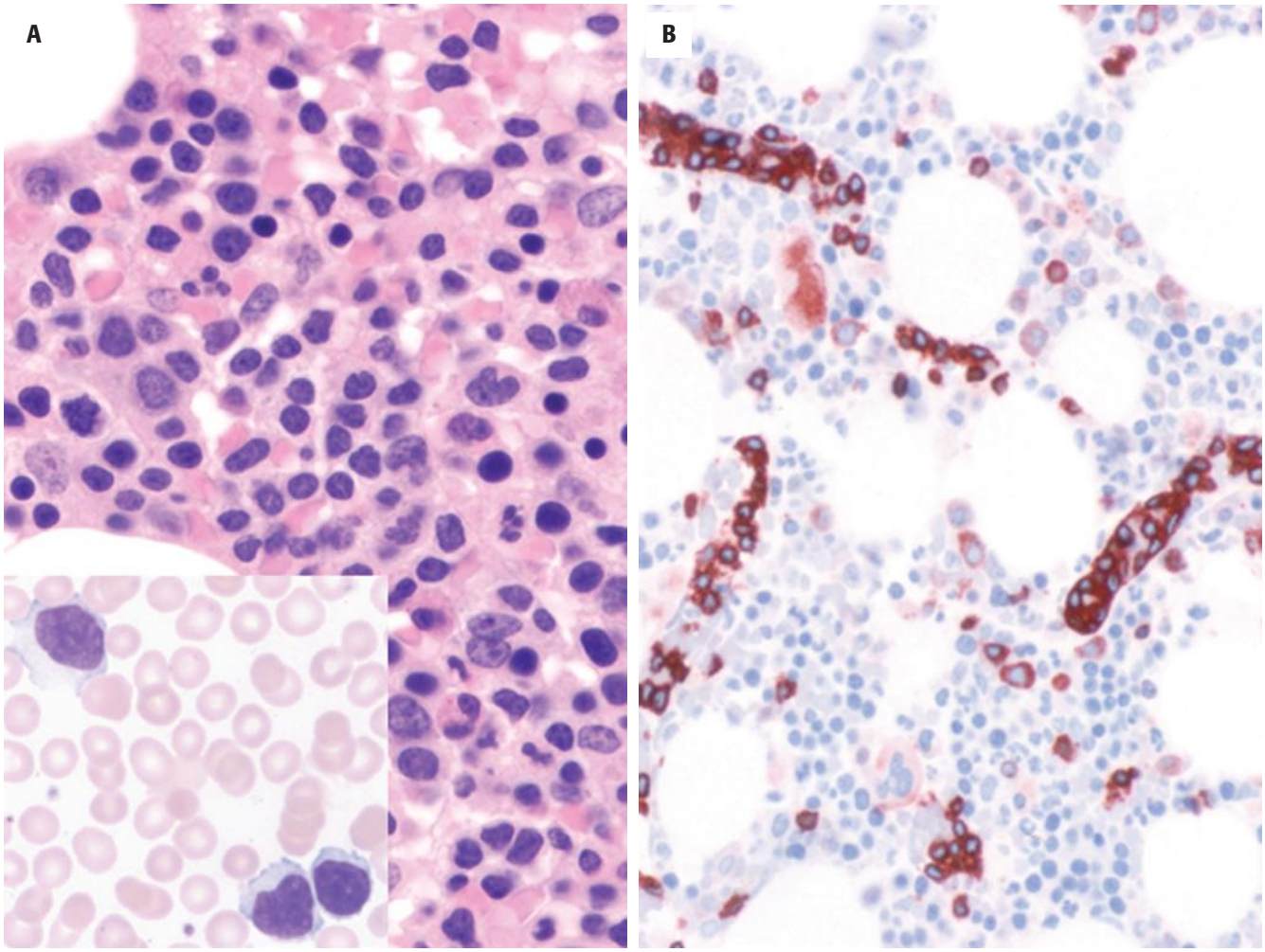


FIG. 7.48

Marginal zone B-cell lymphoma involving bone marrow. The inset shows the cytology of the neoplastic cells with voluminous cytoplasm in the peripheral blood. A, Interstitial involvement of the bone marrow biopsy specimen. B, An immunoperoxidase stain for CD20 highlighting the linearly arranged intrasinusoidal neoplastic cells.

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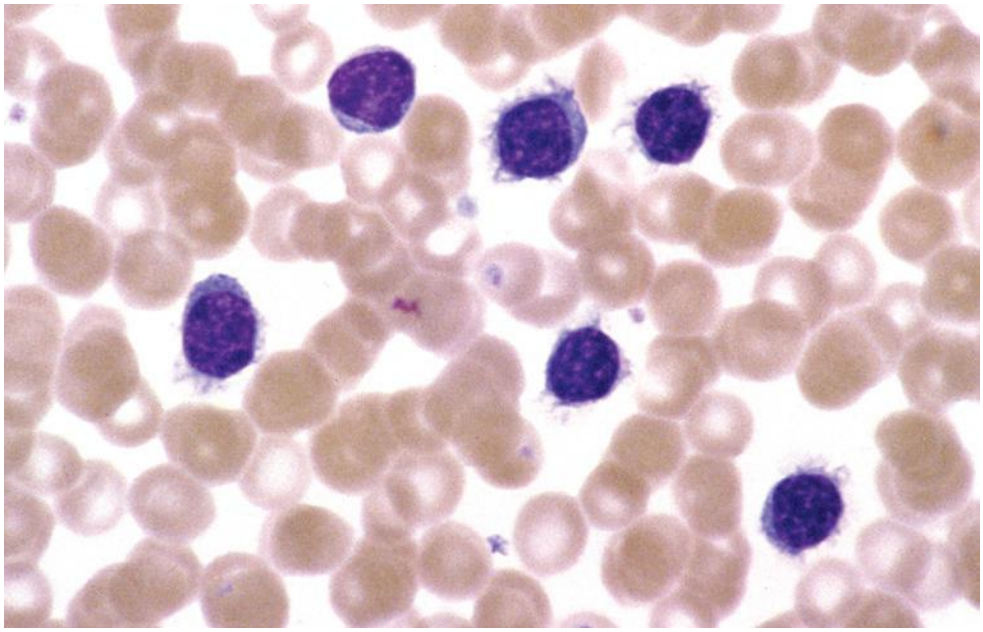


FIG. 7.49
Peripheral blood involvement by splenic marginal zone lymphoma. Note the "villous" cytoplasmic projections.

for CD10, CD21, CD35, BCL6, CD5, SOX11, LEF1, and IRTA1.

Genetics

No single cytogenetic abnormality is exclusively observed in SMZLs. The two most common anomalies are del7q31-32 and trisomy 3. The former is described in up to 40% of SMZL cases and the latter in 15%. In one study, translocations involving 7q31-32 were associated with cyclin-dependent kinase 6 (CDK6) overexpression. But most cases of SMZL with chromosome 7q abnormalities have only deletions of the 7q31-32 region, not translocations. Other translocations that have been described in rare cases of SMZLs involve the immunoglobulin heavy chain locus and the following genes: *PAX5, BCL6, BCL3, IRF4,* and *CCND3.* However, none of these is specific for SMZL.

On a molecular level, clonal immunoglobulin heavy and light chain gene rearrangements can be demonstrated in virtually all cases of SMZL. There is variability in the reported proportions of cases that have somatic hypermutations of *IGH* or point mutations in non-coding sequences of *BCL6*. Some authors have detected frequent somatic hypermutation in the *IGH* loci, whereas others have shown that only a small proportion of cases have point mutations in the 5′ non-coding regions of *BCL6*. Most normal splenic marginal zone B cells have immunoglobulin somatic hypermutations and are thought to represent post-germinal center memory B cells. The data seem to suggest that SMZL, like other low-grade B-cell lymphomas, is also heterogeneous with respect to immunoglobulin and BCL6 gene mutations and represents a mixture of cases analogous to either pre- or post-follicular B cells.

Large-scale genomic studies have identified mutations throughout the NF-κB pathway and those involved in *NOTCH* signaling, implicating aberration in these pathways to be critical for SMZL pathogenesis. Mutations in NF-κB regulators occur in approximately 35% of SMZL cases, with a number of genes targeted including *IKBKB*, *TNFAIP3*, and the *TRAF3/MAP3K14-TRAF2/BIRC3*-negative regulatory complex. The *NOTCH* family of genes, which encode transmembrane proteins that function as ligand-activated transcription factors, shows mutations in 40% of SMZL cases. Most of these mutations lead to sustained *NOTCH* signaling via impaired degradation of *NOTCH* proteins. *NOTCH2* is mutated in 10% to 25% of cases, and *NOTCH1* in approximately 5%. Of these, *NOTCH2* may have diagnostic utility, as mutations in this gene appear highly specific for SMZL among the small B-cell lymphomas. Additionally, cases with *NOTCH2* mutations may have inferior prognosis.

Another gene, *KLF2*, a member of the *KLF* family of zinc finger transcription factors, is mutated in 20% to 42% of SMZL, making this possibly the most commonly mutated gene in SMZL. Most *KLF2* mutations disrupt its nuclear localization, resulting in deregulation of the transcription program orchestrated by this protein. The result here is again constitutive activation of the NF-κB pathway. Interestingly, *KLF2* mutations may subdivide SMZL into two genetic groups: one with *KLF2* mutations, *IGV1-2* rearrangements, and 7q deletion, and one without *KLF2* mutations, with higher rates of *MYD88* and *TP53* mutations. The prognostic significance of these two genetic groups is uncertain at this time.

Differential Diagnosis

Reactive follicular hyperplasia in the spleen can be associated with prominent marginal zone B-cell populations. Up to 10 layers of marginal zone cells surround the follicular mantle in reactive conditions. The borders of the reactive

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marginal zone cells with the adjacent red pulp are sharply defined, with few to no marginal zone cells infiltrating the adjacent red pulp. Non-neoplastic marginal zone B cells are polytypic with respect to kappa and lambda light chain staining.

In the spleen, the differential diagnosis includes grades 1 and 2 FL and MCL. These lymphomas selectively involve the splenic white pulp and can exhibit marginal zone B-cell differentiation. A morphologic and phenotypic approach is used to separate them from one another. In FL, neoplastic centrocytes with a variable mix of centroblasts are present in the centers of the white pulp nodules. They are monotypic for either kappa or lambda light chains, and they express CD10, BCL6, and BCL2. The cells in the regions of marginal zone differentiation can lose CD10 and BCL6 positivity, so it is important to focus on the centers of the nodules. In SMZL with atrophic germinal centers, the non-neoplastic germinal center cells make up a smaller proportion of the total white pulp cell population, they are polytypic for kappa and lambda light chains, and they are BCL2−. In difficult cases one can demonstrate the t(14;18)(q32;q21) by either cytogenetics or FISH in a high proportion of splenic FLs.

MCLs also selectively involve splenic white pulp with central atrophic germinal centers and often a dimorphic cytologic appearance due to marginal zone differentiation at the periphery of the nodules. They are CD5+, CD23−, and very importantly positive for cyclin D1, whereas all SMZLs lack cyclin D1 expression. Although rarely required for diagnosis cytogenetics or FISH showing t(11;14)(q21;q32), *CCND1/IGH,* points to a diagnosis of MCL.

Especially critical in the bone marrow, the differential diagnosis of SMZL includes LPL. Both can have plasmacytic differentiation, and both typically lack a specific phenotypic marker. Intrasinusoidal B-cell infiltrates in the bone marrow together with a history of splenomegaly favor a diagnosis of SMZL. del7q31-32 is also very rare in LPL and, if present, would favor SMZL. Demonstrating *MYD88* L265P mutations in LPL aids in making this distinction. Approximately 90% of LPL cases have these mutations in contrast to less than 10% of SMZL.

Mutational analysis for *NOTCH2* or *KLF2* mutations may prove clinically useful in confirming the diagnosis of SMZL, although additional studies are warranted.

A provisional entity in the WHO lymphoma classification scheme is termed *splenic diffuse red pulp small B cell lymphoma*. It has many characteristics in common with SMZL. It occurs in older adults with a male predominance, produces splenomegaly with frequent involvement of blood and bone marrow. Circulating lymphocytes in some cases have been described as "villous," similar to SMZL. In the spleen this lymphoma produces diffuse red pulp infiltrates with obliteration of the white pulp. The cells are medium size with round to irregular nuclei, condensed chromatin, inconspicuous nucleoli, and abundant pale cytoplasm (Fig. 7.50). In immunohistochemical or flow cytometry analyses, the tumor cells are CD19 and CD20+, they often express CD11c, CD22, and DBA.44, but they are negative for CD103, cyclin D1, and annexin A1, features that separate them from hairy cell leukemia (Fig. 7.51). Although this lesion seems related to SMZL, it is histopathologically distinct, and neither del7q31-32 nor trisomy of chromosomes 3 or 18 has been confirmed in this condition, suggesting that it is a separate disorder. It is clearly distinguishable from hairy cell leukemia, both by pathologic features and clinical features (including failure to respond to purine analog therapy). But, its relationship to hairy cell leukemia variant (provided that it is considered a distinct entity) remains uncertain. Additional studies are needed to better define this provisional entity.

In blood, hairy cell leukemia can morphologically and phenotypically resemble SMZL. However, in the bone marrow, hairy cell leukemia infiltrates occur almost exclusively in the interstitium with hemorrhage and disproportionate suppression of hematopoiesis relative to the degree of involvement by the leukemia. Nodular and intravascular infiltrates are uncommon in hairy cell leukemia and should suggest SMZL. In the spleen, hairy cell leukemia involves red pulp with blood lakes. The white pulp is atrophic in contrast to the prominent white pulp involvement by SMZL. Hairy cell leukemia is associated with *BRAF1* mutations in a high proportion of cases, and this is an unusual finding in SMZL.

Prognosis and Therapy

With an indolent clinical course SMZL has an overall 5-year survival rate of up to 92%. Thus, asymptomatic patients can be followed without treatment. Symptomatic splenomegaly associated with varying degrees of anemia, thrombocytopenia, and neutropenia is often treated by splenectomy. Rituximab monotherapy is also an option, as this has been shown to have results similar to splenectomy. Combination chemotherapy with standard regimens can be used in addition to rituximab in more disseminated disease. A subset of patients with SMZL has a more aggressive clinical course. Risk factors for more guarded prognosis include failure to achieve a complete remission with first-line therapy, involvement of nonhematopoietic sites at diagnosis, high performance status scores, and TP53 expression by the neoplastic cells.

Nodal Marginal Zone B-Cell Lymphoma

Clinical Features

Nodal marginal zone B-cell lymphomas (NMZL) are uncommon. They occur with a median age of 60 to 65 years, but pediatric patients with this lymphoma type have been reported. The pediatric type of NMZL remains a provisional entity in the 2016 WHO classification. Most studies note a striking

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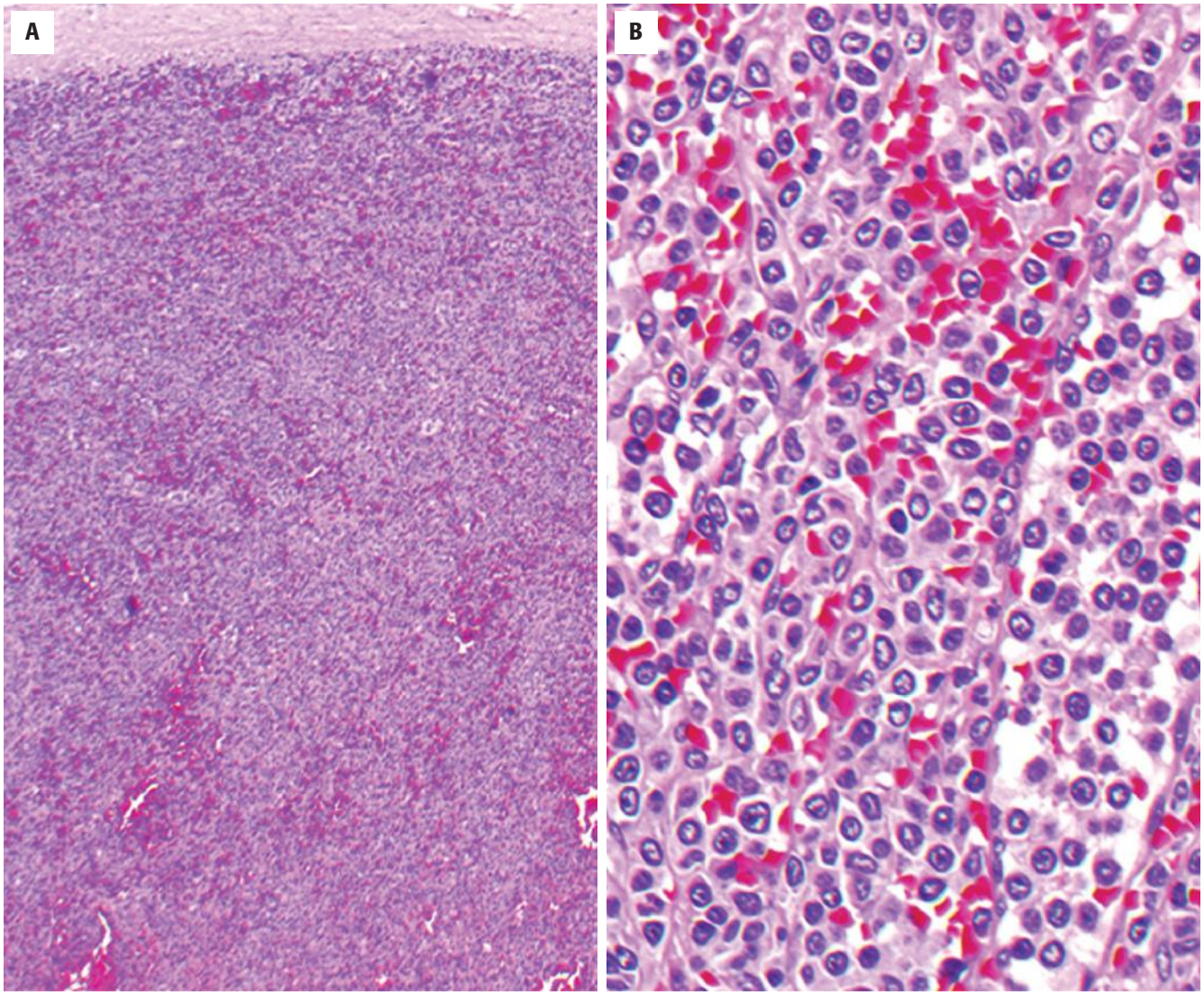


FIG. 7.50

Splenic diffuse red pulp small B-cell lymphoma. A, Diffuse red pulp infiltration of the spleen. B, Typical cytology. Neoplastic cells expand both splenic cords and sinusoids

female predominance (male-to-female ratio 1–1.5:5), with the exception of the pediatric type, which is more common in males. NMZL presents with painless, very slowly progressive or waxing and waning lymph node enlargement. Cervical region lymph nodes are most commonly affected, followed by inguinal and retroperitoneal lymphadenopathy. Although most reports suggest that 50% to 65% of patients with this lymphoma type have Ann Arbor stage I or II disease, some reports indicate advanced stage in up to 71% of patients, with bone marrow involvement in 32%. B symptoms are unusual. Because there is considerable morphologic and phenotypic overlap among nodal, splenic, and extranodal MALT-type marginal zone B-cell lymphomas, involvement of the spleen and of anatomic sites commonly affected by MALT lymphoma should be excluded by staging before a case is accepted as a nodal marginal zone B-cell lymphoma.

Pathologic Features

Morphology

In prototypic cases, abnormal lymphocytes distributed immediately adjacent to the lymph node sinuses and surrounding the residual germinal centers alter, but do not completely efface, the lymph node architecture (Fig. 7.52). From there the tumor spreads in a diffuse pattern to totally replace the underlying lymph node structure and is associated with variable degrees of capsular and trabecular fibrosis. The neoplastic lymphocytes usually are monomorphous and medium sized with round to irregular nuclear contours, condensed chromatin, inconspicuous nucleoli, and voluminous clear cytoplasm with distinct cell membranes (Fig. 7.53). They are mixed with variable numbers of neutrophils, large transformed lymphocytes resembling

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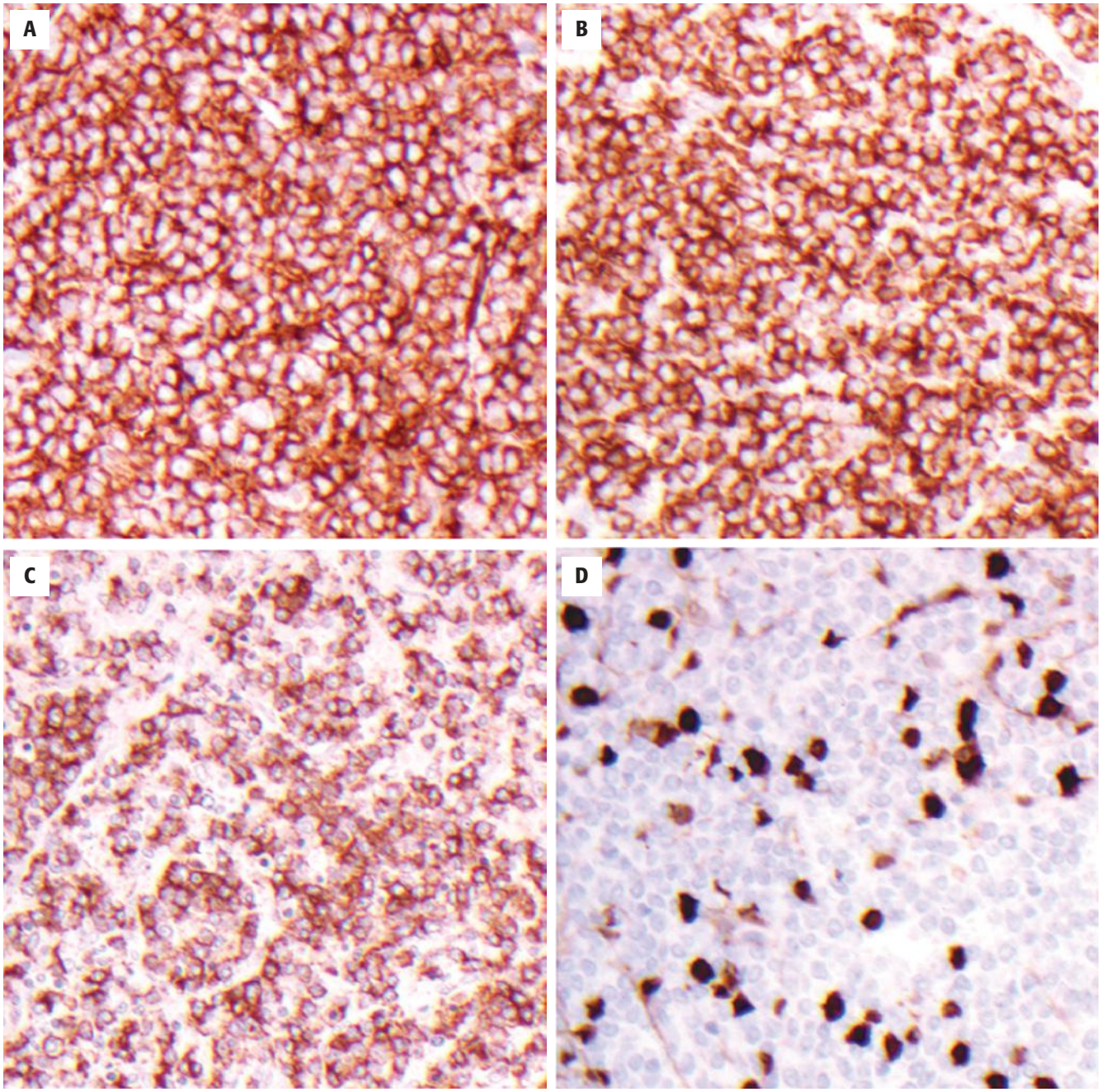


FIG. 7.51

Phenotype of splenic diffuse red pulp small B-cell lymphoma. The neoplastic cells express CD22 (A), CD11c (B), and DBA.44 (C) and are negative for Annexin A1 (D). The Annexin A1–positive cells are granulocytes.

immunoblasts or centroblasts, and very few T cells. The pediatric variant of NMZL is rare, but cases characteristically are thought to have a progressive transformation of germinal center (PTGC)-like pattern.

Cytologic variation of NMZL has been described. In some cases the neoplastic lymphocytes have sparse cytoplasm together with greater nuclear irregularity, and thus the neoplastic cells resemble the small centrocytes of FLs. In other cases admixed monotypic plasma cells, clonally related to the neoplastic lymphocytes, are present. In the purest examples of this disorder, plasma cell differentiation is not present.

In the bone marrow, MZL of nodal, splenic, and MALT types are similar to one another. They can produce intertrabecular nodules, paratrabecular aggregates, or interstitial infiltrates. However, a characteristic feature of MZL in bone marrow is intrasinusoidal involvement (see Fig. 7.48).

Phenotype

The cells of NMZL express the pan B-lymphocyte antigens CD19, CD20, CD22, and PAX-5 and show either kappa or lambda immunoglobulin light chain restriction. They express

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NODAL MARGINAL ZONE B-CELL LYMPHOMA—FACT SHEET

Clinical Features
- 1.8% of lymphomas
- Median age 8th decade
- Female predominance
- Slowly progressive or waxing and waning lymphadenopathy

Morphology
- Perisinusoidal and interfollicular infiltrates
- Medium size lymphocytes with round to irregular nuclei and voluminous clear cytoplasm mimicking nodal monocytoid B cells

Immunophenotype
- CD19+, CD20+, sIg+, IgM without IgD, rarely IgG or IgA
- Monotypic plasma cells in 30%
- CD10 and CD5 negative
- CD43 variably positive
- BCL2 frequently positive

Genetics
- Clonally rearranged immunoglobulin genes
- Trisomies 3, 8, 18 present in some cases
- *PTPRD* mutations
- *NOTCH2* mutations
- *KLF2* mutations

Prognosis and Therapy
- Low-intensity single or multiple agent chemotherapy plus rituximab
- Excellent prognosis for Ann Arbor stage I and II patients
- Median survival 5 to 6 years for Ann Arbor stage III and IV patients

Differential Diagnosis
- Reactive lymphoid hyperplasia, particularly toxoplasma lymphadenitis, cytomegalovirus, lymphadenitis, human immunodeficiency virus–associated lymphadenitis, and cat-scratch disease
- Chronic lymphocytic leukemia/small lymphocytic lymphoma
- Mantle cell lymphoma
- Follicular lymphoma, particularly those with marginal zone B-cell differentiation
- Splenic marginal zone B-cell lymphoma
- Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue
- Lymphoplasmacytic lymphoma
- Hairy cell leukemia

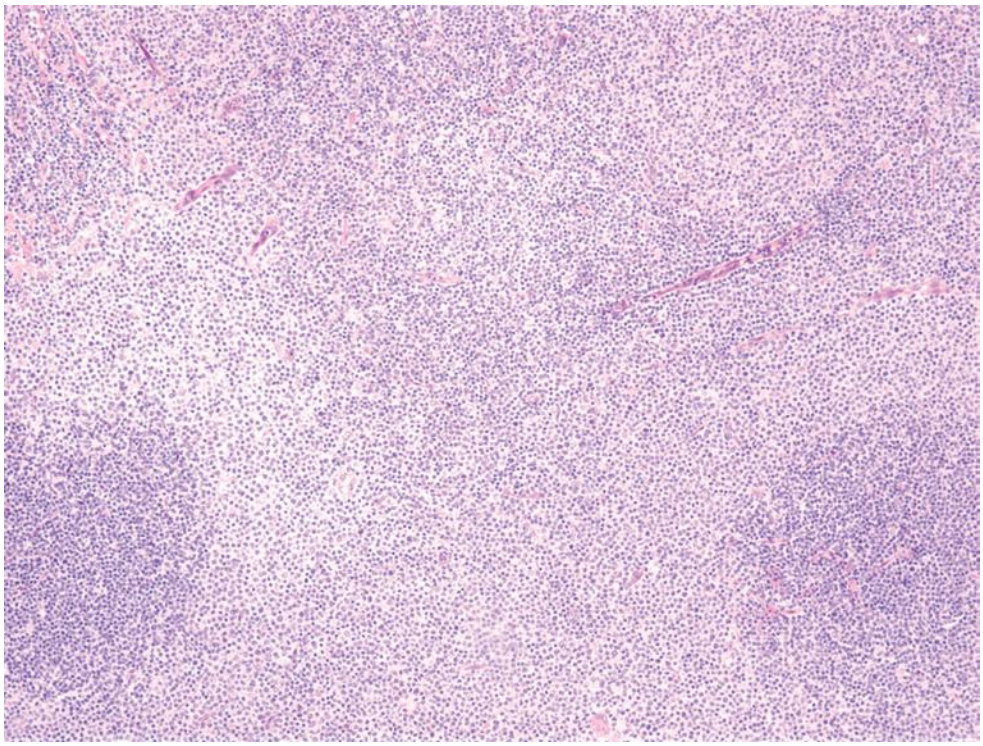


FIG. 7.52
Nodal marginal zone B-cell lymphoma. The neoplastic cells *(pale zones)* are situated adjacent to the lymph node sinusoids and completely surround non-neoplastic primary follicles *(dark zones)*.

IgM and rarely IgG or IgA, but, in contrast to SMZL, they are usually negative for IgD. When the intermixed plasma cells are part of the lymphoma, they too express the same immunoglobulin light chain as the neoplastic small lymphocytes. In contrast to normal monocytoid B cells that do not express BCL2 or CD43, NMZL cells are BCL2 and/or CD43+ in many cases. Typically the cells of this lymphoma type are negative for CD10, CD23, CD35, BCL6, CD5, SOX11, and LEF1. CD5 expression, however, is not uncommonly seen, sometimes along with CD23 expression, creating diagnostic challenges in some instances.

Immunoglobulin superfamily receptor translocation–associated 1, IRTA1, has been published as a potential specific

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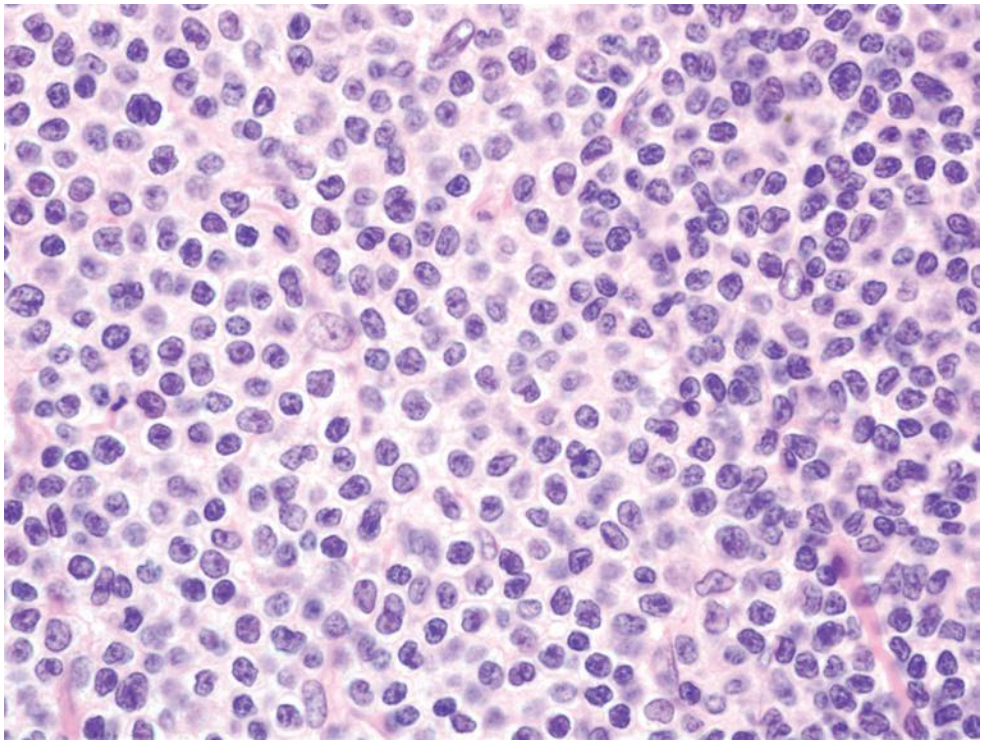


FIG. 7.53
Nodal marginal zone B-cell lymphoma. Typical cytology.

immunohistochemical marker for MZLs of both nodal and extranodal types. This protein is expressed also in normal marginal zone B cells in extranodal sites, as well as in monocytoid B cells. Interestingly, both SMZL and other small B-cell lymphomas overwhelmingly lack expression of this marker.

Genetics

No NMZL-specific chromosomal abnormalities have been detected. Trisomy 3 is the most common abnormality associated with NMZL, but this finding is also present in a variety of other lymphoma types, including splenic and MALT lymphoma. The chromosome translocations found in MALT lymphoma are not detected in rigidly defined NMZL using the criteria described above for prototypic cases without plasma cell differentiation.

Clonal immunoglobulin gene rearrangements occur in NMZL. Somatic hypermutations in the immunoglobulin genes have been described in some cases, but others have non-mutated immunoglobulin genes, indicating that some NMZLs appear to be more similar to naive B cells (no point mutations) and others to post-follicular memory B cells (mutated immunoglobulin genes).

Recently whole exome sequencing studies have identified that, in general, the mutational landscape of NMZL is similar to SMZL, showing mutations in *NOTCH2* and *KLF2*. However, mutations in *MLL2 (KMT2D)* appear enriched in NMZL compared with SMZL, and *PTPRD* mutations seem exclusive to NMZL, albeit only in 14% of cases. *PTPRD* is a receptor-type protein tyrosine phosphatase that functions as a tumor suppressor and has been implicated in solid tumor oncogenesis as well.

Differential Diagnosis

Nodal marginal zone lymphomas must be distinguished from monocytoid B-cell hyperplasia. Monocytoid B-cell hyperplasia rarely occurs as the sole abnormality in the lymph node. It is usually accompanied by follicular hyperplasia with or without epithelioid macrophage clusters that impinge on follicles. Hyperplastic monocytoid B cells do not completely surround reactive follicles or bridge between adjacent completely surrounded germinal centers. Reactive marginal zone B cells are polytypic with respect to kappa and lambda light chain staining, and they are BCL2 negative. NMZL cells show immunoglobulin light chain restriction, and they are BCL2 positive. Immunophenotyping and often gene rearrangement studies to demonstrate light chain restriction and clonality are especially critical in pediatric cases, which can show marked overlap with marginal zone hyperplasia.

Clues to the diagnosis of other low-grade lymphoma types easily confused with NMZL are presented in Tables 7.1, 7.2, and 7.3. It should be noted that currently NMZL is partly a diagnosis of exclusion. One must exclude marginal zone B-cell differentiation in FL and MCL (done by recognizing the specific features of the other lymphoma types in addition to the marginal zone B cells), and one must also exclude MALT lymphoma by staging studies that show no involvement of extranodal sites.

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In the bone marrow, NMZL must be distinguished from other disorders that produce intrasinusoidal B-cell infiltrates: polyclonal B-cell lymphocytosis and intravascular lymphomatosis. Polyclonal B-cell lymphocytosis produces peripheral blood lymphocytosis, but the abnormal B cells show polytypic expression of kappa and lambda immunoglobulin light chains. *Intravascular lymphomatosis* is a term that is usually reserved for large B-cell lymphoma selectively involving vascular spaces without infiltration into the adjacent parenchyma of the involved organ. The large size and prominent nucleoli of the neoplastic cells in intravascular lymphomatosis should be obvious clues to this diagnosis once the intravascular infiltrates are recognized by morphology and staining for CD20. T-cell and NK-cell granular lymphocytic leukemia and hepatosplenic T-cell lymphoma also produce intrasinusoidal bone marrow infiltrates, but the cytolytic T-cell or NK-cell phenotype of these disorders allows them to be readily distinguished from bone marrow involvement by marginal zone B-cell lymphoma.

Prognosis and Therapy

Treatment of patients with NMZL is approached in a manner similar to patients with extra-gastric MALT lymphomas. If necessary, single or multiple agent low-intensity chemotherapy is used. For those patients with stage I or II disease, there is an excellent prognosis. As with pediatric-type FL, patients with pediatric MZL tend to present with localized disease and have a very favorable prognosis. For those with more advanced stage disease the literature on outcome is inconsistent. Some reports document a very indolent clinical course with long survivals, others a clinical course that is more aggressive with more limited median survivals.

LYMPHOPLASMACYTIC LYMPHOMA

Clinical Features

Lymphoplasmacytic lymphoma is an uncommon neoplasm of older adults with a slight male predominance. Usually, LPL affects the bone marrow with less frequent involvement of spleen and lymph nodes. Most commonly, it is associated with mutations in the myeloid differentiation primary response gene 88 *(MYD88)*, IgM paraproteinemia, and manifestations of hyperviscosity (Waldenström macroglobulinemia [WM]). Although WM is defined as LPL with an IgM paraproteinemia of any level, the two terms LPL and WM are not synonymous, and, rarely, LPL can present with non-IgM paraproteins. Some cases of LPL may arise in association with hepatitis C, although other lymphoproliferative disorders can also arise in this setting. Primary cold agglutinin-associated

LYMPHOPLASMACYTIC LYMPHOMA—FACT SHEET

Clinical Features
- 1.5% of nodal lymphomas
- Median age 7th decade
- Male predominance
- IgM paraproteinemia with or without symptoms of hyperviscosity (Waldenström macroglobulinemia) and bone marrow involvement in most cases
- Slowly progressive or waxing and waning lymphadenopathy in uncommon subset of patients with primarily lymph node–based disease

Morphology
- Paracortical infiltrates with open lymph node sinuses
- Diffuse architectural effacement of lymph node
- Spectrum of small lymphocytes, plasmacytoid lymphocytes, plasma cells, and immunoblasts in varying proportions in each case
- Abnormal immunoglobulin production: Dutcher bodies, Russell bodies, amyloid or free immunoglobulin light chain deposits
- Some cases with epithelioid macrophage clusters that obscure presence of lymphoma

Immunophenotype
- CD19+, CD20+, sIg+, IgM without IgD, rarely IgG or IgA
- Monotypic plasma cells in all (defining feature)
- CD10 and CD5 negative
- CD43 variably positive

Genetics
- *MYD88* L265P mutations

Prognosis and Therapy
- Low-intensity single- or multiple-agent chemotherapy
- Plasma exchange for transient relief of symptoms of hyperviscosity
- Survival
  - Asymptomatic Waldenström macroglobulinemia patients—similar to age-matched controls
  - Symptomatic Waldenström macroglobulinemia patents—median survival, 5 to 7 years
  - Lymph node–based disease without Waldenström macroglobulinemia—median survival, 4 years

Differential Diagnosis
- Reactive lymphoid hyperplasia, particularly autoimmune disease–associated lymphadenitis, IgG4-related sclerosing/autoimmune disorder, and Castleman disease types with abundant plasma cells
- Chronic lymphocytic leukemia/small lymphocytic lymphoma
- Mantle cell lymphoma
- Follicular lymphoma, particularly those with plasma cell differentiation
- Nodal marginal zone B-cell lymphoma
- Splenic marginal zone B-cell lymphoma
- Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue

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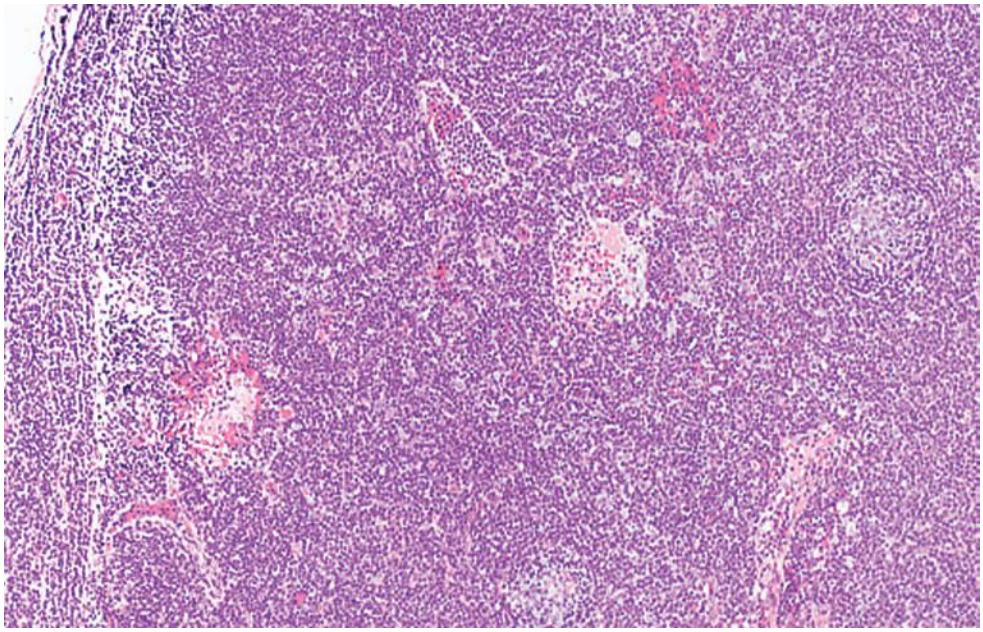


FIG. 7.54

Lymphoplasmacytic lymphoma in a lymph node, infiltrating the capsule and present in the interfollicular zones, but sparing the patent lymph node sinusoids.

lymphoproliferative disease was previously thought to be a form of LPL. This is no longer the case because newer molecular markers, such as *MYD88,* are negative in most cold agglutinin-associated lymphoproliferations, suggesting they are a distinct entity.

Pathologic Features

Morphology

In lymph nodes and bone marrow, the cytologic features of LPL are similar. In lymph nodes, the neoplastic cells infiltrate in the paracortex and hilum, often with sparing or dilatation of the subcapsular and marginal sinuses (Fig. 7.54). Because of this distribution, LPL involvement of lymph nodes can be very subtle. In more advanced cases there is diffuse effacement of the lymph node architecture (Fig. 7.55). The neoplastic cells span a spectrum from small lymphocytes, through lymphoplasmacytoid cells with more abundant cytoplasm ("plasmacytoid lymphocytes"), to mature plasma cells (Fig. 7.56). Often there is a concentration of the plasma cells near blood vessels and lymph node sinuses. As with any neoplastic plasmacytic disorder, Dutcher bodies and Russell bodies can found in the tumor cells in many cases, but finding them may require a persistent search. Other features of abnormal immunoglobulin production, such as crystalline immunoglobulin deposits in the tumor cells (Fig. 7.57A) or in macrophages (Fig. 7.57B) and focal or extensive deposition of free immunoglobulin light chains or amyloid, occur in a small fraction of the cases. An increase in reactive mast cells, clusters of epithelioid macrophages that can obscure the presence of lymphoma, and focal deposits of hemosiderin often accompany the lymphoma cells, especially in bone marrow infiltrates. By convention, proliferation centers as seen in SLL are absent. In the spleen LPLs preferentially involve the red pulp but occasionally are more white pulp based. In bone marrow the abnormal lymphoplasmacytic infiltrates can form any combination of paratrabecular or intertrabecular lymphoid aggregates or involve the bone marrow interstitium (Fig. 7.58). The cytologic spectrum of lymphocytes, plasmacytoid lymphocytes, and plasma cells is probably best appreciated in this disease in Wright-Giemsa–stained bone marrow aspirates (Fig. 7.59).

Phenotype

The neoplastic lymphocytes and plasmacytoid lymphocytes express the pan B-lymphocyte antigens CD19, CD20, and PAX-5 and show either kappa or lambda immunoglobulin light chain restriction. The cells in up to 61% of cases express CD23, and cases with expression of CD5 or CD10 are also not infrequent. The neoplastic plasma cells express CD138 and express the same immunoglobulin light chain type as the neoplastic lymphocytes. Unlike in multiple myeloma, neoplastic plasma cells in LPL typically show at least partial retention of CD45 and CD19 expression. As mentioned above, LPL is usually positive for IgM and lacks staining for IgD but can express IgG or IgA.

Genetics

Karyotypic and FISH studies have repeatedly demonstrated deletions in the long arm of chromosome 6 in many samples from patients with LPL. Thus, though not specific for LPL, del(6)(q21) is the most common structural abnormality in LPL. Compared to other B-cell lineage neoplasms, including

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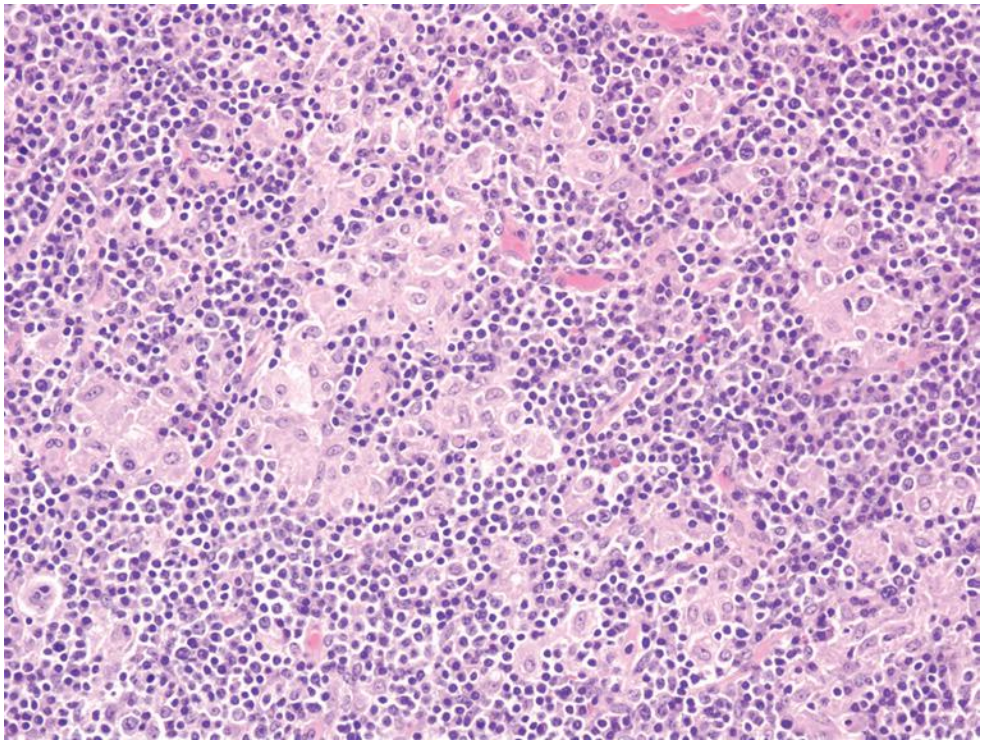


FIG. 7.55
Lymphoplasmacytic lymphoma diffusely effaces lymph node architecture and is associated with clustered epithelioid macrophages.

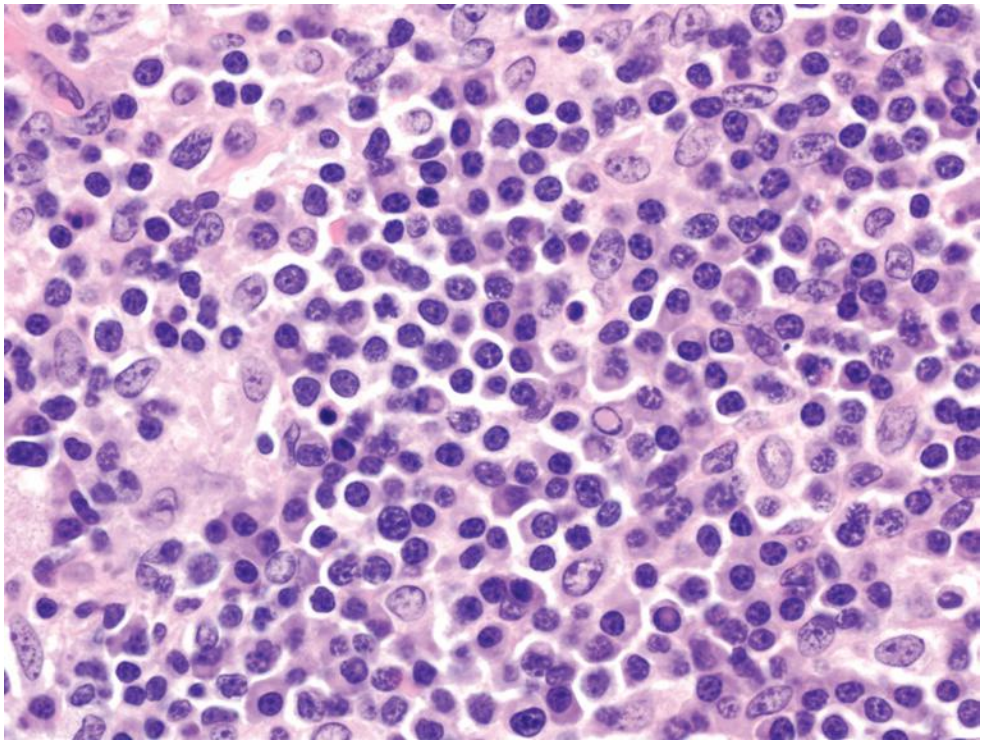


FIG. 7.56
Lymphoplasmacytic lymphoma. Note the cytologic spectrum: small lymphocytes, plasmacytoid lymphocytes and plasma cells, and the Dutcher body in the center of the field.

multiple myeloma, LPL is unusual for its lack of chromosomal translocations involving the immunoglobulin heavy chain gene locus (14q32).

The discovery of the *MYD88* L265P mutation in at least 90% of cases of LPL has changed the way hematopathologists diagnose and view this disease. *MYD88* L265P not only now aids in defining this disease but also may have prognostic value and impact therapy selection. Recently, recurrent mutations in the C-X-C chemokine receptor type 4 *(CXCR4)* gene, similar to those present in WHIM syndrome, were identified in 30% to 40% of LPL cases, essentially always in concert with an *MYD88* mutation. Although the prognostic and

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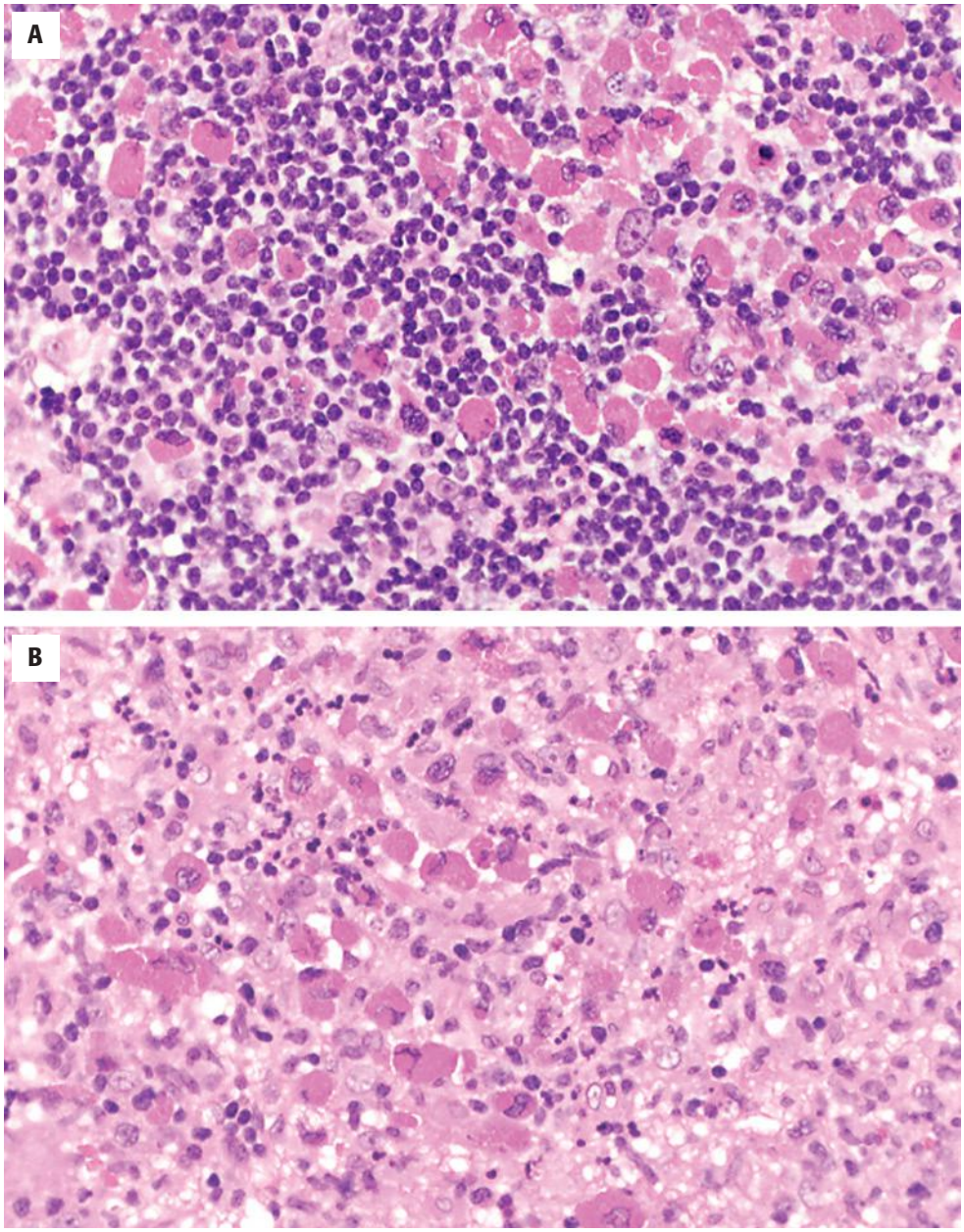


FIG. 7.57

Immunoglobulin inclusions in lymphoplasmacytic lymphoma. A, Abnormal globules and crystals of immunoglobulin in neoplastic plasma cells. B, Cells containing the abnormal immunoglobulin inclusions are associated with a prominent infiltrate of non-neoplastic macrophages.

diagnostic utility of this marker has yet to be fully investigated, early studies suggest a role for predicting response to BTK inhibitors such as ibrutinib.

Differential Diagnosis

Non-specific reactive conditions associated with marked plasma cell infiltrates are in the differential diagnosis of LPL. These reactive cases can be readily identified using paraffin section immunohistochemistry and showing that the plasma cells have a polytypic staining pattern for kappa and lambda immunoglobulin light chains. Localized plasma cell variant Castleman disease (PCCD) is very rare, but it should be considered any time there is a striking interfollicular plasmacytosis in a specimen resembling a lymph node. Clinically, PCCD produces a single dominant mass, sometimes with enlarged adjacent lymph nodes. Although serum gamma globulin levels may be elevated in PCCD, there is polyclonal hypergammaglobulinemia, not a monoclonal protein. Histologically, PCCD is characterized by regressive transformation of germinal centers and sheets of interfollicular plasma cells that at least in areas overrun the lymph node sinuses. The plasma cells are polyclonal with respect to light chain staining, and they are usually associated with abundant Russell bodies. Multicentric Castleman disease (MCD) is characterized by regressive transformation of germinal centers, preserved lymph node sinuses, and increased interfollicular plasma cells, which in some cases (particularly those associated with POEMS

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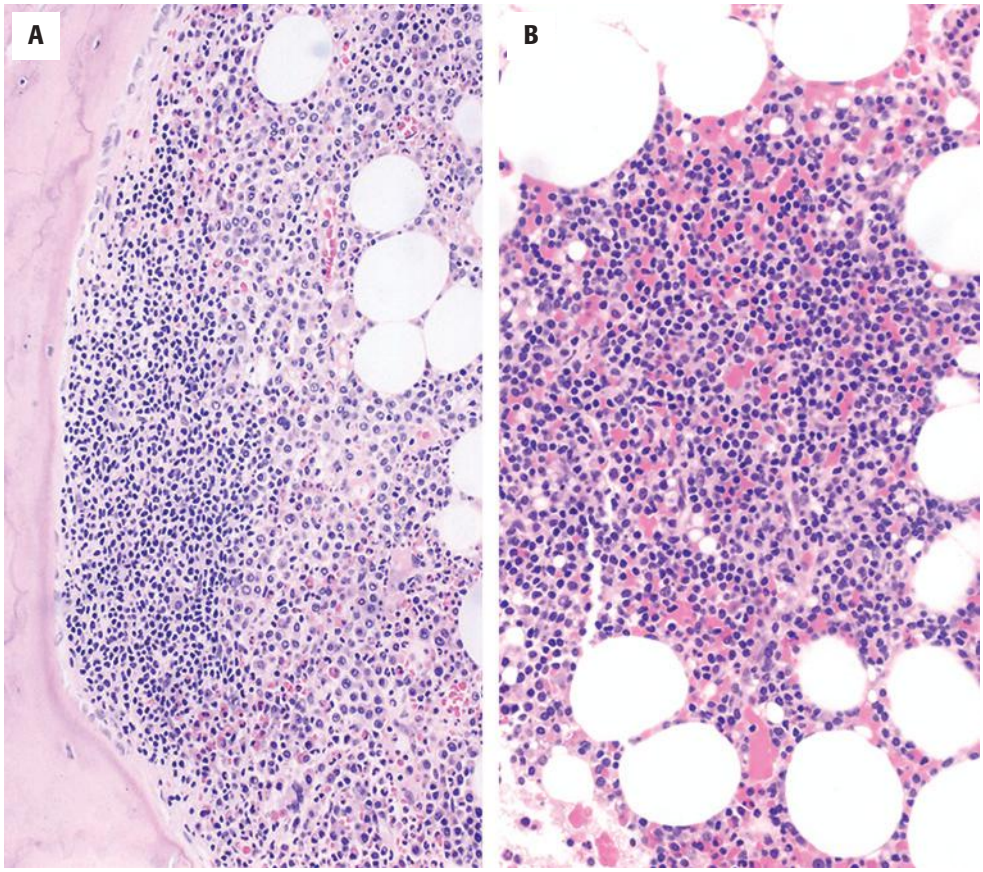


FIG. 7.58

Bone marrow involvement by lymphoplasmacytic lymphoma. A, Paratrabecular pattern. B, Intertrabecular aggregate.

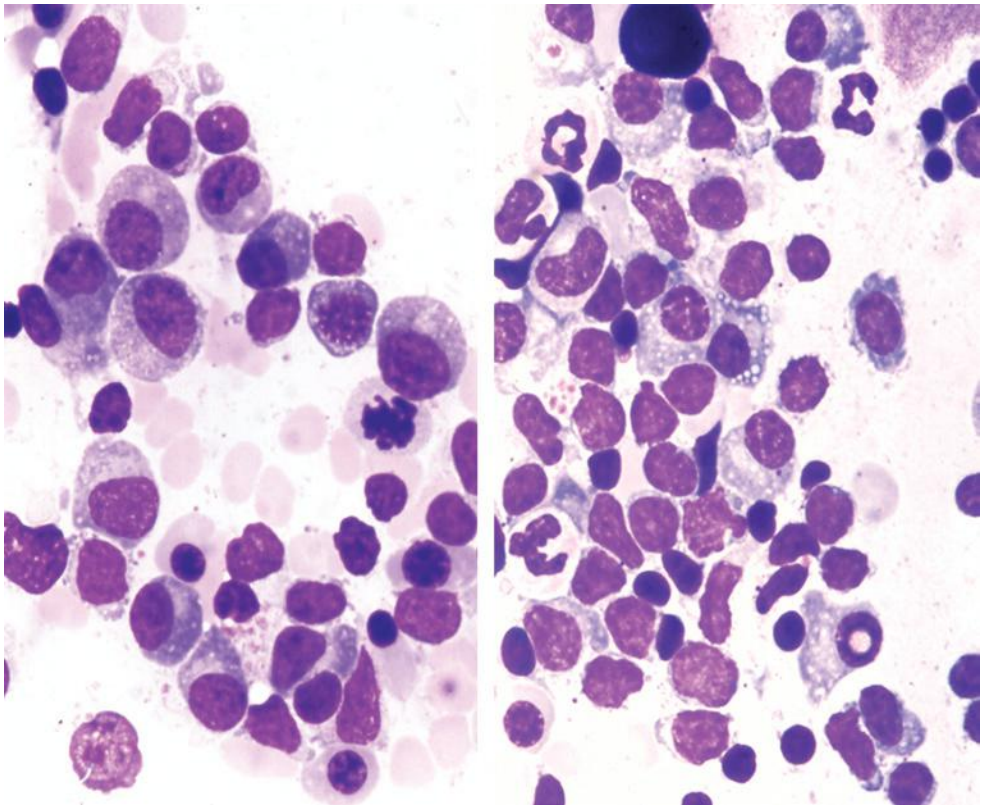


FIG. 7.59

Bone marrow involvement by lymphoplasmacytic lymphoma—aspirate smears stained with Wright-Giemsa. In both of these cases the cell spectrum of small lymphocytes, plasmacytoid lymphocytes, and plasma cells is readily apparent.

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[**p**eripheral neuropathy, **o**rganomegaly, **e**ndocrinopathy, **m**onoclonal plasma cell proliferation, and **s**kin changes] syndrome) are lambda light chain restricted. However, the B cells in MCD are polyclonal. Human herpesvirus-8 (HHV-8)-positive cells are present in MCD cases that arise in immunosuppressed patients. All of the Castleman disease-related lymphoproliferations featuring abundant plasma cells have distinctive clinical features that also help distinguish them from LPL.

Lymphadenopathy, characterized by expanded paracortical regions rich in IgG4-positive plasma cells have been described as part of the IgG4-related sclerosing/autoimmune disease. In these cases, the patients have other autoimmune phenomena, such as pancreatitis, sialadenitis, and pulmonary infiltrates and high serum levels of IgG4. In the lymph nodes and other sites involved by this disorder, the plasma cells are polytypic for kappa and lambda light chains, and thus it can be distinguished from LPL.

The other low-grade lymphomas that can be confused with LPL can be distinguished from one another by using the criteria in Tables 7.3. In particular, prominent monocytoid B-cell populations are rare in LPL and, if present, should suggest marginal zone B-cell lymphoma, particularly MALT-type lymphoma. *MYD88* should be absent in the majority of other low-grade B-cell lymphomas with plasmacytic differentiation and in IgM multiple myeloma. However, up to 10% of MZLs can show *MYD88* mutations, so mutational analysis should not be interpreted in isolation of the pathologic and clinical features.

Plasmacytoma and multiple myeloma involving lymph nodes produce sheets of plasma cells that efface the architecture without intermixed small or plasmacytoid lymphocytes. The neoplastic plasma cells in less than 1% of plasmacytomas and cases of multiple myeloma express IgM, so assessing the immunoglobulin isotype by immunohistochemistry or by serum or urine immunoelectrophoresis in patients with paraproteins can help to distinguish LPL from these other plasma cell dyscrasias. *MYD88* can be useful in this setting as well. Because cyclin D1 is often expressed in myelomas with a lymphoplasmacytoid morphology, confirming lack of cyclin D1 expression in a suspected case of LPL in the bone marrow is also helpful. Finally, in difficult cases, deferring to the clinical distribution of disease can help define the underlying disorder: lymph node and/or spleen only favors LPL; lytic bone lesions, hypercalcemia, and renal failure favor plasma cell myeloma.

Prognosis and Therapy

Therapy for LPL has been an area of intense study in recent years with the identification of new mutations that helped to better define this disease entity. Standard therapy for most patients includes rituximab and either cyclophosphamide/dexamethasone, bendamustine, or bortezomib/dexamethasone. The approach takes into account the overlap of this neoplasm between a low-grade B-cell lymphoma and a plasma cell neoplasm. The BTK inhibitor, ibrutinib, was recently approved for treatment in both initial and relapse settings.

Symptoms of severe hyperviscosity syndrome due to high IgM levels can be effectively, but transiently, treated with plasma exchange. For Waldenström macroglobulinemia, the prognosis primarily depends on whether or not the patient is symptomatic. Survival in asymptomatic patients is similar to that for age- and gender-matched control patients. There is median survival of 7 years and a five-fold risk of death compared to age- and gender-matched control patients for symptomatic patients.


The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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8

Diffuse Aggressive B-Cell Lymphomas

■ Graham W. Slack, MD ■ Eric D. Hsi, MD

INTRODUCTION

This chapter covers diffuse aggressive B-cell lymphomas in immunocompetent patients. In the 2016 revision of the World Health Organization (WHO) classification of lymphoid neoplasms concepts regarding diffuse and aggressive B-cell lymphomas have been further revised to reflect the current understanding of these diseases. Box 8.1 shows the current entities considered under this topic, most of which will be covered in this chapter. The immunodeficiency-related diffuse aggressive lymphomas (plasmablastic lymphoma in human immunodeficiency virus [HIV], Epstein-Barr virus–positive [EBV+] mucocutaneous ulcer) will also be briefly discussed in Chapter 10. De novo diffuse large B-cell lymphoma (DLBCL), which represents the most common type of non-Hodgkin lymphoma, is a clinically and biologically heterogeneous disease. For the purposes of this discussion, DLBCL can be divided into morphologic variants, cell of origin variants, and clinically and biologically distinct subtypes (Box 8.2). DLBCL can also result from transformation of a preexisting low-grade lymphoma, such as chronic lymphocytic leukemia–small lymphocytic lymphoma (i.e., CLL/SLL), follicular lymphoma, or marginal zone lymphoma. Unless the history is known or a low-grade component is represented in the biopsy (so-called composite histology), it might not be possible to distinguish these transformed lymphomas from de novo DLBCL.

Other types of large B-cell lymphomas (LBCLs) are recognized (Box 8.3) and include EBV+ DLBCL, EBV+ mucocutaneous ulcer, DLBCL associated with chronic inflammation, primary mediastinal LBCL, intravascular LBCL, lymphomatoid granulomatosis, *ALK*-positive LBCL, plasmablastic lymphoma, primary effusion lymphoma, and herpesvirus 8–positive (HHV8+) DLBCL, not otherwise specified (NOS). The latter two entities are discussed with the immunodeficiency-related lymphomas (see Chapter 10) and will not be reviewed here. This chapter also discusses Burkitt lymphoma (BL) and the newly created diagnostic entities Burkitt-like lymphoma with 11q aberration, high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements, and high-grade B-cell lymphoma, NOS.

DIFFUSE LARGE B-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED

Clinical Features

De novo DLBCL, NOS is the most common type of non-Hodgkin lymphoma, representing 30% to 40% of all non-Hodgkin lymphomas. Although it can occur in children, this disease occurs primarily in adults, with a median age of onset in the seventh decade. As with most lymphomas, there is a male predominance. Patients may have a rapidly enlarging mass. This usually represents a lymph node; however, occurrence at an extranodal site is common (40%). The most common extranodal site is the gastrointestinal tract, followed by the skin, although essentially any extranodal site can be involved. B symptoms (weight loss, fever, night sweats) are seen in approximately 30% of patients.

Pathologic Features

Histopathology

By definition, the malignant cells proliferate and infiltrate in a diffuse pattern. Usually there is complete effacement of the lymph node architecture, although occasionally there is partial involvement. In the lymph node, partial involvement can manifest as interfollicular or sinusoidal infiltration. Sclerosis can be seen in some cases and may even impart a pseudonodular pattern. Lack of true follicle formation can be confirmed with immunostaining showing lack of a follicular dendritic cell meshwork that is typical of follicular lymphomas. In extranodal sites, obliteration of the normal epithelial components is seen. In a mucosal site, such as the stomach, epithelial invasion by large cells with formation of lymphoepithelial lesions is sometimes seen. Although this is a feature of mucosa-associated lymphoid tissue (MALT)-type lymphomas, such LBCLs are currently considered and classified as extranodal DLBCL, NOS. In such cases an effort should be

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Box 8.1 Large B-Cell Lymphomas in the 2016 Revision of the WHO Classification

Diffuse large B-cell lymphoma, not otherwise specified
  Germinal center B-cell type
  Activated B-cell type
T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the central nervous system (CNS)
Primary cutaneous DLBCL, leg type
EBV+ DLBCL, NOS
EBV+ mucocutaneous ulcer
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK+ large B-cell lymphoma
Plasmablastic lymphoma
Primary effusion lymphoma
HHV8+ DLBCL, NOS
Burkitt lymphoma
Burkitt-like lymphoma with 11q aberration
High-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements
High-grade B-cell lymphoma, NOS
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic Hodgkin lymphoma

Provisional entities in *italics*.

Box 8.2 Variants and Subtypes of DLBCL

DLBCL, Not Otherwise Specified, Morphologic Variants

Centroblastic
Immunoblastic
Anaplastic

DLBCL, Not Otherwise Specified, Cell of Origin Variants

Germinal center B-cell type
Activated B-cell type

DLBCL, Subtypes

T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the CNS
Primary cutaneous DLBCL, leg type
EBV+ DLBCL of the elderly

made to determine whether a conventional low-grade lymphoma (MALT lymphoma) is also present in the biopsy, indicating transformation rather than a de novo DLBCL, NOS.

DLBCL, NOS is cytologically heterogeneous, with morphologic variants recognized. Common to all is a nuclear chromatin pattern that is open and vesicular compared to the condensed chromatin pattern of small B-cell lymphomas. Nucleoli may be single or multiple and variably sized. Cells are usually large (compared to the nuclear size of a benign histiocyte) but occasionally can be intermediate in size (approximately the same size as a benign histiocyte). Three morphologic variants are recognized in the WHO classification,

Box 8.3 Other Large B-Cell Lymphomas Seen in Immunocompetent and Immunocompromised Populations

LBCL, Immunocompetent

EBV+ DLBCL, NOS
EBV+ mucocutaneous ulcer
DLBCL associated with chronic inflammation
Primary mediastinal large B-cell lymphoma
Intravascular large B-cell lymphoma
Lymphomatoid granulomatosis
ALK-positive large B-cell lymphoma

LBCL, Immunocompromised

Plasmablastic lymphoma
Primary effusion lymphoma
HHV8+ DLBCL, NOS

DIFFUSE LARGE B-CELL LYMPHOMA—FACT SHEET

Clinical Features

- Most common type of non-Hodgkin lymphoma
- Median age of onset in 7th decade, males > females
- Rapidly enlarging mass

Morphology

- Diffuse infiltrate of large cells
- Morphologic variants
  - Centroblasts
  - Immunoblasts
  - Anaplastic

Immunophenotype

- CD19+, CD20+, sIg+
- CD10+ (subset), BCL2+ (subset), BCL6+ (subset), MUM1+ (subset)
- Germinal center B-cell type:
  - Hans: CD10+ or CD10–/BCL6+/MUM1–
  - Choi: GCET1+/MUM1–, GCET1–/CD10+, or
  - GCET1–/CD10–/BCL6+/FOXP1–
- Non-germinal center B cell:
  - Hans: CD10–/BCL6– or CD10–/BCL6+/MUM1+
  - Choi: GCET1+/MUM1+, GCET1–/CD10–/BCL6– or
  - GCET1–/CD10–/BCL6+/FOXP1+

Molecular Genetics

- Rearranged immunoglobulin genes
- 20% *IGH/BCL2*
- 30% 3q27 abnormalities
- Gene expression profiling suggests germinal center, activated B cell and "type 3" types

Treatment/Outcome

- Anthracycline-containing multi-agent chemotherapy + rituximab
- 55% 5-year survival, outcome can be stratified based on the International Prognostic Index

Differential Diagnosis

- Peripheral T-cell lymphoma, Burkitt lymphoma, lymphoblastic lymphoma, blastoid or pleomorphic mantle cell lymphoma, myeloid sarcoma, carcinoma, melanoma

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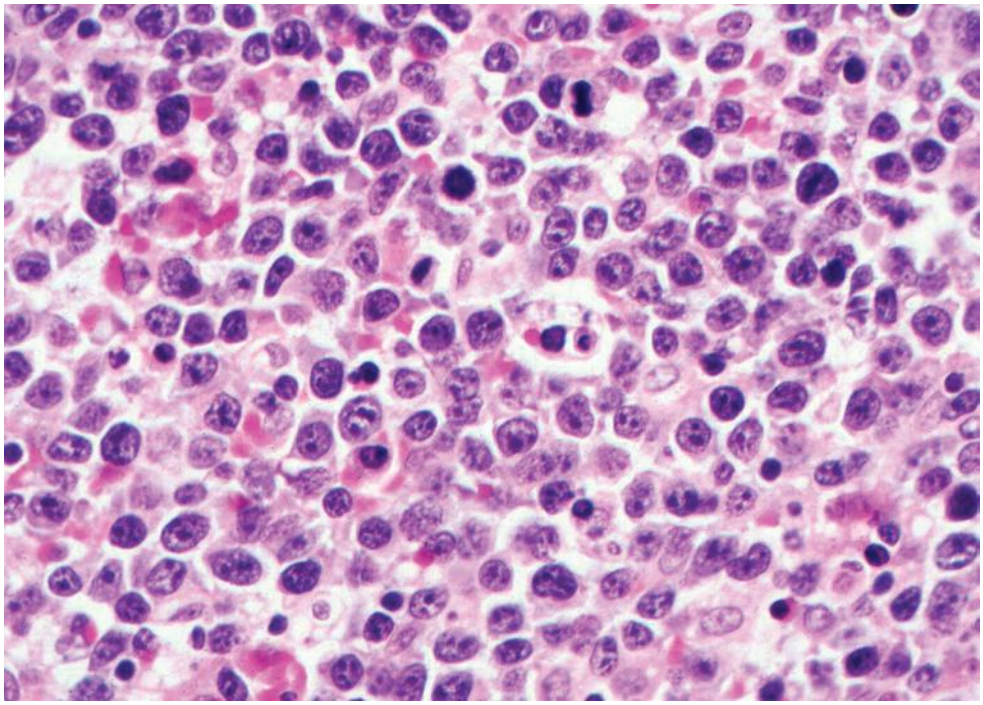


FIG. 8.1
Diffuse large B-cell lymphoma, centroblastic type. The cells are round with multiple small nucleoli.

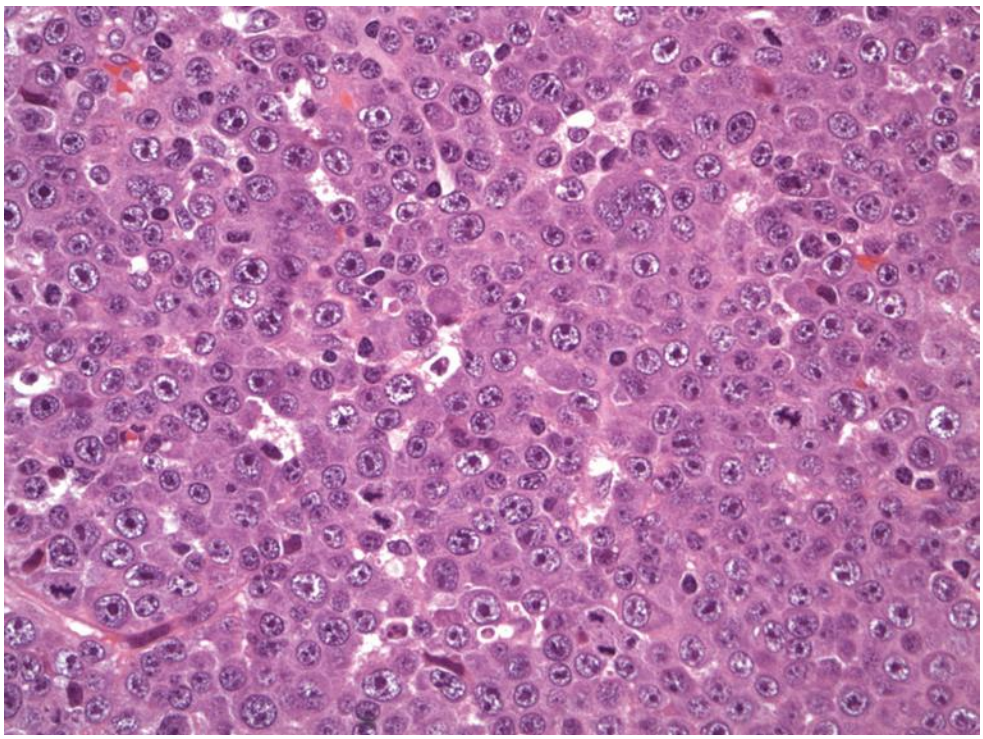


FIG. 8.2
Diffuse large B-cell lymphoma, immunoblastic type. Many cells have large prominent central nucleoli.

but it is not required to report them. They are the centroblastic, immunoblastic, and anaplastic variants (Figs. 8.1 and 8.2). Centroblastic cells are characterized by round to oval large nuclei with vesicular chromatin and multiple (two to four) small nucleoli that are closely applied to the nuclear membrane. Cytoplasm is scant, but visible, particularly on Giemsa staining, in which it has a basophilic quality. In some cases, polylobated nuclei predominate as opposed to the typical round contours. The occurrence of polylobated nuclei typically imparts a smaller cell size and can make distinction from small B-cell lymphomas difficult. Multilobated cells are common in some extranodal lymphomas, such as primary lymphoma of bone. Immunoblasts are large cells with a single, prominent central nucleolus and more abundant basophilic

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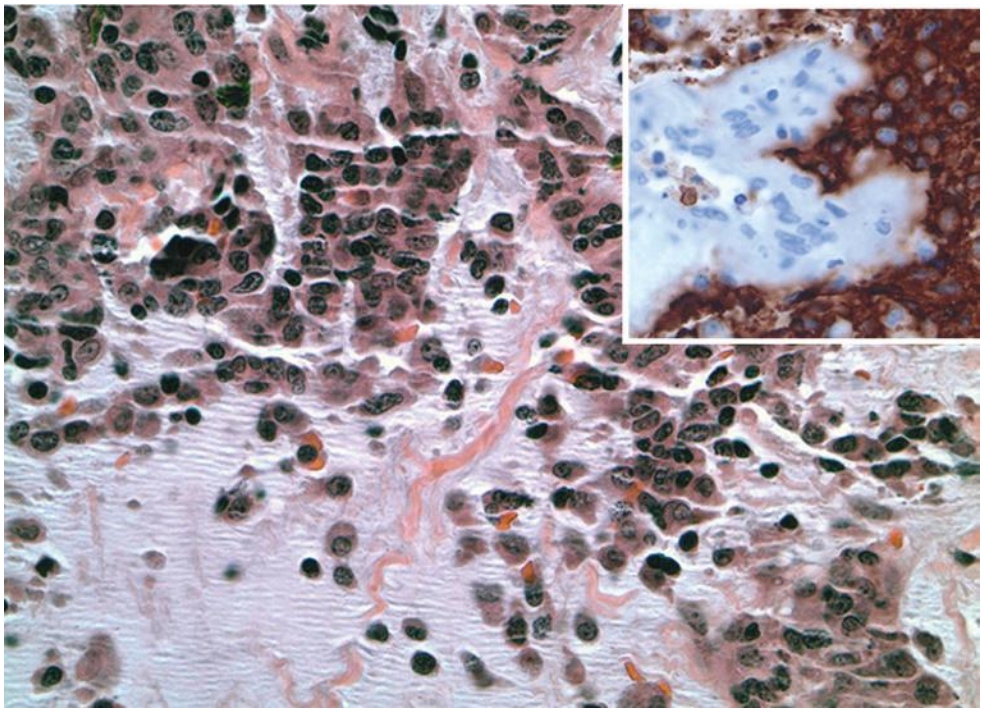


FIG. 8.3

Myxoid variant of diffuse large B-cell lymphoma. The lymphoma cells have vesicular chromatin and moderate amounts of eosinophilic cytoplasm. The *inset* shows the CD20 immunostain, confirming a B-cell lineage.

cytoplasm. Plasmacytoid differentiation can be seen. Diagnosis of the immunoblastic variant is appropriate when more than 90% of cells are of this type. Some studies have suggested a worse outcome for patients with this variant. The anaplastic variant is composed of sheets of anaplastic, atypical cells with bizarre multilobated–multinucleated cells that may resemble Reed-Sternberg cells. This term reflects both cytologic features and pattern of growth. For example, anaplastic DLBCL, NOS may grow within lymph node sinuses, reminiscent of an epithelial tumor. *Pleomorphic* may be a better term than *anaplastic,* because the latter can be confused with anaplastic large-cell lymphoma of T-cell lineage.

Other unusual morphologic variants have been described, and it is useful to be aware of them primarily because they can initially be mistaken for other non-hematolymphoid tumors until immunophenotyping is performed. These variants include signet ring cell, microvillus, myxoid, and spindle cell variants (Fig. 8.3).

Approximately 5% to 15% of patients with DLBCL, NOS have bone marrow involvement, and this is associated with an adverse outcome. The histology, however, is highly variable. The pattern can be paratrabecular, interstitial, or diffuse. The diffuse pattern is uncommon and is usually seen when the lymphomatous infiltrate is composed of large cells. The cytology is often discordant (approximately half of the cases), meaning the marrow infiltrate is composed of small lymphocytes or mixed small and large lymphocytes. Studies have shown that patients with concordant large cells in the bone marrow have a poor response to therapy and poor overall survival. This finding is independent of the International Prognostic Index (IPI) score. The presence of discordant marrow involvement at the time of staging (small B cells in the marrow) has been shown to be a predictor of inferior progression-free survival, but it is not independent of the clinical IPI variables. Discordant disease travels with older age, elevated serum lactate dehydrogenase (LDH), advanced stage, and increased number of extranodal sites.

Immunophenotype

Immunophenotypically, the malignant B cells of DLBCL, NOS express the pan B-cell markers CD19, CD20, CD22, CD79a, and PAX5 in the vast majority of cases. Although lack of CD20 expression is rarely encountered in pretreatment cases, and CD20 may be lost in cases occurring following rituximab therapy. Beyond this DLBCL, NOS is an immunophenotypically heterogeneous disease. CD10 is expressed in 25% to 50% of cases, BCL6 is expressed 50% to 90% of cases, and MUM1 is expressed in 35% to 65% of cases. Surface immunoglobulin (Ig) is expressed in most cases, but in approximately 10% of cases it is undetectable by flow cytometry. Other variably expressed antigens include CD30 (10% to 40% of cases), CD5 (<10% of cases), and cyclin D1 (~2% of cases with weak and focal staining). Expression of Ki-67 is highly variable and can range from 30% to more than 95%.

Gene expression profiling (GEP) has shown that DLBCL, NOS can be classified into prognostically significant subtypes based on the cell of origin (COO)—namely, germinal center B-cell type (GCB) and activated B-cell (ABC) type—with the ABC-type DLBCL, NOS exhibiting a different biology and worse overall response to cyclophosphamide, doxorubicin, vincristine, and prednisone plus rituximab (R-CHOP) therapy compared to the GCB type. For this reason the current WHO classification requires the identification of the COO subtype.

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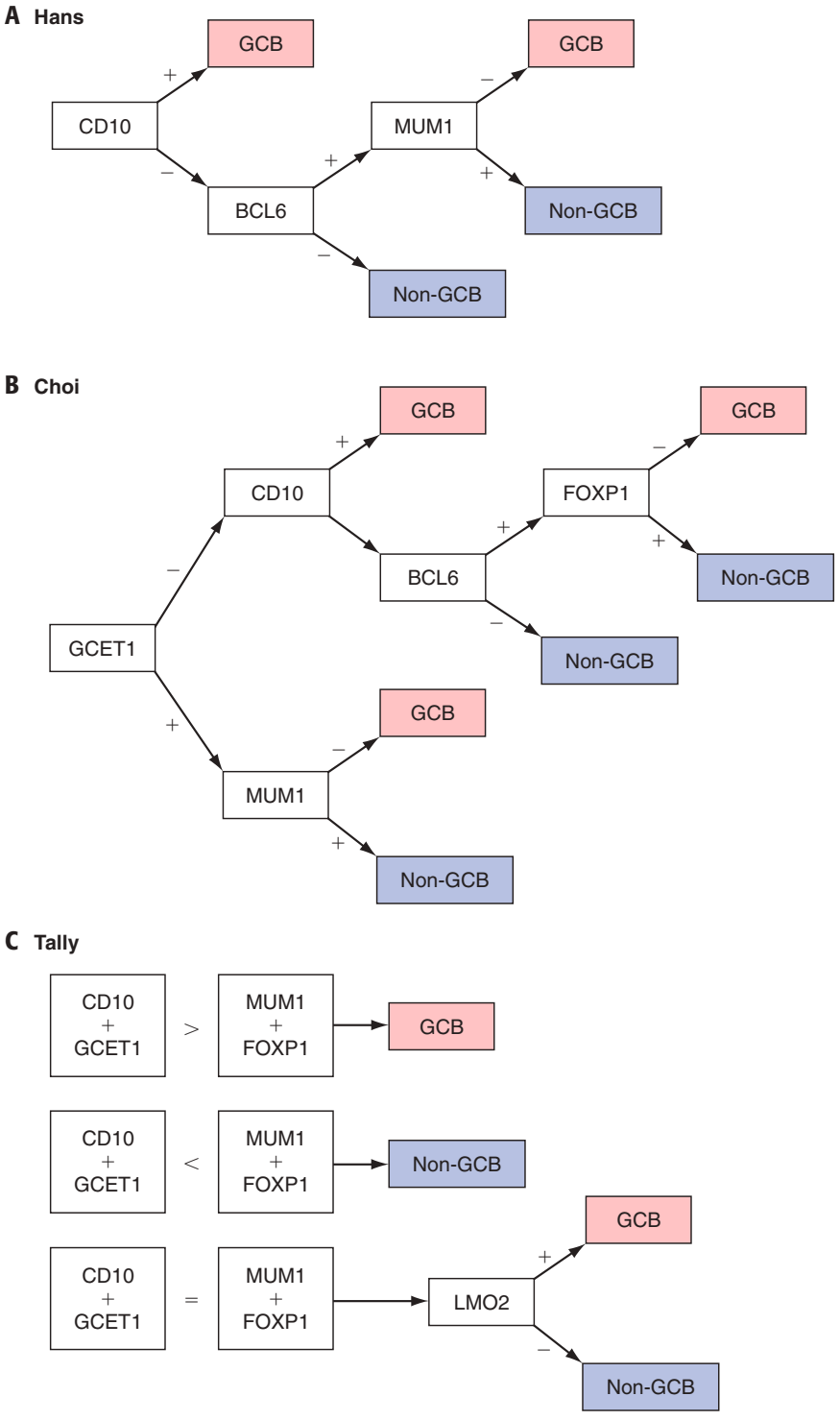


FIG. 8.4

Algorithms for immunohistochemical discrimination between germinal center B-cell type and activated B-cell/non–germinal center B-cell type in diffuse large B-cell lymphoma, NOS. A, Hans algorithm. Positivity is defined as ≥30% positive cells. B, Choi algorithm. Positivity for CD10 and BCL6 is defined as ≥30% positive cells. Positivity for MUM1, GCET1, and FOXP1 is defined as ≥80% positive cells. C, Tally algorithm. Positivity for CD10, GCET1, MUM1, FOXP1, and LMO2 is defined as ≥30% positive cells. Positive stains are assigned a score of 1, negative stains a score of 0. *(A, From Hans CP, Weisenburger DD, Greiner TC, et al: Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using tissue microarray.* Blood *103:275–282, 2004. B, From Choi WWL, Weisenburger DD, Greiner TC, et al: A new algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy.* Clin Cancer Res *15:5494–5502, 2009. C, From Meyer PN, Fu K, Greiner TC. et al: Immunohistochemical methods for predicting cell of origin and survival in patients with diffuse large B-cell lymphoma treated with rituximab.* J Clin Oncol *29:200–207, 2011.)*

Until recently GEP has required the use of fresh frozen tissue and has not been widely used in clinical laboratories. However, algorithms based on immunohistochemical (IHC) staining have been devised that can be routinely performed and can differentiate the COO subtypes of DLBCL, NOS with close concordance to the GEP classification scheme. The Hans algorithm uses the expression pattern of CD10, BCL6, and MUM1 and has approximately 80% to 86% concordance with the GEP classification. The Choi algorithm uses the expression pattern of CD10, BCL6, MUM1, GCET1, and FOXP1 and has approximately 93% concordance with the GEP classification (Fig. 8.4). Another approach referred to as the tally algorithm uses a combination of IHC antibodies but without regard to order and shows the highest concordance with GEP. Germinal center markers (CD10 and GCET1) and activated B-cell markers (MUM1 and FOXP1) are scored

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(≥30% of cells), and cases are classified based on whether more germinal center or ABC markers are present. In case of a tie, LMO2 is used to determine subtype (≥30% + Cells = GCB; <30% + Cells = non-GCB).

MYC and BCL2 are two other proteins that are also variably expressed in DLBCL, NOS and approximately 25% to 30% of cases express both when using cut-offs of ≥40% and ≥50% for MYC and BCL2, respectively. These so-called dual expressers (also sometimes referred to as "double expressers") are enriched in the ABC type and are associated with worse response to R-CHOP therapy, independent of the IPI, COO type, and underlying *MYC* and *BCL2* gene rearrangements. Dual expression of MYC and BCL2 also defines a group of patients at high risk of central nervous system relapse. Importantly, dual expression of MYC and BCL2 is not a surrogate marker for dual rearrangement of the *MYC* and *BCL2* genes (so-called double-hit lymphoma; see discussion below). Molecular genetic testing by fluorescence in situ hybridization (FISH) analysis is required to exclude underlying gene rearrangements of the *MYC* and *BCL2* genes.

Molecular Genetics

Because DLBCLs are monoclonal B-cell neoplasms, Ig genes are usually clonally rearranged whereas T-cell receptor genes are typically in the germline configuration.

Our understanding of the molecular heterogeneity of DLBCL, NOS has been advanced by gene expression profiling. DLBCL, NOS can be segregated into at least three subgroups based on similarities to normal cell types: GCB (45% to 55%), ABC (35% to 45%), and the unclassifiable "Type 3" group (10%), which shows a probability of less than 90% based on gene expression of belonging to either GCB or ABC subtypes (Fig. 8.5). The IHC classifiers use binary GCB versus ABC/non-GCB categories only, and thus the unclassifiable cases may be variably distributed within these two groups, making these algorithms less biologically meaningful. However, GEP has recently become possible in formalin-fixed paraffin-embedded (FFPE) tissue specimens, and assays, such as Lymph2Cx, have been developed to determine COO in DLBLC, NOS using FFPE with near equivalent concordance to the fresh frozen tissue–based assays. These FFPE-based assays are likely to find their way into routine clinical practice in the near future, particularly if the results of ongoing clinical trials with targeted drugs indicate that therapeutic decisions should be made based on COO. Mini-gene predictors, using two to six genes, have also been described that appear to be independent of the clinical IPI variables.

The cell-of-origin distinctions also have underpinnings in biology, with molecular genetics and next-generation sequencing having been helpful in furthering our understanding of the molecular alterations underlying these disease subtypes. GCB-type DLBCL harbors rearrangements of *BCL2* exclusively, usually in the form of a t(14;18)(q32q21) (*IGH-BCL2*), which is seen in approximately 35% to 45% of cases. *MYC* rearrangements are also largely restricted to the GCB-type occurring in approximately 10% of all DLBCL, NOS cases. Co-occurrence of *BCL2* and *MYC* rearrangements is seen in approximately 5% of all DLBCL cases, called double-hit lymphoma, and is associated with aggressive behavior. DLBCL cases that harbor a double-hit abnormality are re-classified as high-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements (discussed later); thus laboratories must have a strategy to find these cases. Some have elected to preform FISH testing on all cases of diffuse aggressive B-cell lymphomas, whereas others have elected screening strategies that involve MYC IHC. GCB-type DLBCL is also enriched with recurrent mutations or deletions in genes associated with epigenetic regulation (*EZH2*, *CREBBP*, *EP300*, *MLL2*, *MLL3*, *MEF2B*, and *TET2*). Recurrent mutations in genes associated with B-cell receptor, Toll-like receptor, and NF-κB signaling pathways (*CARD11, CD79A*, *CD79B*, *MYD88, TNFPAI3*, and *NFKBIE*) are also seen in a minority of cases. In addition, recurrent mutations in DNA repair/genomic integrity pathways are also identified (*TP53*) in 10% to 20% of cases. The *EZH2* mutation is exclusive to GCB-type DLBCL, NOS and is found in approximately 20% of cases. It is a gain-of-function mutation that leads to increased expression of the EZH2 protein, which is responsible for methylation of histone H3 at lysine 27 leading to transcriptional repression and gene silencing. ABC-type DLBCL is enriched with 3q27/*BCL6* gene rearrangements as well as gains/amplifications of *BCL2* and *MYC*; double-hit lymphoma is rare in this type of DLBCL. ABC-type DLBCL, NOS is enriched with recurrent mutations or deletions in genes associated with Toll-like receptor signaling, NF-κB signaling, and B-cell receptor signaling pathways (*MYD88*, *TNFPAI3*, *CD79A*, *CD79B*, *CARD11,* and *NFKBIE*), although recurrent mutations and deletions in genes related to epigenetic modification (*CREBBP*, *TET2*, *EP300*, *MLL2*, and *MLL3*) are occasionally observed. Recurrent mutations in DNA repair/genomic integrity pathways are also identified (*TP53*) in 10% to 25% of cases. *PRDM1/BLIMP1* mutations are seen exclusively in ABC-type DLBCL, NOS and are found in approximately 25% of cases. It is a loss-of-function mutation that prevents terminal differentiation of the malignant B cells.

In aggregate, the survival data and molecular genetic findings underscore the rationale behind distinguishing DLBCL, NOS cases based on cell of origin. Constitutive alterations in specific pathways in these subtypes, such as epigenetic regulation and NF-κB signaling in GCB and ABC-type lymphomas, respectively, support the potential effects of specific biological therapies for patients with GCB versus ABC-type DLBCLs.

Differential Diagnosis

The differential diagnosis of DLBCL, NOS includes other hematopoietic neoplasms of intermediate to large cells, including T-cell lymphomas, Burkitt lymphoma (BL), blastoid and pleomorphic variants of mantle cell lymphoma, lymphoblastic lymphoma, and myeloid sarcoma. Usually these neoplasms

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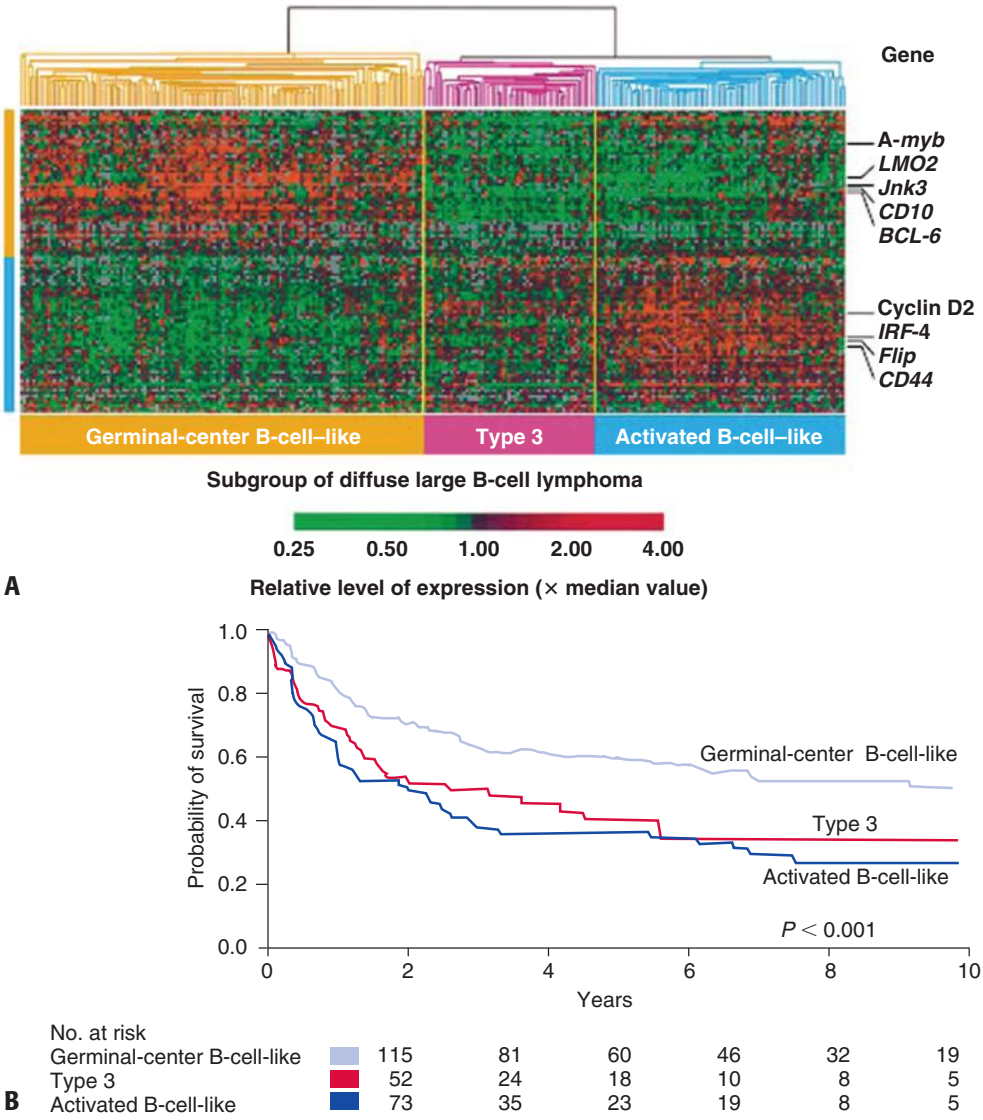


FIG. 8.5

Classification of diffuse large B-cell lymphoma, NOS. A, Diffuse large B-cell lymphoma (DLBCL) gene expression profiling showed cases can be subclassified based on their differential expression of a specific set of genes. B, This type of subclassification has prognostic significance with germinal center types of DLBCLs having a favorable prognosis independent of the International Prognostic Index.

can be easily distinguished from DLBCL, NOS by routine immunophenotyping. T-cell lymphomas can be identified because of a lack of B-cell markers and the expression of T-cell–associated antigens such as CD2, CD3, CD5, and CD7 by the malignant cells. BL generally has a high mitotic index with a starry-sky pattern and is composed of medium-sized cells with multiple small nucleoli. *MYC* translocations are virtually always present in BL; however, this finding is not specific because *MYC* translocation can be seen as a primary or secondary event in DLBCL, NOS. Importantly, BL cells have a characteristic immunophenotype with expression of CD20, CD10, and BCL6, an absence of BCL2 expression, and a high proliferative rate in nearly all cases. Therefore consideration of morphologic, immunophenotypic, and molecular genetic features is required for accurate diagnosis.

Blastoid and pleomorphic mantle cell lymphoma can resemble DLBCL, NOS but will typically express CD5 and cyclin D1, with the latter in a strong and uniform pattern not seen in DLBCL, NOS. Lymphoblastic lymphoma cells have a fine, blastic chromatin pattern unlike the more vesicular chromatin of DLBCL, NOS, and the neoplastic cells will usually be positive for terminal deoxynucleotidyl transferase (TdT) or CD34, or both. Myeloid sarcoma will lack B-cell markers and express myeloid markers such as CD13, CD33, lysozyme, myeloperoxidase, or CD68. Pleomorphic examples of DLBCL may mimic other anaplastic neoplasms (carcinomas, including small-cell carcinoma, melanomas, or sarcomas). Expression of B-cell markers and lack of markers expected in these other nonlymphoid malignancies, such as keratins or melanoma-associated antigens, will aid in correct diagnosis.

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TABLE 8.1
International Prognostic Index

Prognostic Factors	Risk Score[a]		5-Year OS (mo.)
Age (>60)	Low	0, 1	73
LDH (nl)	Low Int	2	51
Performance status	High Int	3	43
Stage (III, IV)	High	4, 5	26
Extranodal (>1 site)			

[a]The risk score is the sum of the number of prognostic factors (0-5).

Prognosis and Therapy

The current standard-of-care treatment for DLBCL, NOS combines the multiple-agent chemotherapy regimen CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) with humanized anti-CD20 monoclonal antibody, rituximab (R-CHOP). With this type of therapy, patients can be cured in approximately 60% to 70% of cases, representing a significant improvement over CHOP alone. Prognosis of patients can also be stratified by the five clinical factors of the IPI score (Table 8.1). Segregation of DLBCL, NOS into GCB and ABC types provides further prognostic information, as does identifying the presence of dual MYC and BCL2 protein expression. Identification of these additional prognostic factors may help determine alternative therapies in the future.

T-CELL/HISTIOCYTE-RICH LARGE B-CELL LYMPHOMA

T-CELL/HISTIOCYTE-RICH LARGE B-CELL LYMPHOMA—FACT SHEET

Clinical Features
- Middle-aged adults, males > females
- Lymph node, spleen, liver, and bone marrow involvement common

Morphology
- Large, atypical lymphocytes rare (<10% of cells)
- Numerous small lymphocytes and histiocytes (>90% of cells)
- Diffuse pattern of infiltration

Immunophenotype
- Large, atypical lymphocytes: CD45+, CD20+ B cells
- Background small lymphocytes: CD3+ T cells

Molecular Genetics
- Gains of 2p11, 4q13, 18q21, Xq, Xp21
- Loss of 9p11, 17p

Treatment/Outcome
- Anthracycline-containing multi-agent chemotherapy + rituximab
- 45% to 75% 5-year survival

Differential Diagnosis
- Classic Hodgkin lymphoma, lymphocyte-predominant Hodgkin lymphoma, peripheral T-cell lymphoma

Clinical Features

T-cell/histiocyte-rich LBCL (TC/HRBCL) is primarily a disease of young to middle-aged adults, with a median age of onset of 30 years; it affects men more often than women. Patients usually present with B symptoms, advanced stage disease (III and IV), and a high IPI score. Extranodal disease is common, with the spleen, liver, and bone marrow being the most commonly involved extranodal sites.

Pathologic Features

Histopathology

Lymph nodes involved by TC/HRBCL show architectural effacement by a diffuse or vaguely nodular cellular infiltrate consisting predominantly (>90%) of small T cells and non-epithelioid histiocytes. Malignant cells are scattered singly among the cellular background, although in some cases they may cluster. They are large, highly atypical cells that are often multilobated with variably prominent nucleoli (Fig. 8.6). In some cases the cells can resemble the lymphocyte-predominant (LP) cells characteristic of nodular lymphocyte predominance Hodgkin lymphoma (NLPHL) or Hodgkin and Reed-Sternberg cells characteristic of classic Hodgkin lymphoma (HL). Some cases may have cells that have more conventional immunoblastic or centroblastic features.

Splenic involvement occurs in a multifocal and micronodular pattern with expansion of the white pulp by an infiltrate of cells similar in composition to that described in the lymph node. The red pulp is not usually involved. Within the liver the lymphomatous infiltrate is confined principally to the portal tracts. Bone marrow involvement in TC/HRBCL occurs at a significantly higher rate than de novo DLBCL, NOS (60%). Involvement is often paratrabecular with a polymorphous infiltrate of small T cells, histiocytes, and scattered large atypical CD20+ B cells. Small B cells are typically scarce.

Immunophenotype

As noted, most cells in the infiltrate are reactive CD3+ T cells or CD68+ histiocytes. Only occasional CD20+ large B cells should be seen, and small CD20+ B cells should be

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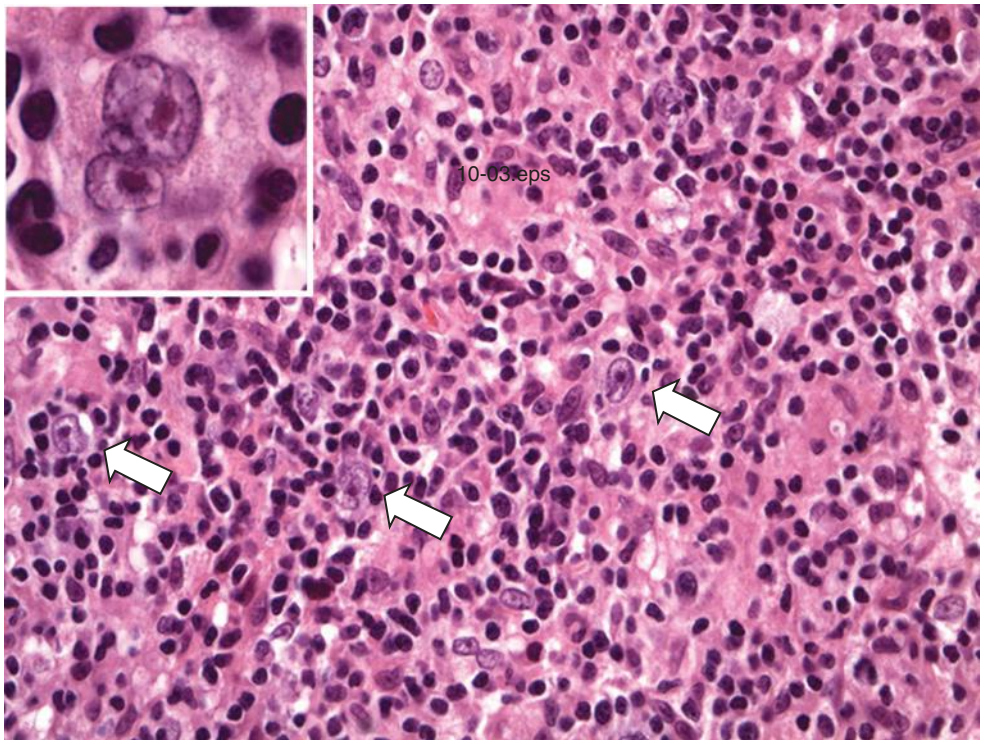


FIG. 8.6

T-cell/histiocyte-rich large B-cell lymphoma. Low magnification shows predominance of small lymphocytes, scattered histiocytes, and occasional atypical large cells that represent the malignant cells *(arrows)*. The *inset* shows one of these atypical lobulated cells.

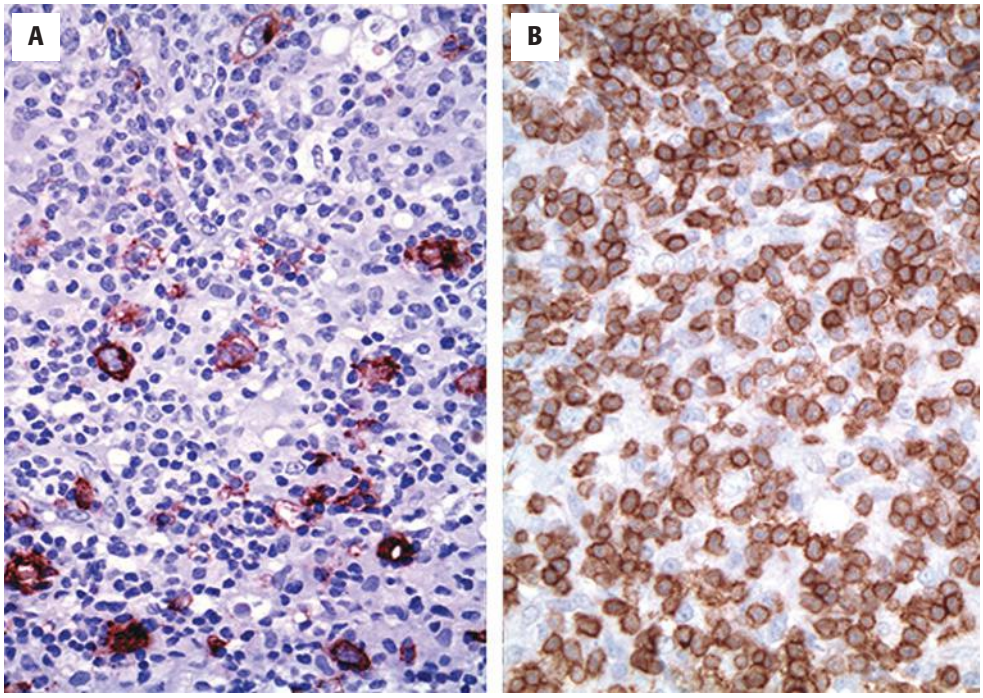


FIG. 8.7

T-cell/histiocyte-rich large B-cell lymphoma. Immunostains for CD20 (A) and CD3 (B) show that these large cells are CD20+ B cells. The small lymphocytes are reactive T cells.

absent or only rarely seen (Fig. 8.7). The immunophenotype of the malignant cells is somewhat heterogeneous. The malignant cells are positive for CD45 and the pan B-cell markers CD20, CD79a, and PAX5; however, expression of CD10, BCL6, BCL2, and EMA is variable. Some have suggested, based on BCL6 expression, that a germinal center origin can be entertained, but others could not replicate these findings. It is likely that TC/HRBCL, as currently described, is heterogeneous, in part because of a lack of precise diagnostic criteria. Many believe that a subset of cases within the category of TC/HRBCL might define a specific entity, closely related to NLPHL. Evidence in support of this theory includes the occurrence of the two lymphomas in the same patient and an identical immunophenotype of large B cells in both (CD20+, CD79a+, BCL6+, EMA+, and CD75+). EBV is absent, as are follicular dendritic cell meshworks when using

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stains for CD21, CD23, or CD35. Background T cells are typically CD8+ and express cytotoxic markers, including TIA1, granzyme B, and perforin. The distinguishing features between TC/HRBCL and NLPHL rely primarily on the surrounding non-neoplastic microenvironment.

Molecular Genetics

Immunoglobulin genes are clonally rearranged and somatically hypermutated in the majority of cases suggestive of a germinal center B-cell stage of differentiation. Few studies have investigated the molecular genetics landscape of TC/HRBCL, but there appears to be an average of approximately five genetic abnormalities per tumor, with reported abnormalities including gains of 2p11, 4q13, 18q21, Xq, and Xp21 and losses of 9p11 and 17p.

Differential Diagnosis

TC/HRBCL must be distinguished from NLPHL, classic HL, and peripheral T-cell lymphomas. In differentiating NLPHL from TC/RBCL, a diffuse pattern and lack of a follicular dendritic cell meshworks supports a diagnosis of TC/HRBCL over NLPHL. Furthermore, NLPHL contains large numbers of small non-neoplastic IgD+ B cells and increased numbers of CD57+, PD1+ T cells within the nodules; these are not features of TC/HRBCL. Strong expression of CD20 and CD45 in the atypical large cells with lack of CD15 helps to exclude classic HL and T-cell lymphoma.

Prognosis and Therapy

T-cell/HRBCL is an aggressive lymphoma. Treatment is usually with a multiple-agent anthracycline-containing regimen (CHOP) plus rituximab. Five-year overall survival rates with CHOP alone are reported to be in the range of 45% to 58%, similar to DLBCL, NOS. The effect of adding rituximab is not well established, but one small study has identified a similar 3-year overall survival to DLBCL, NOS. Distinguishing TC/HRBCL from NLPHL and classic HL is important because therapies for HL do not adequately treat TC/HRBCL.

PRIMARY DIFFUSE LARGE B-CELL LYMPHOMA OF THE CENTRAL NERVOUS SYSTEM—FACT SHEET

Clinical Features
- Rare tumor but most common type of primary CNS lymphoma
- Supratentorial location most common
- Median age at diagnosis 55 years
- Presenting symptoms include mental status changes, headache, focal neurologic deficit

Morphology
- Sheets of large centroblastic or immunoblastic cells
- Perivascular distribution

Immunophenotype
- CD20+, BCL2+/–, BCL6–/+, MUM1+, MYC+/–, EBV–

Molecular Genetics
- Rearranged immunoglobulin genes
- del6q21-22 and *BCL6* rearrangement
- *MYD88*, *TBL1XR1*, *CD79B* mutations

Treatment/Outcome
- Multi-agent therapy with high-dose methotrexate and radiation
- Aggressive lymphoma, median survival approximately 3 years (age >60 is poor prognostic factor)

Differential Diagnosis
- Burkitt lymphoma, metastatic carcinoma, melanoma, inflammatory reaction (encephalitis)

PRIMARY DIFFUSE LARGE B-CELL LYMPHOMA OF THE CENTRAL NERVOUS SYSTEM

Clinical Features

Primary DLBCL of the central nervous system (PCNSL) is a rare tumor accounting for 1% of non-Hodgkin lymphomas and 5% of brain tumors. The most common type of primary central nervous system (CNS) lymphoma, it encompasses all cases of DLBCL that arise in the brain, spinal cord, meninges, or eye parenchyma. DLBCL occurring in the CNS in the setting of immunodeficiency is not included in this diagnostic category. The median age of onset is 55 years, with a slight male predominance. Patients may have headache, mental status changes, or focal neurologic symptoms related to mass effect at the site of involvement. In one series, 81% of cases occurred in a supratentorial location, 7% infratentorially, and 12% in both locations. Because this neoplasm grows rapidly, the duration of signs and symptoms is on the order of weeks.

Pathologic Features

Histopathology

The tumor resembles other DLBCLs cytologically. In a series of 62 PCNSL cases in immunocompetent patients, 87% were centroblastic and 13% were immunoblastic. The pattern of involvement is diffuse with a propensity for perivascular involvement (Fig. 8.8). A diffuse pattern is more

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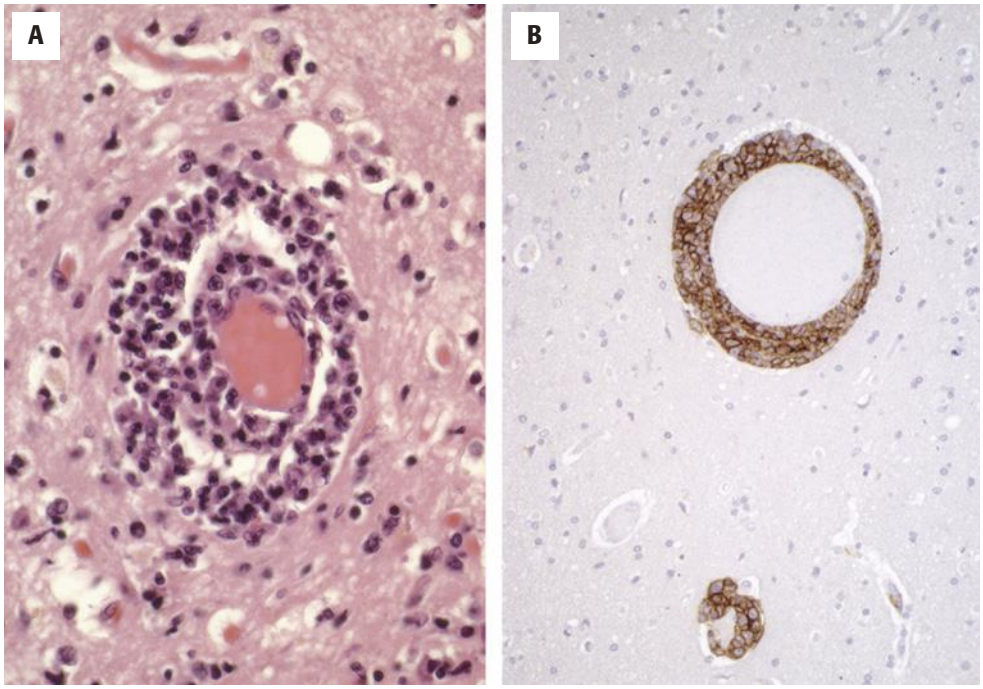


FIG. 8.8
Primary diffuse large B-cell lymphoma of the CNS. A, Lymphoma cells often show a perivascular distribution. B, CD20 stain confirms B-cell lineage.

readily seen when a large mass is biopsied; perivascular involvement may be the only pattern seen in smaller stereotactic biopsy specimens. Necrosis and surrounding gliosis are commonly associated features.

Immunophenotype

The lymphoma cells express pan B-cell markers CD19, CD20, CD79a, and PAX5. CD10 is expressed in less than 20% of cases, BCL6 in 60% to 80% of cases, and MUM1 in more than 90% of cases. The reported expression of BCL2 is variable but is seen in at least 50% of cases, with dual expression of MYC and BCL2 identified in upward of 80% of cases in some series. Using the Hans algorithm, approximately 80% to 95% of cases are classified as ABC/non-GCB type; this is due to the frequent expression of MUM1. Because many cases co-express BCL6, it has been suggested that this tumor is derived from a B cell in the late germinal center to early post-germinal center stage of development. EBV is usually absent.

Molecular Genetics

Monoclonal immunoglobulin gene rearrangement is present. There are no specific diagnostic molecular genetic abnormalities identified in PCNSL, but abnormalities of 1q21, 6q, 7q, and 14q are reported. Comparative genomic hybridization studies have shown that gains outnumber losses, with chromosome 12q showing the most frequent gains (63%). The most commonly deleted locus is located at chromosome 6q (47%), with 6q21-22 being the commonly deleted region. Rearrangement of the *BCL6* gene is also seen in a significant minority of cases (23% to 37%). Gene expression profiling shows that PCNSL is a tumor of late germinal center B cells, and its transcriptional signature closely resembles that of systemic DLBCL, NOS. Using an integrative analysis combining GEP and high-resolution array comparative genomic hybridization, it has been shown that 65% of PCNSLs harbor deletions of 9p21.3 involving *CDKN2A* as well as a number of previously unrecognized deletions associated with diminished expression of the candidate genes. Several recent studies have identified highly recurrent somatic mutations affecting the NF-κB pathway in the majority of PCNSL, including mutations in *MYD88*, *TBL1XR1*, and *CD79B*. These mutations occur with greater frequency than is seen in DLBCL, NOS, likely reflecting the paucity of antigen stimuli from the surrounding immune-privileged environment and serving as a genetic hallmark for this disease.

Differential Diagnosis

The differential diagnosis is similar to that of DLBCL, NOS at any site. Given the perivascular arrangement of lymphoid cells, inflammatory conditions such as encephalitis should be considered. However, the cytologic atypia and predominance of B cells help differentiate PCNSL from inflammatory conditions. EBV studies are helpful for distinguishing PCNSL from lymphomatoid granulomatosis involving the brain.

Prognosis and Therapy

Therapy is generally multiple-agent therapy with high-dose methotrexate combined with radiotherapy. The latter is

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generally withheld in older patients because of unacceptable toxicity. This lymphoma is aggressive, with a median overall survival of 37 months. An important prognostic factor is age. Patients older than 60 years fare substantially worse than those younger than 60 years. Deletion of chromosome 6q21-22 and rearrangement of *BCL6* have also been shown to correlate with a decreased overall survival.

PRIMARY CUTANEOUS DIFFUSE LARGE B-CELL LYMPHOMA, LEG TYPE

Clinical Features

Primary cutaneous DLBCL, leg type, is primarily a disease of elderly adults (median age, 77 years) with a female predominance. It usually manifests with localized disease of the skin with a single or multiple, localized, rapidly growing erythematous or violaceous plaques or nodules, often with ulceration. The lower leg is the most frequent site of involvement, but other sites can be involved as well. Dissemination to nodal or extranodal sites is common with disease progression.

PRIMARY CUTANEOUS DIFFUSE LARGE B-CELL LYMPHOMA, LEG TYPE—FACT SHEET

Clinical Features
- Presents most commonly in elderly, females > males
- Rapidly enlarging mass on the lower extremities

Morphology
- Diffuse sheets of large centroblasts and immunoblasts
- Absent or minimal stromal response

Immunophenotype
- CD20+, CD10–, BCL6+, MUM1+, FOXP1+, BCL2+, cIgM, p63

Molecular Genetics
- Rearranged immunoglobulin genes
- Amplification of 18q21 (*BCL2* and *MALT1*)
- Deletion of 9p21 (*CDKN2A*)
- Translocation of *BCL6, MYC,* and/or *IGH* present in many cases
- *MYD88* mutation

Treatment/Outcome
- Aggressive lymphoma with 50% 5-year survival
- Treatment with anthracycline-containing multi-agent chemotherapy ± radiation

Differential Diagnosis
- Secondary involvement in skin of systemic DLBCL, NOS
- Cutaneous lymphoblastic lymphoma

Pathologic Features

Histopathology

The tumor infiltrates the dermis and hypodermis and is composed of diffuse sheets of medium to large centroblasts and immunoblasts without admixed small centrocytes (Fig. 8.9). The cells have a characteristic round nuclear shape. There is little stromal reaction, and few infiltrating, small non-neoplastic lymphocytes are present. Mitoses are easily seen. The epidermis is uninvolved in most cases and separated from the tumor by a Grenz zone.

Immunophenotype

The tumor is positive for the pan B-cell markers CD20, CD79a, and PAX5. Most cases (>85%) express BCL2, MUM1, and FOXP1 and lack expression of CD10. BCL6 expression is seen in a variable number of cases. p63 is also variably expressed but seen in most cases. Cytoplasmic IgM with or without coexpression of IgD is reported in virtually all cases. EBV is negative. Follicular dendritic cell meshworks are absent.

Molecular Genetics

Monoclonal *Ig* rearrangement is present. The tumor lacks t(14;18); however, translocations involving *BCL6*, *MYC,* and *IGH* are common. Recurring genetic abnormalities include amplification of chromosome 18q21, where the *BCL2* and *MALT1* genes are located; deletion of chromosome 9p21.3, where the *CDKN2A* gene is located; deletion of 6q, where the *BLIMP1* gene is located; and recurrent somatic mutation of *MYD88*, which is seen in approximately 60% to 70% of cases. Amplification of *BCL2* is believed to be the mechanism responsible for the frequent expression of BCL2 protein seen in this tumor. Gene expression profiling shows that the tumor is similar to activated B-cell–like DLBCL, NOS.

Differential Diagnosis

The main differential diagnosis of DLBCL, leg type, is cutaneous involvement by systemic DLBCL, NOS. This can be excluded only by careful staging procedures and knowledge of the clinical history. Another B-cell lymphoma that must be excluded is B-cell lineage lymphoblastic lymphoma. Cutaneous lymphoblastic lymphoma is rare. Unlike primary cutaneous DLBCL, leg type, lymphoblastic lymphomas usually express CD10 and TdT. Primary cutaneous follicle center lymphoma (described in Chapter 7) may be composed of large cells, but they contain centrocytic cells, often lack BCL2, express germinal center markers such as CD10, and uncommonly express cytoplasmic IgM and p63.

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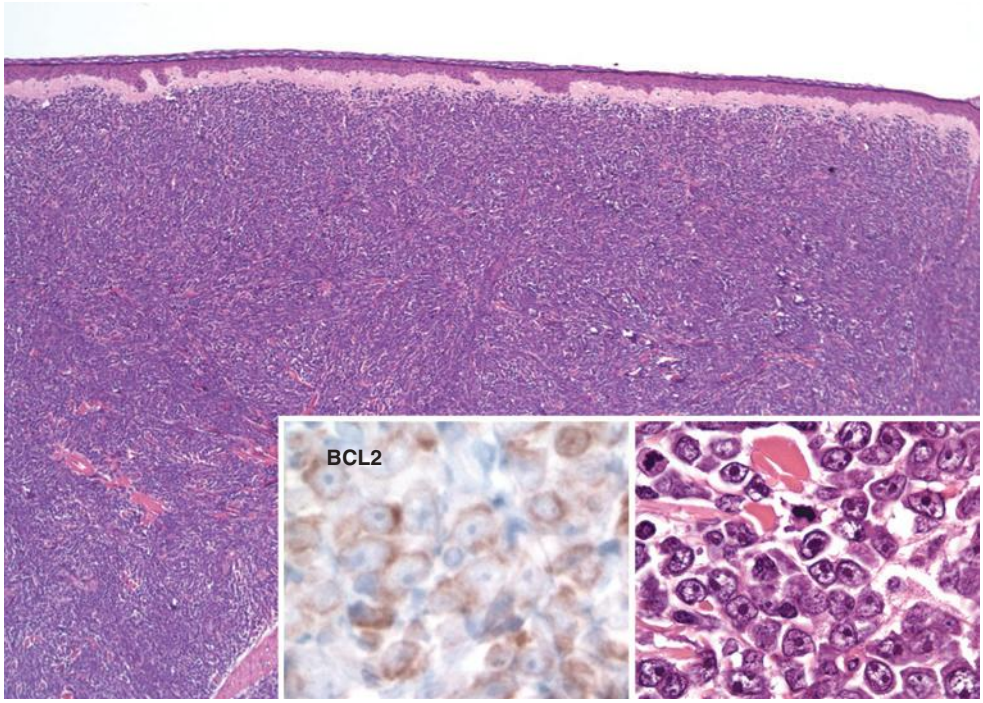


FIG. 8.9

Primary cutaneous diffuse large B-cell lymphoma, leg type. Low magnification shows a diffuse infiltrate of atypical large cells that express CD20. The *inset* in the lower right corner shows a predominance of large, round immunoblastic cells. Expression of BCL2 is characteristic.

Prognosis and Therapy

Systemic anthracycline-containing multiple-agent immunochemotherapy with the selective addition of radiotherapy is the preferred type of treatment for this lymphoma. Primary cutaneous DLBCL, leg type, has an intermediate clinical course with a 50% 5-year survival. Adverse prognostic factors include advanced age, multiple skin lesions, and disease localized to the leg. Some authors have suggested that it is the expression of BCL2 protein, a known poor prognostic indicator in other lymphomas, along with other clinical factors that contribute to the poor outcome of this lymphoma, rather than location on the leg. Other studies have shown that deletion of 9p21.3, methylation of the promoter region of *CDKN2A*, and *MYD88* mutation are associated with an unfavorable prognosis.

Epstein-Barr Virus–Positive Diffuse Large B-Cell Lymphoma, Not Otherwise Specified

Clinical Features

Epstein-Barr virus (EBV)–positive diffuse large B-cell lymphoma, not otherwise specified (EBV+ DLBCL, NOS) is a monoclonal counterpart of a related group of diseases collectively known as *EBV-positive B-cell lymphoproliferative disorders* that does not meet the criteria for a diagnosis of a more specific EBV+ disease entity. It is seen most commonly in Southeast Asia, particularly Japan, and Latin America, and it is less commonly seen in Western countries. The disease is thought to result from age-associated immune senescence and affects patients without known immunodeficiency or previous history of an EBV-associated lymphoproliferative disorder (i.e., DLBCL associated with chronic inflammation, lymphomatoid granulomatosis, primary effusion lymphoma, or plasmablastic lymphoma). EBV positivity in DLBCL is proportionately more common with increasing age, reaching a peak after the age of 90 years. Although peak incidence occurs in the eighth decade, cases occurring in patients younger than 50 years of age have been increasingly recognized. Hence, the prior nomenclature of "EBV + diffuse large B-cell lymphoma of the elderly" has been dropped in favor of the current name. It is important to exclude other causes of underlying immunodeficiency before making this diagnosis.

EBV+ DLBCL, NOS affects men slightly more frequently than women and appears in lymph nodes and at extranodal sites, including the skin, gastrointestinal tract, lung, and tonsil. Greater than 50% of patients exhibit B symptoms, elevated low-density lipoprotein (LDH), and advanced-stage disease, although solid organ and bone marrow involvement is relatively infrequent.

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EBV-POSITIVE DIFFUSE LARGE B-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED—FACT SHEET

Clinical Features
- Typically affects patients >50 years of age with no history of immunosuppression but can be seen in younger patients
- Usually presents with B symptoms and advanced stage
- Nodal and extranodal disease common; solid organs and bone marrow not usually involved
- Rare in Western countries; more common in Southeast Asian and Latin American populations

Morphology
- Polymorphic or monomorphic infiltrate of immunoblast-like transformed cells
- Hodgkin and Reed-Sternberg–like cells
- Mixed inflammatory cells in polymorphic infiltrate
- Can resemble TC/HRLBCL in younger patients

Immunophenotype
- CD20+, CD79a+, CD10–, BCL6–, MUM1+, CD30±, CD15–, EBV+

Molecular Genetics
- Clonally rearranged *IGH* gene
- Clonal EBV genome
- NF-κB and JAK/STAT pathway activation
- Gains of 9p24 (PD-L2)

Treatment/Outcome
- Combination immunochemotherapy (CHOP-R) ± radiation
- 2-year median survival in older patients; younger patients, better prognosis

Differential Diagnosis
- Infectious mononucleosis
- Classic Hodgkin lymphoma
- EBV+ mucocutaneous ulcer

Pathologic Features

Histopathology

Epstein-Barr–positive DLBCL, NOS is morphologically heterogeneous. It can be divided into polymorphous and large-cell lymphoma subtypes; however, these are of no clinical significance, and usually both morphologies can be seen in the same specimen when examined thoroughly. In all cases, the tumor consists of a polymorphic or monomorphic infiltrate of immunoblast-like transformed cells and Hodgkin and Reed-Sternberg (HRS)-like cells that efface normal architecture (Fig. 8.10). Inflammatory cells, including small reactive lymphocytes, plasma cells, histiocytes, and epithelioid cells, are admixed in polymorphic areas. Foci of geographic necrosis are also common and reminiscent of posttransplant lymphoproliferative disorder. In younger patients, cases often resemble T-cell/histiocyte-rich large B-cell lymphoma.

Immunophenotype

The malignant cells in EBV+ DLBCL, NOS express the pan B-cell antigens CD20, CD79a, and PAX5 and exhibit a nongerminal center immunophenotype, being negative for CD10 and BCL6 and positive for MUM1. Approximately three fourths of cases express CD30, and this is more commonly seen in Southeast Asian and Latin cases. CD15 is universally absent. PD-L1 is frequently expressed. EBV is present in all cases, typically seen in more than 50% of malignant cells, and best detected by in situ hybridization for EBV-encoded RNA (EBER).

Molecular Genetics

Epstein-Barr virus–positive (EBV+) DLBCL, NOS is a monoclonal disorder, and a clonal rearrangement of the *IGH* gene is usually detectable. A clonal EBV genome is also usually identified. Rearrangements of *BCL2*, *BCL6*, and *MYC* are absent. GEP studies have identified enhanced activity of the NF-κB and JAK/STAT pathways within this disease; however, mutations affecting these pathways (*CD79B*, *CARD11*, *MYD88*) are only rarely detected, which is suggestive of EBV-mediated activation of these pathways. In addition, gains of 9p24 (*PD-L2*) have been identified, which promote immune evasion.

Differential Diagnosis

The main differential diagnosis includes infectious mononucleosis and EBV+ classic Hodgkin lymphoma (classic HL). Unlike EBV+ DLBCL, NOS, infectious mononucleosis shows preservation of lymphoid architecture with preferential expansion of the paracortex by a polymorphous cellular infiltrate. Zonal necrosis is uncommon, and a monoclonal B-cell population is not detected. The HRS cells of classic HL usually differ from the HRS-like cells of EBV+ DLBCL, NOS in that they do not express CD79a, usually lack CD20, and are positive for CD15 in most cases. In addition, reactive T cells with an activated immunophenotype (TIA1+/perforin+) are common in EBV+ DLBCL, NOS but infrequent in classic HL.

Prognosis and Therapy

Standard treatment of EBV+ DLBCL, NOS consists of combination immunochemotherapy, usually R-CHOP with selective use of radiotherapy. Younger patients appear to exhibit a better response to therapy than older patients, where it is an aggressive disease with a median survival of approximately 2 years.

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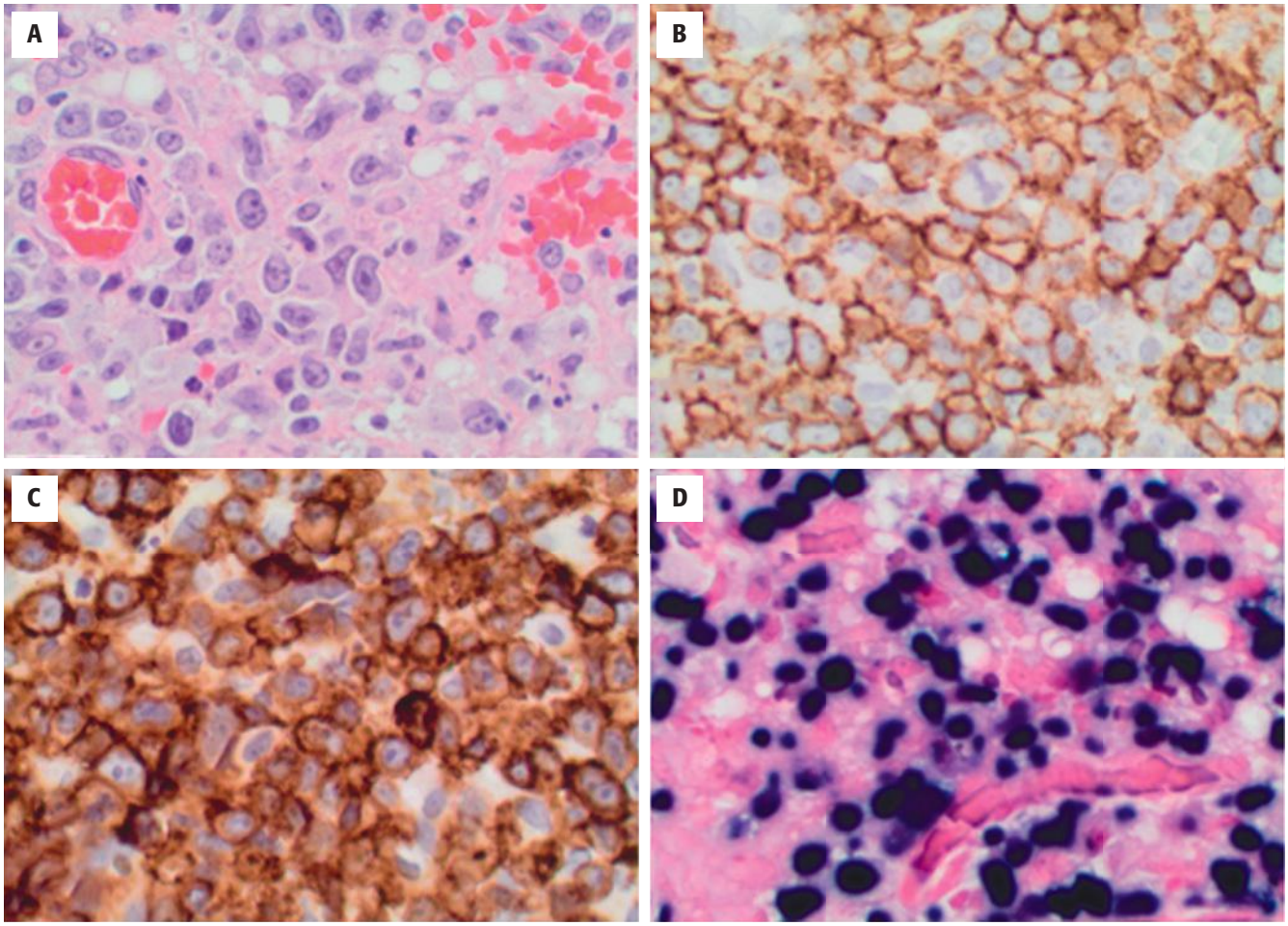


FIG. 8.10

EBV+ diffuse large B-cell lymphoma, NOS. A, The tumor is composed of a monomorphic proliferation of large, atypical lymphocytes with immunoblastic features. The lymphoma cells are positive for CD20 (B), CD30 (C), and frequently exhibit nuclear expression of EBV-encoded RNA (EBER) (D).

Indicators of poor prognosis include advanced age, the presence of B symptoms, advanced stage, multiple extranodal sites of involvement, an elevated LDH, and CD30 expression.

EPSTEIN-BARR VIRUS–POSITIVE MUCOCUTANEOUS ULCER

Clinical Features

Epstein-Barr virus–positive mucocutaneous ulcer (EBV+ MCU) is a disease of older people, presenting at a median age of 77 years. It affects females more than males and is associated with various types of immunosuppression, including age-related immune senescence as well as immunosuppressive therapy with agents such as azathioprine, methotrexate, and cyclosporine. It presents with localized and discrete mucocutaneous ulcers involving the oral cavity, skin, or gastrointestinal tract.

Pathologic Features

Histopathology

Involved tissue shows sharply demarcated surface ulceration with infiltration by a polymorphous cellular infiltrate composed of large atypical B cells, resembling immunoblasts or Reed-Sternberg cells, in a background of abundant small lymphocytes, plasma cells, and histiocytes. Lesions are usually well circumscribed with a rim of small lymphocytes at the base.

Immunophenotype

The atypical B cells show strong expression of CD30 and usually express the pan B-cell antigens CD20, CD79a, and PAX5, with some cases showing reduced expression of CD20. B cells also express CD15 in a minority of cases. Background small lymphocytes are CD3+ T cells and predominantly CD4+. EBV is positive in atypical B cells as well as some background

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EBV-POSITIVE MUCOCUTANEOUS ULCER—FACT SHEET

Clinical Features
- Affects older patients, females > males
- Prior immunosuppression or age-related immune senescence
- Presents with localized discreet mucocutaneous ulcers of oropharynx, skin, or GI tract

Morphology
- Surface ulceration with polymorphous cellular infiltrate
- Atypical lymphocytes with immunoblast or Reed-Sternberg–like features
- Admixed small lymphocytes, plasma cells, and histiocytes

Immunophenotype
- Atypical cells CD20+, CD30+, EBER+ B cells; variably positive for CD15
- Background lymphocytes are CD3+, CD4+ T cells

Molecular Genetics
- Rearranged immunoglobulin and T-cell receptor genes in a minority of cases

Treatment/Outcome
- Usually self-limited disease requiring only conservative management

Differential Diagnosis
- EBV+ diffuse large B-cell lymphoma, NOS
- EBV+ classic Hodgkin lymphoma

small lymphocytes. Thus, a range of EBV+ cells is typically seen.

Molecular Genetics

Epstein-Barr virus–positive MCU shows clonal rearrangements of immunoglobulin genes in approximately 40% of cases. Clonal T-cell populations are also identified in a minority of cases.

Differential Diagnosis

The main differential diagnosis includes EBV+ DLBCL, NOS and EBV+ classic Hodgkin lymphoma (cHL). cHL generally has a mixed inflammatory background with HRS cells exhibiting an immunohistochemical profile as expected, and its presence at a mucosal site would be highly unusual. The EBV in cHL is typically seen only in the HRS cells as opposed to a spectrum of cells as seen in EBV+ MCU. EBV+ DLBCL also does not usually present as ulcers and tends to be more monomorphic. Distinguishing EBV+ MCU from these two diseases requires close attention to the clinical features at presentation as well as the cytologic features and immunophenotype of the atypical B cells.

Prognosis and Therapy

Epstein-Barr virus–positive MCU is usually a self-limited disease with an indolent clinical course that responds well to conservative management. Spontaneous regression is seen in nearly half of patients, whereas a small proportion of patients experience a relapsing and remitting course. This latter group of patients may benefit from therapeutic intervention, including surgical excision, radiation therapy, chemotherapy, or immunotherapy.

DIFFUSE LARGE B-CELL LYMPHOMA ASSOCIATED WITH CHRONIC INFLAMMATION

Clinical Features

Diffuse large B-cell lymphoma associated with chronic inflammation is a rare lymphoma occurring at sites of long-standing chronic inflammation within body cavities and enclosed spaces. It is associated with latent EBV infection (type III latency) and is believed to arise as a result of acquired local immunosuppression within the inflammatory milieu.

The most commonly affected site is the pleural cavity (called *pyothorax-associated lymphoma* [PAL]). PAL usually occurs in the setting of an artificial pneumothorax created to treat pulmonary or pleural tuberculosis. It is seen most frequently in Japan, where artificial pneumothorax is common; it is seen only occasionally in Western countries. The delay in lymphoma development after the onset of inflammation is greater than 20 years. As such, PAL tends to be a disease of older adults, with the median age occurring in the seventh decade (61 to 70 years). Men are affected more frequently than women (>5 : 1 male-to-female ratio). Patients usually have a large pleural or lung-based mass, or both, with associated chest pain, respiratory symptoms, or hemoptysis. Fever, elevated LDH, and low-stage disease are also common.

Other affected sites include bone with osteomyelitis, periarticular soft tissue and joints with metallic implants, chronic skin ulcers, sites of surgical mesh implants, atrial myxomas, and cardiac prostheses. Lymphoma usually develops at these sites after at least 10 years of chronic inflammation. Patients complain of a mass or localized pain or both. Recently, DLBCL associated with chronic inflammation has been recognized in cystic and pseudocystic lesions, including hydroceles, and cysts of the ovary, kidney, adrenal gland, and spleen, further expanding the clinicopathologic spectrum of this disease.

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DLBCL ASSOCIATED WITH CHRONIC INFLAMMATION—FACT SHEET

Clinical Features
- Occurs in the setting of long-standing chronic inflammation in body cavities or enclosed spaces
- Pleural cavity most commonly affected (pyothorax-associated lymphoma)
- Predominantly older men (M:F >5:1)
- Fever, chest/back pain and respiratory symptoms
- Low-stage disease
- Other affected sites include bone, joints and skin, and cysts

Morphology
- Diffuse infiltrate of large immunoblastic or plasmacytic cells

Immunophenotype
- CD20+, CD79a+, PAX5+, CD10–, BCL6–, MUM1+, CD138–/+, CD30±, EBV+, HHV8–

Molecular Genetics
- Clonally rearranged *IGH* gene
- ~80% gain/amplification of *MYC*, 67% mutated *TP53*, 30% deletion *TNFAIP3* (A20)
- Unique gene expression profile with overexpression of *IFI27*

Treatment/Outcome
- Chemotherapy, radiation therapy, and/or surgery
- 5-year overall survival is 22% to 35%
- Cystic cases more indolent behavior

Differential Diagnosis
- Primary effusion lymphoma
- DLBCL, NOS
- Non-hematopoietic malignancy

Pathologic Features

Histopathology

Morphologically indistinguishable from DLBCL, NOS, DLBCL, NOS usually consists of large, atypical lymphocytes with immunoblastic features. Plasmacytic differentiation or large anaplastic cells are encountered occasionally. Cases occurring in cystic lesions show infiltration by large atypical B cells in a background of inflammation and fibrosis, but they do not form discrete masses. Cases associated with cardiac/vascular prostheses may occur as single or clustered cells embedded in fibrin.

Immunophenotype

The malignant lymphocytes express the pan B-cell antigens CD20, CD79a, and PAX5. They exhibit a nongerminal center immunophenotype, being negative for CD10 and BCL6 and positive for MUM1. CD138 and CD30 are variably expressed. Occasionally tumor cells are positive for T-cell–associated antigens CD2, CD3, or CD4. EBV is positive and is best detected by in situ hybridization for EBER. HHV8 is absent.

Molecular Genetics

A monoclonal B-cell lymphoma and clonal rearrangement of Ig genes is present in DLBCL associated with chronic inflammation. Approximately 80% of PAL cases show gains/amplifications of *MYC*, 67% of cases are associated with mutation of *TP53*, and 30% exhibit deletion of *TNFAIP3* (A20). Compared to DLBCL, NOS, DLBCL associated with chronic inflammation exhibits a unique gene expression profile with overexpression of genes involved in apoptosis, signal transduction, and interferon response, with the *IFI27* (interferon-α-inducible protein 27) gene being one of the most overexpressed. Clonal genomic EBV DNA is also detected.

Differential Diagnosis

The differential diagnosis includes PEL, DLBCL, NOS, and nonhematopoietic malignancies. DLBCL associated with chronic inflammation differs and can be distinguished from PEL because it forms a solid mass, expresses pan B-cell antigens (CD20 and CD79a), and is negative for HHV8. The distinction between DLBCL associated with chronic inflammation and DLBCL, NOS requires an adequate history and knowledge of whether there is a history of long-standing chronic inflammation at the biopsied site. Anaplastic-appearing nonhematopoietic malignancies, such as carcinoma, mesothelioma, or sarcoma, may exhibit some similarities with DLBCL associated with chronic inflammation; however, the nonhematopoietic tumors lack CD45 and CD20 and express other tumor-specific antigens (e.g., keratin, calretinin) that are not expressed by DLBCL.

Prognosis and Therapy

Treatment of DLBCL associated with chronic inflammation may consist of any combination of chemotherapy (R-CHOP), radiotherapy, or surgery. Despite treatment, the disease is clinically aggressive, with overall 5-year survival ranging from 22% to 35%. Indicators of poor prognosis include male gender, poor performance status, advanced-stage disease, and elevated LDH. Cases occurring in cystic lesions or isolated in fibrin are associated with a more indolent clinical course, and conservative management may be appropriate.

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PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA

Clinical Features

Primary mediastinal large B-cell lymphoma (PMBCL) appears most commonly in young to middle-aged women (median age 39 years; male-to-female ratio, 1:2) as a bulky mediastinal mass arising from the thymus with or without invasion of local structures and regional adenopathy. Most patients have stage I or II disease. Bone marrow involvement is extremely uncommon. Symptoms are usually related to mass effect and include cough, dyspnea, or superior vena cava syndrome.

Pathologic Features

Histopathology

This lymphoma is characterized by sheets of large, centroblastic cells, often with abundant clear cytoplasm. Heterogeneity can be seen, with some cases containing medium-sized cells and others containing large atypical polylobated cells, sometimes resembling Hodgkin and Reed-Sternberg cells. There is usually a fine sclerotic stroma present that can result in compartmentalization of the tumor cells (Fig. 8.11). Occasionally, interpretation of biopsy specimens is difficult because of the crush artifact inherent in sclerotic tissue that distorts the appearance of cells. Moreover, the small biopsy size and crush artifact associated with mediastinoscopy techniques further contribute to diagnostic difficulty. Close comparison of atypical cells to the small reactive lymphocytes in the same area will aid in the recognition of the malignant cells.

PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA—FACT SHEET

Clinical Features
- Young to middle-aged adults, females > males
- Mediastinal mass may result in compression (superior vena cava syndrome) of local structures

Morphology
- Large centroblastic or polylobated cells with clear cytoplasm
- Fine compartmentalizing alveolar fibrosis
- Some cells may resemble Hodgkin and Reed-Sternberg cells

Immunophenotype
- CD19+, CD20+, CD10±, BCL2±, BCL6±, CD30± (weak, focal), CD45+, MAL±, nuclear cREL+, Grb2+, CD23, TRAF, CD200, PD-L1

Molecular Genetics
- Rearranged immunoglobulin genes
- Gains of 9p, amplification of 2p involving *cREL*

Treatment/Outcome
- Anthracycline-based multi-agent chemotherapy ± radiation therapy
- 75% event-free survival can be achieved and maintained without late relapses

Differential Diagnosis
- Classic Hodgkin lymphoma
- Other large cell neoplasms (carcinoma, germ cell tumor)

Immunophenotype

The lymphoma cells express pan B-cell markers such as CD19, CD20, CD79a, and PAX5 and usually lack expression of immunoglobulin and HLA antigen class I/II molecules. CD5 is not expressed, but CD10 can be seen in a minority (20%) of cases. BCL6 is expressed in approximately 50% of cases. BCL2 and MUM1 have been reported in approximately 75% of cases. Many of these lymphomas lack detectable surface Ig, although B-cell transcription factors Oct-2 and BOB.1 are present. CD30 is commonly expressed (up to 85% of cases), but usually in a weak or focal pattern. CD30 expression should not be considered definitive evidence of classic HL. Unlike classic HL, CD45 is expressed in PMBCL. The neoplastic cells in PMBCL typically lack expression of CD15. CD200 is expressed in 80% of cases but is not specific, being seen in 10% to 15% of DLBCL, NOS. CD23 and PD-L1 are expressed in approximately 70% of cases, and TNF receptor-associated factor 1 (TRAF-1) is expressed in 60% of cases. EBV is absent.

Expression of myelin and lymphocyte protein (MAL), a lipid-raft protein involved in intracellular transport and T-cell development, has been shown to be specific for this type of lymphoma and can be seen in approximately 70% to 80%% of cases (Fig. 8.12). c-REL is also characteristically expressed in its active form (nuclear), similar to classic HL.

Molecular Genetics

These lymphomas have clonally rearranged Ig genes and germline T-cell receptor genes. Rearrangements of *BCL2, BCL6,* and *MYC* are not seen. Translocations involving the *CIITA* gene, the master regulator of major histocompatibility complex (MHC) class II expression (HLA-DR), are seen in 38% of cases. The fusion partners are promiscuous but involve PD-1 ligands in 50% of cases. PMBCL does have characteristic molecular genetic features with gains of chromosome 9p24 encompassing *JAK2, PD-L1, PD-L2,* and amplification of chromosome 2p involving *c-REL.* Inactivating mutations of *PTPN1*, resulting in increased JAK/STAT signaling, have also been identified in approximately 20% of cases. As with other lymphomas, gene expression profiling has revealed interesting features. The overall expression profile shows that PMBCL has a unique signature but suggests it is closely related to classic HL. In particular, as noted previously, MAL is noted to be overexpressed, a feature peculiar to PMBCL and not

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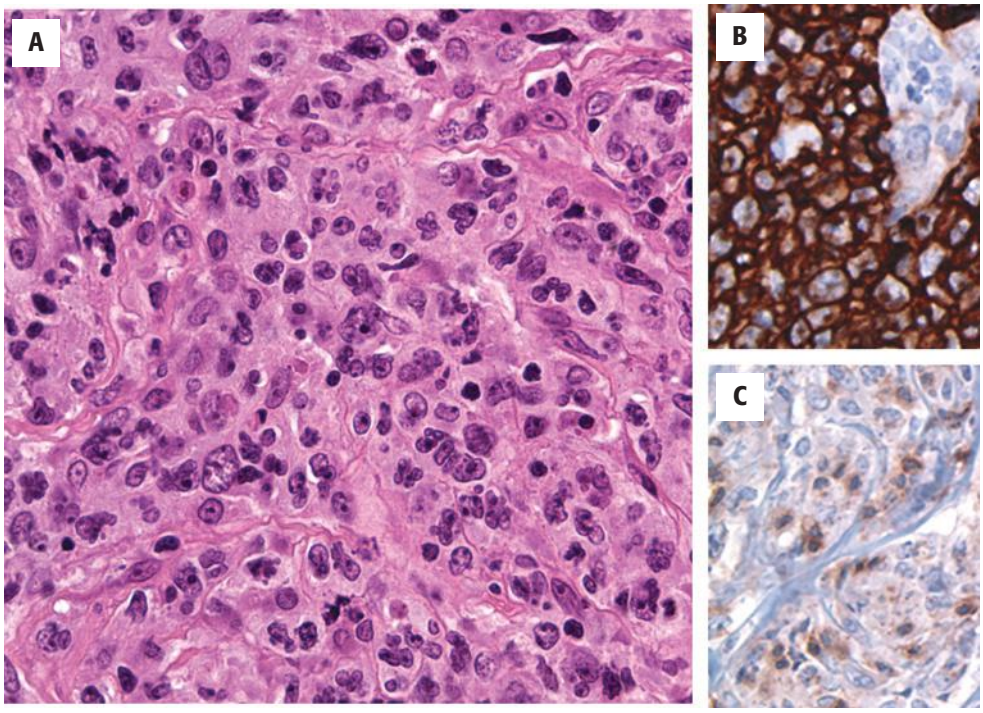


FIG. 8.11

Primary mediastinal large B-cell lymphoma. A, The lymphoma cells are large with lobulated nuclei set in a background of fine sclerotic stroma. The cells are positive for CD20 (B) and negative for CD3 (C).

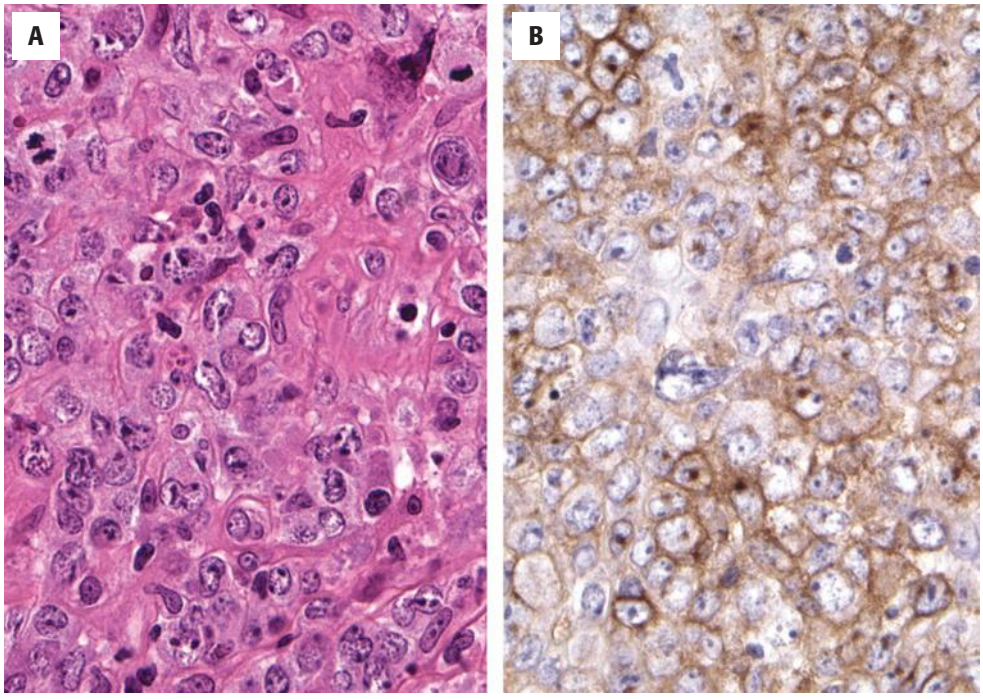


FIG. 8.12

Another case of primary mediastinal large B-cell lymphoma. A, Characteristic morphologic features. B, Myelin and lymphocyte (MAL) protein is also characteristic.

typically seen in DLBCL, NOS. As might be expected from *c-REL* amplification, NF-κB transcriptional targets have also been shown to be activated in this lymphoma, resulting in an antiapoptotic effect. Additionally, inactivating mutations of *TNFAIP3* (A20) have been identified in this disease. Similarly, amplifications involving 9p24 appear to preferentially lead to overexpression of PD-L2>PD-L1. Overexpression of *JAK2* can also lead to increased expression of the PD-1 ligands and strongly suggest that a dominant pathogenetic mechanism in PMBCL is escape from immunosurveillance.

Differential Diagnosis

The main differential diagnostic considerations for PMBCL are DLBCL, NOS arising in mediastinal lymph nodes and classic HL. Regarding DLBCL, NOS, the histologic features of centroblastic cells with clear cytoplasm in a sclerotic stroma all support the diagnosis of PMBCL. Currently, however, PMBCL is a clinicopathologic diagnosis, and awareness of the clinical scenario is required—specifically, the presence

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of a mediastinal mass without significant systemic disease or a prior history of lymphoma. Expression of CD23 as well as nuclear c-REL and MAL will strongly support the diagnosis of PMBCL. Similarly, expression of STAT1 and TRAF1, particularly coexpressed with nuclear c-REL, can be useful for distinguishing PMBCL. Because expression of CD30 is seen in PMBCL, classic HL can be a diagnostic consideration in a mediastinal biopsy. Unlike classic HL, PMBCL expresses CD45 and lacks strong expression of CD15. MAL expression supports PMBCL; however, a small minority of cases of classic HL (10% to 20%) express MAL. Expression of growth factor receptor-bound protein 2 (Grb2) has been shown to be useful in distinguishing PMBCL from classic HL, because it is universally expressed by PMBCL but only expressed by the neoplastic cells in classic HL in 10% of cases.

Rare cases may have morphologic and phenotypic features that overlap between a diagnosis of PMBCL and classic HL and are now recognized as mediastinal gray-zone lymphomas. These cases warrant a diagnosis of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic HL. Translocations involving *CIITA* occur in 24% of these cases, further testament to the relatedness of these borderline lesions.

Prognosis and Therapy

Therapy for PMBCL consists of multiple-agent anthracycline-containing regimens, such as CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone), MACOP-B (methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone), or DA-EPOCH (dose-adjusted etoposide, prednisone, vincristine, cytophosphamide, doxorubicin) with rituximab followed by involved field radiation. Using this approach, an 80% complete remission rate and 75% event-free survival can be achieved. Infusion therapy EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab) has also shown promising results in treating PMBCL. The disease course following immunochemotherapy appears distinct from DLBCL, NOS. PMBCL does not appear to result in late relapses, a feature that is sometimes seen in DLBCL, NOS, although cases with low or absent PD-L1 expression have been reported to have a worse progression-free survival.

INTRAVASCULAR LARGE B-CELL LYMPHOMA

Clinical Features

Intravascular large B-cell lymphoma (IVLBCL) is an uncommon type of extranodal DLBCL characterized by the selective localization of lymphoma cells to the intravascular space, most commonly involving the small and medium-sized blood vessels. Patients are typically older adults (median age, 71 years), with men and women affected equally. Distinct clinical entities are currently recognized and are distinguished by the geographic origin of the patient. Patients originating from Southeast Asian countries typically exhibit B symptoms, hepatosplenomegaly, bone marrow involvement, thrombocytopenia, and hemophagocytic syndrome. Patients from Western countries exhibit B symptoms and disease localized to the central nervous system and skin. A cutaneous variant is also recognized. The cutaneous variant is seen predominantly in young women from Western countries. Patients with cutaneous variant IVLBCL generally have a good performance status and have disease limited to the skin. Because of the nonspecific nature of the presenting symptoms and the lack of an identifiable mass, the diagnosis of IVLBCL is often delayed, sometimes being made at autopsy. In suspected cases with no localizing findings, random skin biopsies may prove helpful in establishing a diagnosis.

INTRAVASCULAR LARGE B-CELL LYMPHOMA—FACT SHEET

Clinical Features
- Median age at diagnosis 71 years
- Affects men and women equally
- Non-specific dermatologic, neurologic, or constitutional symptoms
- *Asian variant:* patient from Southeast Asian countries with B symptoms, hepatosplenomegaly, bone marrow involvement, thrombocytopenia, hemophagocytic syndrome
- *Western variant:* patients from Western countries with B symptoms, CNS and skin involvement
- *Cutaneous variant:* young women from Western countries with disease localized to the skin

Morphology
- Intravascular localization of large lymphoma cells
- Intrasinusoidal involvement in bone marrow, liver, and spleen

Immunophenotype
- CD20+, CD5±, CD10±, BCL6±, MUM1+, BCL2+

Molecular Genetics
- Clonally rearranged immunoglobulin genes
- No recurrent abnormalities identified

Treatment/Outcome
- *Asian and Western variants:* aggressive course with poor outcome unless early diagnosis and institution of anthracycline-containing multi-agent chemotherapy
- *Cutaneous variant:* significantly longer survival than other variants

Differential Diagnosis
- Non-intravascular non-Hodgkin B-cell lymphoma with minor intravascular component
- Intravascular undifferentiated carcinoma

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Pathologic Features

Histopathology

Although almost any tissue can be involved, the most commonly biopsied site is skin. The unifying feature is the localization of the lymphoma cells to the lumina of small blood vessels and capillaries (Fig. 8.13). Minimal extravasation of tumor cells may be seen. The lymphoma cells are large with vesicular chromatin and prominent nucleoli. Cases composed of anaplastic cells and small atypical cells have also been reported. Mitotic figures are often present. Bone marrow, liver, and spleen show sinusoidal involvement. Hemophagocytosis is seen in some cases, typically in patients of Asian origin. By nature the infiltrate can be subtle, and close attention to contents of vascular spaces is required to avoid overlooking this lymphoma. Particularly in the bone marrow, recognition of subtle involvement may be greatly aided by routine immunohistochemistry (see later discussion).

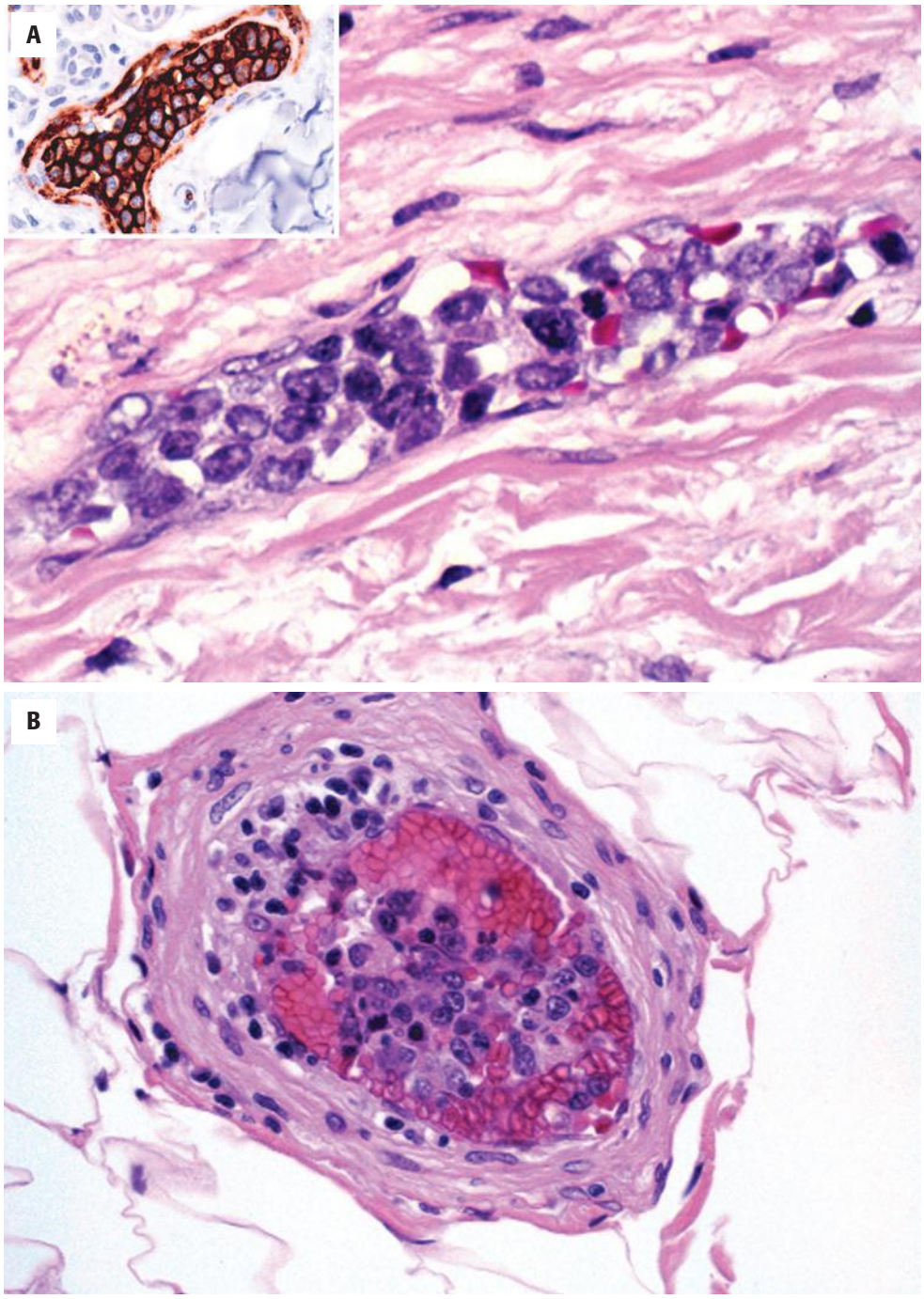


FIG. 8.13

Intravascular lymphoma. A, The lymphoma cells are confined to the lumina of vessels. The *inset* shows expression of CD20. B, This case shows the lymphoma cells admixed with the erythrocytes within the blood vessel. It was the only vessel involved in this biopsy of a peripheral nerve performed for neurologic symptoms related to this undiagnosed lymphoma.

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Immunophenotype

By definition the tumor cells are B cells and express pan B-cell antigens, such as CD20, CD79a, and PAX5. CD5, CD10, BCL6, and MUM1 are expressed in approximately 38%, 13%, 26%, and 95% of cases, respectively. BCL2 is expressed in approximately 90% of cases. CD30 expression is rare. EBV is usually not present in these lymphomas; rare EBV+ cases have been reported in the setting of immunodeficiency. Studies have reported a lack of expression of the adhesion molecules CD29 (β1 integrin), CD18 (β2 integrin), and CD54 (ICAM-1), which might explain the unusual localization of this lymphoma.

Molecular Genetics

These lymphomas have clonally rearranged immunoglobulin genes, although false-negative clonality studies can occur because of sensitivity problems related to small amounts of tumor cells in most biopsies. The t(14;18)(q32;q21) (*IGH-BCL2*) translocation is not identified, but losses at 6q21-23 and gains of 18q13-q23 have been reported.

Differential Diagnosis

The differential diagnosis includes other nonintravascular lymphoma cases of DLBCL with a minor intravascular component. Other lineage lymphomas (very rare intravascular T- and natural killer [NK]-cell lymphomas) should be considered but can be excluded by immunophenotyping. Undifferentiated carcinomas can be seen within blood vessels and may resemble IVLBCL morphologically; however, they will express epithelial antigens and lack of expression of CD45 and B-cell markers, thus excluding a diagnosis of IVLBCL. Vascular tumors can also be considered because of the location of the malignant cells. In fact, before widespread immunophenotyping in paraffin sections, an endothelial origin had been postulated. Differentiation from endothelial tumors is easily accomplished with immunostaining for B-cell markers and endothelial markers such as CD31 and CD34.

Prognosis and Therapy

Given the rarity of this lymphoma, studies with large numbers of patients have not been performed; however, this lymphoma is aggressive with a generally poor outcome. Survival is often measured in weeks to months. When the diagnosis is made early, anthracycline-containing multiple-agent immunochemotherapy has resulted in complete remission and long-term survival. One study reported a 33% 3-year survival. A more favorable outcome is reported in patients with the cutaneous variant of the disease.

ALK-POSITIVE LARGE B-CELL LYMPHOMA

Clinical Features

Anaplastic lymphoma kinase (ALK)-positive LBCL is a rare lymphoma. It occurs most frequently in immunocompetent adult males (male-to-female ratio, 5 : 1) at a median age of 43 years, with only 15% to 20% of cases occurring in the pediatric population. It can be included under the rubric of plasmablastic lymphomas because the morphology and immunophenotype are characteristic. It is primarily a disease of lymph nodes and appears at an advanced stage (III and IV) in the majority of patients, but bone marrow involvement is rare. Extranodal disease is less common but has been reported in the nasopharynx, liver, spleen, gastrointestinal tract, soft tissue, and bone.

Pathologic Features

Histopathology

The tumor typically infiltrates lymph node sinuses. The neoplastic cells are large and immunoblast-like with round nucleoli, prominent central nucleoli, and abundant basophilic

ALK-POSITIVE LARGE B-CELL LYMPHOMA—FACT SHEET

Clinical Features
- Rare disorder affecting males > females
- Median age 43 years
- Rapidly enlarging mass

Morphology
- Sheets of large immunoblast or plasmablast-like cells
- Sinusoidal distribution in lymph nodes

Immunophenotype
- CD45±, CD3–, CD20–, CD30–, CD138+, EMA+, cytoplasmic IgA+, pSTAT3+, ALK+
- EBV–, HHV8–

Molecular Genetics
- Clonally rearranged immunoglobulin genes
- t(2;17)(p23;q23) in majority of cases

Treatment/Outcome
- Anthracycline-containing combination chemotherapy
- 12-month median survival

Differential Diagnosis
- Other non-Hodgkin lymphomas with plasmablastic morphology
- ALK+ anaplastic large-cell lymphoma
- Epithelial malignancies

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cytoplasm. Plasmablastic differentiation is common, and Reed-Sternberg–like cells or multinucleated anaplastic large cells may be seen (Fig. 8.14). Foci of necrosis are often present. Extranodal sites show a similar cellular infiltrate but lack the sinusoidal pattern of involvement seen in lymph nodes.

Immunophenotype

This lymphoma has a distinctive immunophenotype. Tumor cells are plasmablastic and negative for pan B-cell markers CD19, CD20, CD22, and CD79a, as well as T-cell–associated antigens CD2, CD3, CD5, CD7, and CD8, although CD4 can be weakly positive in a subset of cases. CD45 is only weakly and focally positive or completely absent. The neoplastic cells express the plasma T-cell–associated antigens CD138 and EMA and are positive for monoclonal cytoplasmic Ig, usually IgA. pSTAT3 is present in all cases. CD30 is negative or only focally and weakly positive. ALK is expressed in all cases. Similar to ALK-positive anaplastic large-cell lymphoma (ALCL, ALK-positive), the pattern of ALK expression correlates with the underlying *ALK*-associated genetic abnormality (see later discussion). Most cases express ALK in a granular cytoplasmic pattern with the cytoplasmic, nuclear, and nucleolar patterns much less frequently described. EBV and HHV8 are not detected.

Molecular Genetics

The lymphoma has monoclonal rearrangement of the *IGH* gene. Genetic studies show that most cases harbor the t(2;17)(p23;q23), which fuses the clathrin heavy chain gene *(CLTC)* at chromosome 17q23 with the *ALK* gene at chromosome 2p23. This genetic abnormality is associated with the granular cytoplasmic pattern of ALK protein expression seen by immunohistochemical staining. Fewer cases harbor the t(2;5)(p23;q35) commonly seen in ALCL, ALK-positive. This abnormality is associated with the cytoplasmic, nuclear, and nucleolar pattern of ALK protein expression seen by immunohistochemical staining. Other less common translocations have been described involving *SEC31A* on chromosome 3q27 and *SQSTM1* on chromosome 5q35.1. *MYC* rearrangements are not seen but gains/amplifications are reported in approximately 50% of cases.

Differential Diagnosis

ALK-positive LBCL shares many morphologic and immunohistochemical features with other tumors including plasmablastic lymphoma; primary effusion lymphoma; ALCL, ALK-positive; and epithelial tumors. Careful application and interpretation of immunohistochemical stains (e.g., CD138, EMA, ALK, EBV, HHV8, CD30, Igs) will help to reveal the true nature of the neoplastic B cells. Laboratories that use screening immunohistochemical approaches should be cautious, because this lymphoma is easily confused with epithelial tumors showing sinusoidal lymph node infiltration.

Prognosis and Therapy

Patients with ALK-positive LBCL typically follow an aggressive clinical course. Treatment usually consists of anthracycline-containing multiple-agent chemotherapy, such as CHOP. Rituximab is not usually included because of the lack of CD20 expression by the tumor. Despite therapeutic intervention, the prognosis remains poor, and nearly half of the patients die within the first year, with a median survival of 12.2 months.

Lymphomatoid Granulomatosis

Inclusion of lymphomatoid granulomatosis (LyG) into this chapter is somewhat arbitrary given the heterogeneity of histopathology and behavior; however, because higher grade lesions can be considered aggressive B-cell neoplasms it is covered here. Our concept of this disease has changed over time. Originally it was described in the context of studies of Wegener's granulomatosis. In 1972, Liebow used the term LyG to emphasize the similarity of LyG to Wegener's granulomatosis; however, LyG was not believed to be caused by granulomatous inflammation. Instead it was thought to represent a destructive lymphoid proliferation. Because T cells predominate in the infiltrate it has also been considered a T-cell disorder, often along the spectrum of what is known as extranodal NK/T-cell lymphoma, nasal type (so-called angiocentric immunoproliferative lesions). It is currently known that most cases are EBV-driven B-cell lymphoproliferative disorders with variable histology and outcomes.

Clinical Features

Because LyG is a rare disorder with few published case series and case reports, making generalization difficult. LyG can be seen at almost any age, but most cases occur in the fourth to sixth decades, with a male predominance (male-to-female ratio, 2 : 1). Patients are almost always symptomatic and appear most commonly with fever, cough, dyspnea, weakness, and weight loss. The chest radiograph usually shows multiple bilateral rounded masses. Besides the lung, other commonly involved sites include skin, central nervous system, kidney, spleen, liver, and lymph node. The disorder generally produces nodular masses in these tissues. Although not associated with autoimmune diseases or frank immunodeficiency, some studies have suggested T-cell defects in cellular immunity. For this reason some have considered this disorder

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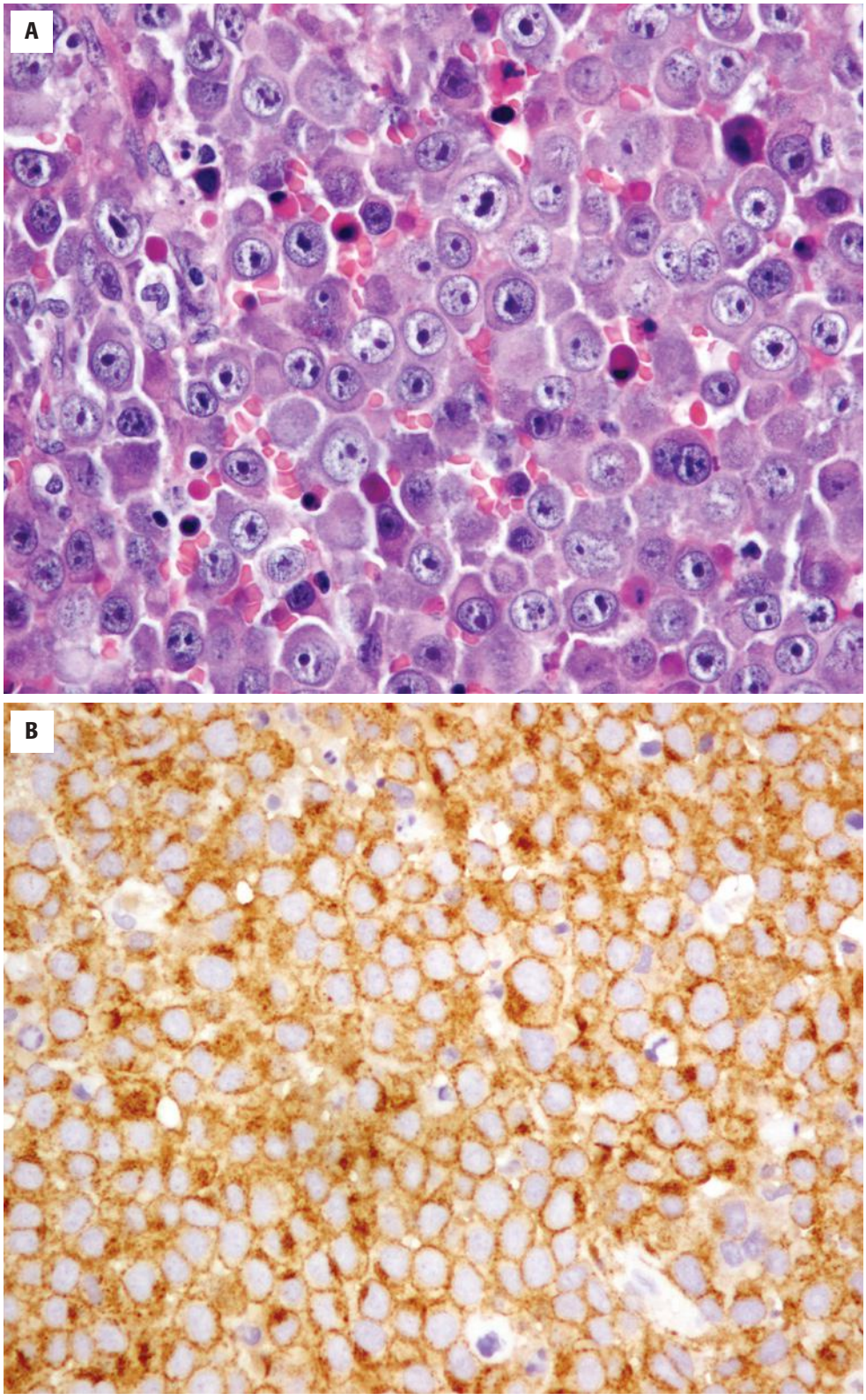


FIG. 8.14

Anaplastic lymphoma kinase (ALK)-positive large B-cell lymphoma. A, The cells have immunoblastic morphology with plasmacytoid features. The cells usually lack expression of B-cell markers such as CD20. B, ALK staining shows the characteristic cytoplasmic granular staining pattern. This is in contrast to the nuclear and cytoplasmic staining seen in most anaplastic large-cell lymphomas.

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LYMPHOMATOID GRANULOMATOSIS—FACT SHEET

Clinical Features
- Rare disorder
- Most commonly presents in adults (4th-6th decades) with fever, cough, dyspnea, and weight loss
- Manifests as lung mass (bilaterally) on chest X-ray

Morphology
- Angiocentric and angiodestructive lesions composed of a polymorphous infiltrate of small lymphocytes, histiocytes, plasma cells, and variable numbers of immunoblasts
- Hodgkin-like cells can be seen
- Cytologic grading is done based on the presence of large cells (EBV+)
- Grade I: <5 EBV+ cells/high-power field (hpf)
- Grade II: 5–50 EBV+ cells/hpf
- Grade III: >50 EBV+ cells/hpf
- Necrosis seen in higher grade lesions

Immunophenotype
- Numerous small T cells
- Large atypical immunoblast and Hodgkin-like cells are CD20+ B cells and EBV+

Molecular Genetics
- EBV+ B cells are monoclonal, best detected in higher grade lesions
- *IGH* gene rearrangement studies may be negative in lower grade lesions
- T-cell receptor gene rearrangement usually negative

Treatment/Outcome
- Single or multi-agent chemotherapy, immunomodulatory therapy
- Variable clinical course, higher grade lesions have an aggressive clinical course with median survival <18 months

in a similar light as posttransplantation lymphoproliferative disorders.

Pathologic Features

Histopathology

Lymphomatoid granulomatosis most commonly manifests in the lung as nodules composed of a polymorphous cellular population of small lymphocytes, plasma cells, immunoblasts, and histiocytes. Well-formed granulomas, neutrophils, and eosinophils are not part of the infiltrate. Large, atypical immunoblast-like cells are variably present—ranging from absent or rare to frequent. Some cells may resemble Hodgkin-Reed-Sternberg cells. At the periphery of the nodules, an angiocentric or angiodestructive lymphoid infiltrate can be seen and may contribute to the necrosis that may be present. Lesions of LyG have been graded cytologically based on the presence of large atypical cells. These grading criteria were developed at a time when LyG and angiocentric NK/T-cell lymphomas were often grouped together because of imprecise definitions. Currently, grading based on the number of large EBV+ cells is recommended. Lesions that consist of sheets of large atypical B cells and lack an inflammatory background should be classified as EBV+ DLBCL, arising in the background of LyG.

- *Grade I:* lesions show a polymorphous lymphoid infiltrate without cytologic atypia, little or no necrosis, and rare (fewer than five cells per high-power field [hpf]) EBV+ B cells (by EBER in situ hybridization).
- *Grade II:* lesions show occasional large atypical lymphoid cells with or without necrosis and between 5 and 50 EBV+ B cells per hpf.
- *Grade III:* lesions maintain an inflammatory background, but contain many large atypical cells (including Hodgkin-like cells) that may form clusters. Necrosis and numerous (>50 cells per hpf) EBV+ B cells are present (Fig. 8.15). The EBV+ B cells can be shown to be monoclonal.

In the skin, a commonly involved site, papules or nodules with an angiocentric–angiodestructive dermal lymphoid infiltrate composed predominantly of small T cells are seen. Ulceration is common. EBV+ cells may be present in small numbers but are often absent. Indurated plaques can also develop but are usually EBV−.

Bone marrow involvement is uncommon in LyG. When involvement is present, nonparatrabecular atypical lymphoid infiltrates can be seen. Hemophagocytic syndrome has been reported and manifests similarly as in other settings.

Immunophenotype

The background small lymphocytes of LYG are CD3+ T-cells. CD4+, and CD8+ cells are present, with CD4+ cells predominating. The large atypical cells are CD20+ B cells that express EBV by EBER in situ hybridization and EBV LMP1 by immunohistochemistry. CD30 may be expressed, but CD15 is absent.

Molecular Genetics

Immunoglobulin gene rearrangement can be demonstrated by polymerase chain reaction (PCR) in most cases of grade II and III LyG. Less consistent documentation of a B-cell clone is seen in grade I lesions. When multiple lesions are tested for clonality, different clones may be found, analogous to what may occur in posttransplantation lymphoproliferative disorders. T-cell receptor gene rearrangement is usually negative.

Differential Diagnosis

Wegener's granulomatosis is in the differential diagnosis of LyG; it has a clinical presentation similar to LyG with constitutional symptoms and involvement of the skin and kidney.

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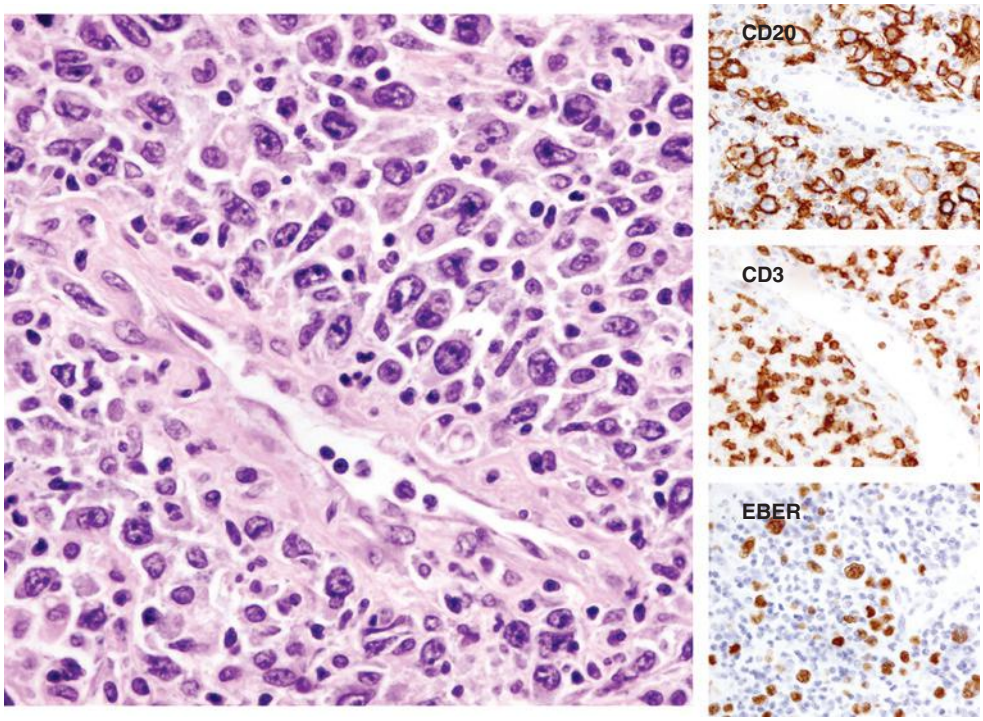


FIG. 8.15

Lymphomatoid granulomatosis, grade III. This open lung biopsy specimen shows an atypical infiltrate composed of large lymphoid cells and admixed small lymphocytes. The large cells are CD20+ B cells, and the small lymphocytes are T cells. EBV-encoded RNA (EBER) is present in the large cells.

The former often involves the upper respiratory tract, unlike LyG, which involves the lower respiratory tract. Histologically, both can have an inflammatory infiltrate with necrosis. Well-formed necrotizing granulomata are not usually seen in LyG, and the presence of large atypical immunoblast or Hodgkin-like CD20+ B cells favors LyG. Likewise, EBV+ atypical cells are seen in LyG. Finally, demonstration of monoclonal *IGH* rearrangement is characteristic of higher grade LyG.

Many older series of LyG likely included cases of extranodal, NK/T-cell lymphoma, nasal type. This lymphoma manifests as an EBV+ angiocentric and angiodestructive lymphoproliferative disorder, and it has similar features to LyG; however, it usually involves the nasal cavity as opposed to the lung. In extranodal NK/T-cell lymphoma, nasal-type cells are cytologically atypical, as opposed to the T cells in cases of LyG. Large atypical EBV+ B cells are not a feature of extranodal NK/T-cell lymphoma, nasal type.

In non-pulmonary sites, the differential diagnosis of LyG is broad and would include other lesions rich in T cells with EBV+ large B cells, including angioimmnoblastic T-cell lymphoma and perhaps EBV+ mucocutaneous ulcer. Clinical presentation and distribution of disease, as well as T-cell phenotyping (Tfh markers) and clonality studies (to exclude a T-cell lymphoma), are essential to make the most reasonable interpretation.

Prognosis and Therapy

The clinical course of LyG is variable. A waxing and waning course can be seen with spontaneous recovery in some cases. Patients with higher-grade lesions follow an aggressive clinical course, with median survival of less than 18 months. Some patients progress to overt DLBCL. Single-agent (cyclophosphamide) or combination immunotherapy and anthracycline-containing multiple-agent chemotherapy (R-CHOP) have been used to treat LyG. Because of the paucity of studies, clear guidelines are lacking. Given the presumed immunodeficiency state, immunomodulatory therapies (interferon-α 2b) have been attempted with some success.

PLASMABLASTIC LYMPHOMA

Clinical Features

Plasmablastic lymphoma (PBL) is a rare lymphoma usually associated with immunosuppression. It occurs most frequently in patients infected with the human immunodeficiency virus (HIV), but is also seen in patients receiving immunosuppressive therapy, the elderly with age-associated immunesenescence, and rarely immunocompetent patients. PBL is primarily a disease of adults, affecting men more often than women. In the setting of HIV infection, the most frequent site of involvement is the oral cavity. It also affects other extranodal sites, with a predilection for mucosal tissues, including the sinonasal cavities, orbit, respiratory and gastrointestinal tracts, skin, and bone. Lymph node involvement is uncommon and is more frequent in immunocompetent patients. In most patients, PBL appears at an advanced stage with an intermediate- to high-risk IPI.

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PLASMABLASTIC LYMPHOMA—FACT SHEET

Clinical Features
- Rare disorder associated with immunosuppression (HIV, iatrogenic, age-associated)
- Affects males > females
- Presents at high stage

Morphology
- Sheets of large immunoblast or plasmablast-like cells
- Mature plasma cells may be admixed

Immunophenotype
- CD45–, CD3–, CD20–, CD38+, CD79a±, CD138+, PAX5–, MUM1+, EMA±, cIg±, CD30±, MYC±, EBV±, HHV8–, ALK–

Molecular Genetics
- Clonally rearranged immunoglobulin genes
- ~50% of cases associated with *MYC* rearrangement, usually t(8;14)

Treatment/Outcome
- Anthracycline-containing combination chemotherapy
- HAART for HIV
- 8- to 14-month median survival

Differential Diagnosis
- Plasmacytoma
- Primary effusion lymphoma
- ALK+ diffuse large B-cell lymphoma

Pathologic Features

Histopathology

Plasmablastic lymphoma infiltrates in a diffuse pattern. The neoplastic cells are large and atypical with immunoblastic, plasmablastic, or plasmacytic features, including eccentric nuclei, prominent central or peripheral nucleoli, basophilic cytoplasm, and a perinuclear hof (Fig. 8.16). Mature-appearing plasma cells may also be present. Mitotic figures, apoptotic bodies, and tingible body macrophages are also featured.

Immunophenotype

The tumor cells of PBL exhibit features of terminally differentiated B cells and express antigens in a pattern similar to mature plasma cells. They are invariably positive for CD38, CD138, and MUM1; variably positive for CD79a, cytoplasmic Ig, EMA, and CD30; and usually negative for CD45, CD20, and PAX5, although these antigens are occasionally weakly expressed. BLIMP1 and XBP1, markers of terminal B-cell differentiation, are also usually expressed. These two markers are not often routinely available but further support the plasmacytic differentiation of these lymphomas. A significant proportion of cases express MYC. PBL usually does not express CD56, distinguishing it from true plasma cell neoplasms. The proliferation index is in excess of 90%, and approximately 70% to 75% of cases are associated with EBV

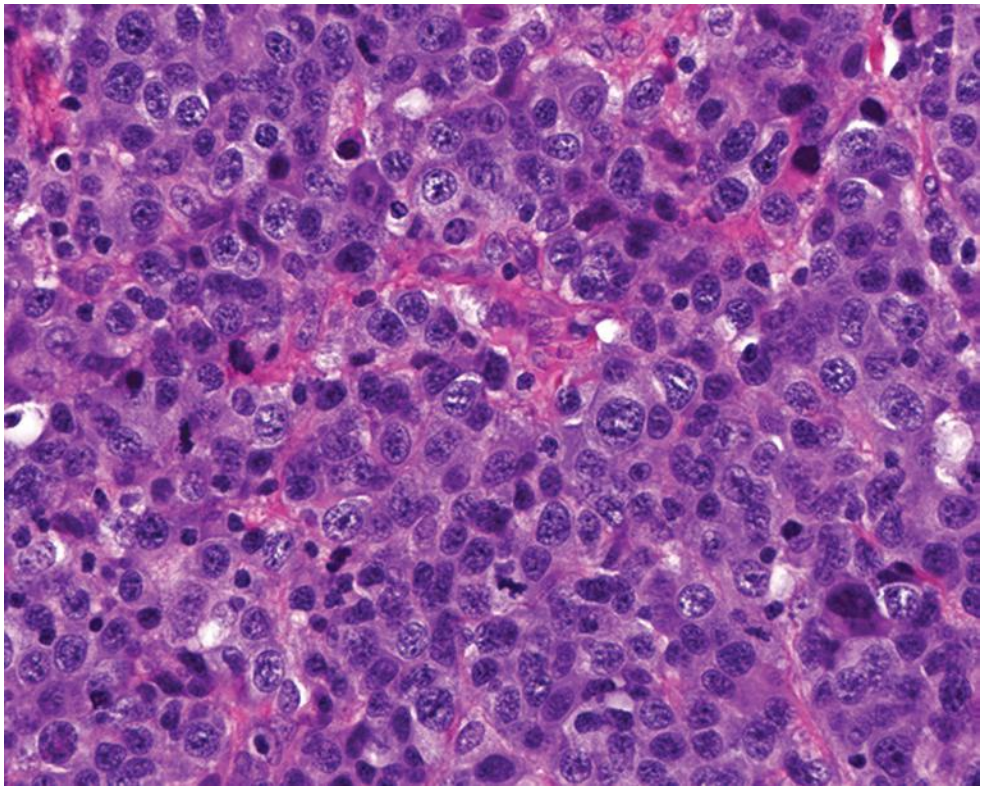


FIG. 8.16
Plasmablastic lymphoma. The malignant lymphocytes are large and atypical with plasmacytic features.

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infection in a type I latency pattern (best detected by in situ hybridization for EBER), which is enriched in oral cases. HHV8 infection is not present.

Molecular Genetics

Plasmablastic lymphoma exhibits monoclonal rearrangement of the *IGH* gene. *MYC* gene rearrangement is a recurring secondary genetic abnormality in PBL, occurring in approximately 50% of cases. The translocation partner is usually *IGH*. It is seen in the setting of a complex karyotype. *MYC* gains are also observed in a small proportion of cases; however, rearrangements of *BCL2* and *BCL6* are not usually seen, and PBL is not considered part of the spectrum of double-hit lymphomas. Recently, gene expression profiling has revealed upregulation of *DNMT3B*, *PTP4A3*, and *CD320* in EBV+ cases arising in the setting of posttransplantation immunosuppression.

Differential Diagnosis

The differential diagnosis includes other lymphoid neoplasms with plasmacytic features such as ALK-positive DLBCL, PEL, PBL associated with HHV8+ multicentric Castleman disease, and plasmacytoma. PBL is distinguished from ALK-positive DLBCL by its lack of expression of the ALK protein, and absence of HHV8 co-infection distinguishes PBL from primary effusion lymphoma and multicentric Castleman-related PBL. Differentiation from plasmacytoma can be challenging. Features that help distinguish PBL from plasmacytoma include its association with an immunodeficient state and the presence of EBV infection. PBL is not typically associated with bone lesions or circulating M-proteins. In addition, the identification of an *MYC* gene rearrangement can help to distinguish PBL from plasmacytoma, as *MYC* rearrangement is less common in the latter disorder.

Prognosis and Therapy

Plasmablastic lymphoma is an aggressive lymphoma, and a standard therapy has not yet been established. Treatment usually consists of chemotherapy with various regimens including CHOP, R-CHOP, and cyclophosphamide, vincristine, doxorubicin, high-dose methotrexate/ifosfamide, etoposide, high-dose cytarabine (CODOX-M/IVAC). The use of a rituximab-containing regimen is of uncertain utility if the neoplastic cells do not express CD20. In the HIV-infected population, the addition of highly active antiretroviral therapy also improves prognosis; however, overall survival is short despite therapeutic intervention, with a median survival of less than 8 to 14 months.

BURKITT LYMPHOMA

Clinical Features

Burkitt lymphoma is a highly aggressive mature B-cell neoplasm occurring primarily in children and young adults. It can manifest as a lymphomatous mass or occasionally as a leukemic process. In former classifications it was considered a form of acute leukemia (e.g., French-American-British Classification L3 ALL); however, it is no longer considered in the spectrum of acute leukemias. Three clinical variants are recognized: endemic, sporadic, and immunodeficiency-associated BL. The latter is treated in detail in Chapter 10.

Endemic BL occurs principally in equatorial Africa and Papua, New Guinea. In Africa it is the most common childhood malignancy and was first described in detail by Dennis Burkitt in 1958. It is seen in early childhood (4 to 7 years old) with a male predominance and has a predilection for extranodal involvement of the jaw, abdomen, orbit, and paraspinal region. The association with EBV infection is strong and is seen in essentially all endemic cases. The sporadic type of BL is seen throughout the world, including Europe and North America. It occurs mainly in children and young adults and decreases in frequency with increasing age. It is a relatively rare lymphoma, accounting for 1% of all lymphoma in adults. In children, however, it is among the most common types. Sites of involvement include the abdomen, nasopharynx, and bone marrow. Lymph nodes are not frequently involved in

BURKITT LYMPHOMA—FACT SHEET

Clinical Features
- Presents most commonly in children and young adults
- Rapidly enlarging mass—extranodal sites common
- Endemic (African), sporadic, and immunodeficiency-related types

Morphology
- Starry-sky pattern
- Intermediately sized cells (small, non-cleaved cells)
- Numerous mitoses
- Characteristic imprint morphology with abundant basophilic cytoplasm and vacuoles (FAB-L3 morphology)

Immunophenotype
- CD19+, CD20+, CD10+, BCL6+, CD5–, BCL2–, surface immunoglobulin + Ki-67 ~100%

Molecular Genetics
- t(8;14)(q24;q32), t(8;22)(q24;q11), or t(2;8)(p12;q24)
- No rearrangement of *CCND1*, *BCL6*, or *BCL2*
- Non-complex karyotype

Treatment/Outcome
- Aggressive, rapidly growing lymphoma
- High-intensity, short-duration therapy such as hyper-CVAD used
- Complete remission possible in 75% to 90%

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younger patients but are more frequently involved in older patients.

Pathologic Features

Histopathology

The typical morphologic feature of BL is a diffuse infiltrate of monomorphous, medium-sized cells. At low magnification there is a starry-sky pattern, which refers to the mottled or moth-eaten appearance produced by the pale tingible body macrophages scattered throughout the lymphomatous infiltrate. These macrophages contain cellular debris produced from phagocytosis of apoptotic lymphoma cells. Rarely, a follicular pattern may be seen, possibly representing colonization of preexisting reactive germinal centers. The cells are monotonous, with nuclei that are approximately the size of benign histiocyte nuclei. The chromatin is vesicular, and there are multiple small basophilic nucleoli (Fig. 8.17). Cytoplasm is basophilic and moderate in amount. The cells often sit slightly apart from one another, imparting a squared-off or cobblestone pattern. The imprint morphology is characteristic, consisting of blastic cells with moderate amounts of deeply basophilic cytoplasm with punched-out vacuoles that contain lipid, which can be demonstrated with oil-red O staining. Vacuoles are not specific and can be seen in non-Burkitt large-cell lymphomas and are particularly frequent in anaplastic large-cell lymphoma. Variant morphologies have been described. Patients with HIV may have more variation in cell size and plasmacytic features. Cases with slightly more morphologic heterogeneity but otherwise exhibiting the immunophenotypic and molecular genetic features of BL should still be diagnosed as BL.

Immunophenotype

Burkitt lymphoma cells express the pan B-cell markers CD19, CD20, and CD79a. They are positive for cytoplasmic IgM and surface Igs, and they universally express CD10 and BCL6 and lack BCL2 in the vast majority of cases. Some cases may show weak and focal expression of BCL2. BL cells do not express MUM1 or cyclin-D1. Cases that are associated with EBV (endemic or sporadic) are positive for Epstein-Barr virus nuclear antigen 1 (EBNA-1) and EBER but lack latent membrane protein 1 (LMP-1) and EBNA-2, which is consistent with the type I latency pattern of EBV infection. Immunostaining for adipophilin, a lipid droplet–associated protein overexpressed in BL, shows strong staining in 95% of BL, with only weak staining in 10% of non-BL cases. Ki-67 staining shows that almost 100% of tumor cells are positive, reflecting the underlying biology of MYC overexpression driving tumor cells into cell cycle. Predominantly nuclear localization of MYC protein by immunohistochemistry and expression of the pre–B-cell receptor protein VpreB3 is seen in the vast majority of BL and may also be useful for detecting *MYC* translocations in DLBCL, NOS and high grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* translocation.

Molecular Genetics

Regardless of the clinical variant, translocation of *MYC* (chromosome 8q24) is considered the primary transforming event. One of three partner genes is involved: Ig heavy chain *(IGH)* at chromosome 14q32, κ light chain *(IGK)* at chromosome 2p11, or λ light chain *(IGL)* at chromosome 22q11. In most cases, fluorescence in situ hybridization can be used to detect these translocations. Cryptic and variant *MYC/IGH* translocations are described that cannot be detected using standard *MYC* break-apart probes alone. To prevent misdiagnosis, fusion probes that target the specific translocations should be used to confirm the presence of an *MYC* translocation. Rarely, *MYC* rearrangement cannot be demonstrated. Some cases may represent false-negative fluorescence in situ hybridization studies. Most cases are true negatives that harbor 11q abnormalities with alternatively activated pathways causing *MYC* deregulation (see later discussion). Cases without demonstrable *MYC* rearrangement that otherwise meet the morphologic and immunophenotypic criteria for a diagnosis of BL should be tested for 11q abnormalities.

There is considerable heterogeneity in the *MYC* breakpoint in BL. In the t(8;14), breakpoints are 5′ to *MYC*, whereas in the t(2;8) and the t(8;22) the breakpoints are 3′ to *MYC*. In the 5′ breakpoint of the t(8;14) there is a propensity for the breakpoint to be far upstream (>100 kB) from *MYC* and also to involve the J (joining) region of *IGH*; this is seen in endemic BL. In sporadic cases, the breakpoint is immediately 5′ to *MYC* and involves the switch regions of *IGH*. Translocations involving the first exon or intron of *MYC* are designated class I; those involving the immediately upstream region as class II; and those far upstream as class III (Table 8.2).

Although *MYC* rearrangement is a diagnostic feature of BL, it is not specific for the disease as other B-cell non-Hodgkin lymphomas, including mantle cell lymphoma, follicular lymphoma, and DLBCL, NOS, can harbor *MYC* rearrangements. In the latter group of lymphomas, *MYC* rearrangement is often a secondary genetic event related to disease transformation. Detection of other recurring genetic abnormalities, including rearrangement of the *CCDN1*, *BCL2,* and *BCL6* genes, precludes a diagnosis of BL.

Secondary genetic changes are common in BL but are usually fewer than two in number and complex karyotypes are unusual. The most frequently observed recurring abnormalities include gains at chromosomes 1q, 7, 9q, 12q, 13q, 20q, 22q, and Xq and losses of 4q, 6q, 13q32-34, and 17p.

Gene expression profiling has identified a unique molecular profile in BL that predicts for good response to therapy and distinguishes BL from DLBCL. Interestingly, some cases with the BL molecular profile meet the diagnostic criteria for DLBCL, NOS by morphology, immunophenotype, and genetic features, whereas some cases that satisfy the criteria for a diagnosis of BL lack the BL molecular profile and have

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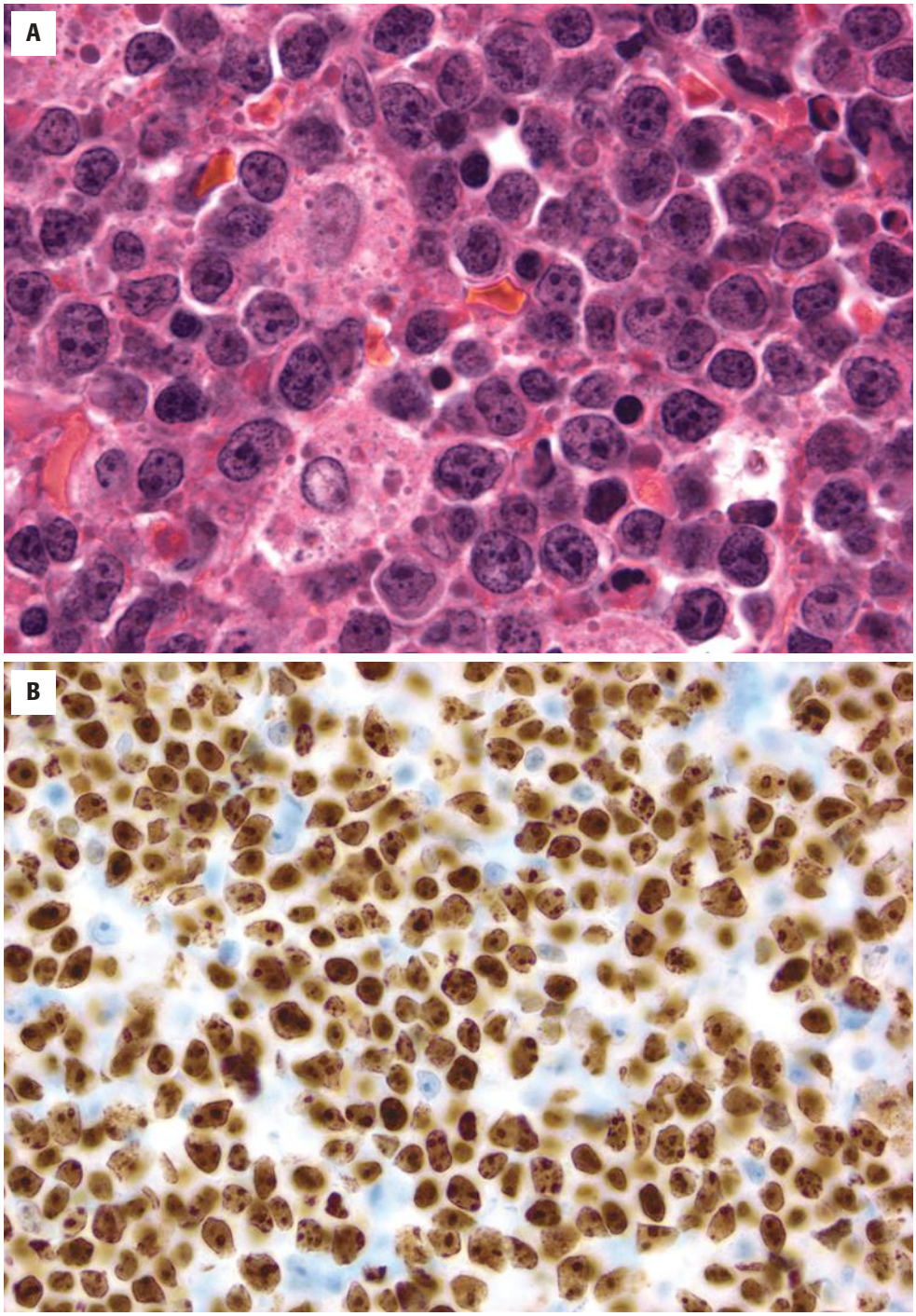


FIG. 8.17

Burkitt lymphoma. A, The cells are monotonous intermediate-sized cells (similar in size to a benign histiocyte) with multiple inconspicuous nucleoli. The characteristic immunophenotype is CD10+, BCL2–. B, The Ki-67 labeling index approaches 100% due to the presence of *MYC* deregulation.

TABLE 8.2
Genetic Features of Burkitt Lymphoma

	Endemic (African) BL	Sporadic BL	HIV-Associated BL
Class	III	I and II	I
MYC breakpoint	>100 kB 5′ of *MYC*	Located in first exon/intron or immediately 5′ of *MYC*	Within first exon/intron of *MYC*
IGH breakpoint	Predominantly J region	Predominantly switch region	Predominantly switch region

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a worse clinical outcome. This finding serves to highlight the imprecise nature of the current diagnostic criteria in BL.

Recent next-generation sequencing studies have revealed recurrent somatic mutations in the transcription factors *ID3* and *TCF3* in approximately 70% of cases. These mutations are enriched in this disease but are not specific for BL, as they have also been rarely seen in other high-grade B-cell lymphomas. Other identified, frequently recurring mutated genes include *PIK3R1*, *ARID1A*, *GNA13*, and *SMARCA4*.

Differential Diagnosis

The differential diagnosis includes other diffuse aggressive B-cell lymphomas, such as Burkitt-like lymphoma with 11q aberration, B-cell precursor lymphoblastic lymphoma, blastoid variant of mantle cell lymphoma, and de novo or transformed DLBCL, NOS. The diagnostic features of these various lymphomas are listed in Table 8.3. The first entity is distinguished from BL on the basis of findings from genetic studies such as high resolution single nucleotide polymorphism (SNP) array. The next two entities are easily distinguished from BL by morphologic and immunophenotypic features. Lymphoblastic lymphoma, like BL, is often composed of medium-sized cells, but it exhibits more evenly dispersed, fine, blastic chromatin. Unlike BL, lymphoblastic lymphoma expresses TdT or CD34, or both, in almost all cases and lacks surface Ig. Blastoid mantle cell lymphoma can mimic BL morphologically but differs from BL by its expression of CD5 and cyclin-D1.

The main differential diagnostic consideration in BL is DLBCL, NOS. DLBCL, NOS is often composed of large atypical cells with centroblastic or immunoblastic morphology but is occasionally composed of medium-sized, centroblast-like cells resembling BL. Failure of the lymphoma to express CD10, expression of BCL2, or a Ki-67 proliferation index less than 95% favors DLBCL, NOS and helps to exclude BL. Absence of an *MYC* rearrangement, or an *MYC* rearrangement in the setting of a complex karyotype, also helps to exclude BL. Rarely, it may not be possible to differentiate between BL and DLBCL, NOS. These cases warrant a diagnosis of high-grade B-cell lymphoma (see later discussion).

Prognosis and Therapy

Therapy consists of aggressive, short-duration chemotherapeutic regimens with CNS prophylaxis. The high-intensity, short-duration strategy is needed because of the high growth fraction and the need to avoid the potential for rapid growth and resistance development between chemotherapy cycles. It is beyond the scope of this chapter to review therapeutic protocols. However, common regimens include CODOX-M/IVAC and hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate, and cytarabine). Complete remissions have been reported in 75% to 90% of patients, with overall survival at 2 years of greater than 70% in some studies. Relapses typically occur within 1 year of treatment.

TABLE 8.3
Differential Diagnosis of Burkitt Lymphoma

	Histology	Immunophenotype	Proliferation Index	Molecular Genetics
Burkitt lymphoma	Intermediately sized monotonous cells with multiple small nucleoli, starry-sky pattern	CD19+, CD20+, CD5–, CD10+, BCL2–, cyclin D1–, TdT–	~100%	*MYC* rearrangement in all cases
Burkitt-like lymphoma with 11q aberration	Intermediately sized monotonous cells with multiple small nucleoli, starry-sky pattern	CD19+, CD20+, CD5–, CD10+, BCL2–, cyclin D1–, TdT–	~100%	*MYC* rearrangement absent 11q aberration (proximal gains, telomeric losses)
DLBCL, NOS (de novo or transformed B-cell NHL)	Large centroblastic, immunoblastic, or intermediately sized "small" centroblastic cells	CD19+, CD20+, CD5±, CD10±, BCL2+, cyclin D1–, TdT–	<90%	*MYC* rearrangement (10%), t(14;18) (20%), *BCL6* abnormality (20%)
Blastoid MCL	Intermediately sized cells resembling lymphoblasts	CD19+, CD20+, CD5+, CD10–, BCL2+, cyclin D1+	<90%	t(11;14) in virtually 100% of cases
Lymphoblastic lymphoma	Lymphoblasts	CD19+, CD20±, CD5–, CD10+, BCL2+, cyclin D1–, TdT+	<90%	Lacks *MYC* rearrangement

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BURKITT-LIKE LYMPHOMA WITH 11q ABERRATION

Clinical Features

Burkitt-like lymphoma with 11q aberration is a new and provisional entity in the 2016 revision of the WHO classification of lymphoid neoplasm. It is a rare disease with few reported cases. The disease tends to occur in younger patients, with a slight male predominance and a predilection for lymph node involvement.

Pathologic Features

Histopathology

The morphologic features appear to be indistinguishable from Burkitt lymphoma, and the tumors are generally composed of a diffuse infiltrate of monomorphous, medium-sized cells that are monotonous, with round nuclei, vesicular chromatin, multiple small basophilic nucleoli, and scant to moderate amounts of cytoplasm. At low-power magnification a starry-sky appearance may be appreciated.

Immunophenotype

The malignant cells are B cells that express pan B-cell antigens CD20, CD79a, and PAX5. They are immunophenotypically indistinguishable from the malignant cells of BL and are positive for CD10 and BCL6 and negative for BCL2 and CD5. They also exhibit a high proliferation index by Ki-67 staining, usually in excess of 95%. EBV is absent.

Molecular Genetics

The genetic hallmark of this disease is an aberration of chromosome 11q, including interstitial gains at 11q23.2-q23.3 and/or telomeric losses of 11q24.1-qter, and the absence of an *MYC* rearrangement. These abnormalities can often be identified by conventional karyotype and/or FISH methods but are best detected using array comparative genomic hybridization (CGH) or single nucleotide polymorphism (SNP) array protocols. This disease appears to be cytogenetically more complex than BL, with approximately 12 cytogenetic abnormalities detected per case. In addition, mutation in *ID3*, which is found in upward of 60% of BL, is not identified.

BURKITT-LIKE LYMPHOMA WITH 11Q ABERRATION—FACT SHEET

Clinical Features
- Occurs in younger patients; males > females
- Lymph node involvement common

Morphology
- Similar to Burkitt lymphoma
- Starry-sky pattern
- Intermediately sized cells with basophilic cytoplasm
- Numerous mitoses

Immunophenotype
- CD20+, CD10+, BCL6+, CD5–, BCL2–, CD5–
- Ki-67 >95%

Molecular Genetics
- Gains of 11q23.2-q23.3; losses of 11q24.1-qter
- MYC rearrangement not identified

Treatment/Outcome
- High-intensity, short-duration therapy such as hyper-CVAD used
- Complete remission with cure possible in most cases

Differential Diagnosis

Burkitt lymphoma is the most likely disease in the differential diagnosis. Distinguishing Burkitt-like lymphoma with 11q aberrations from BL requires thorough evaluation at the cytogenetic level to both identify an 11q abnormality and exclude the presence of an MYC rearrangement. Molecular testing for *ID3* mutation may also be of benefit. Other diseases in the differential diagnosis are similar to those described for Burkitt lymphoma (see previous discussion).

Prognosis and Therapy

Patients with Burkitt-like lymphoma have typically been treated with therapy appropriate for Burkitt lymphoma and show a similarly high rate of survival.

HIGH-GRADE B-CELL LYMPHOMA

High-grade B-cell lymphoma (HGBCL) is a new diagnostic category in the 2016 revision of the WHO classification of lymphoid neoplasms. It replaces B-cell lymphoma, unclassifiable (BCLU), with features intermediate between DLBCL and Burkitt lymphoma and incorporates other rare entities not previously classifiable by the 2008 classification. It is essentially a "wastebasket" category for the high-grade B-cell lymphomas that do not meet the diagnostic criteria for other entities within the classification. It is further divided into two entities according to the presence or absence of specific genetic changes: (1) high-grade B-cell lymphoma, with *MYC* and

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BCL2 and/or *BCL6* rearrangements, and (2) high-grade B-cell lymphoma, NOS.

This category largely has its basis in gene array studies recognizing cases with genetic and histopathologic features that are intermediate between DLBCL, NOS and BL. In the past, diseases that met the criteria for inclusion in this category have been called atypical Burkitt lymphoma; Burkitt-like lymphoma; gray-zone lymphoma; or B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma. This diagnostic category does not represent a well-defined clinicopathologic entity but rather a heterogeneous group of de novo or transformed high-grade B-cell lymphomas that cannot be classified more accurately because they do not meet the diagnostic criteria required for other disease entities.

Clinical Features

A rare disease, HGBCL is usually diagnosed in older adult patients, with men and women affected equally. Patients exhibit a rapidly enlarging mass involving lymph nodes or extranodal sites. There is no predilection for any particular body site. Advanced-stage disease is more common, and bone marrow and CNS involvement are frequently seen.

Pathologic Features

Histopathology

High-grade B-cell lymphoma is a morphologically heterogeneous disease. Some cases closely resemble BL and are composed of a monotonous population of medium-sized cells with vesicular chromatin, small inconspicuous nucleoli, and moderate basophilic cytoplasm. These cases usually have one or more immunophenotypic or karyotypic abnormalities that are not consistent with BL and preclude its diagnosis (see later). Conversely, other cases are reminiscent of DLBCL, NOS, with greater nuclear pleomorphism and increased numbers of large atypical cells within the infiltrate; however, these cases usually have an immunophenotype or karyotype that is consistent with BL or conversely harbor *MYC* and *BCL2* and/or *BCL6* rearrangements. Rarely, cases may exhibit blastoid features and resemble acute lymphoblastic lymphoma.

Immunophenotype

All cases of HGBCL are composed of B cells that express the pan B-cell antigens CD20, CD79a, and PAX5. Cases of HGBCL that morphologically resemble BL usually have an immunophenotype that is inconsistent with BL and precludes its diagnosis. Typical inconsistent features include strong expression of BCL2 and MUM1, absence of BCL6 expression, and a proliferation index less than 95%. Cases of HGBCL that are morphologically in keeping with DLBCL exhibit a phenotype consistent with BL and express CD10 and BCL6, lack expression of BCL2, and have a proliferation index of approximately 100%. If the latter group does not express this immunophenotype or harbor *MYC* and *BCL2* and/or *BCL6* gene rearrangements a diagnosis of DLBCL, NOS is appropriate. Most cases express MYC, typically resulting from underlying *MYC* rearrangement, gain/amplification, or gene mutation. Dual expression of MYC and BCL2 is commonly encountered; however, this is insufficient for a diagnosis of high-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangement as this disease requires demonstration of rearrangement by FISH analysis. Blastoid cases lack expression of TdT as well as cyclin D1 and SOX11 excluding acute lymphoblastic lymphoma and blastoid/pleomorphic mantle cell lymphoma, respectively.

HIGH-GRADE B-CELL LYMPHOMA—FACT SHEET

Clinical Features
- Presents in older patients with advanced stage ± CNS involvement
- Rapidly enlarging mass
- Very aggressive disease

Morphology
- Burkitt-like morphology
- DLBCL-like morphology
- Blastoid morphology

Immunophenotype
- Burkitt lymphoma–like: CD20+, CD10+, BCL6±, BCL2+, MIB1 <95%
- DLBCL-like: CD20+, CD10+, BCL6+, BCL2–, MIB1 ~100%
- Blastoid: does not express TdT or cyclin D1/SOX11
- MYC expression is common

Molecular Genetics
- Clonally rearranged immunoglobulin genes
- Complex karyotype common
- Recurrent mutations in *ID3*, *MYC*, *BCL2*, *CREBBP/EP300, EZH2*

High-Grade B-Cell Lymphoma, With MYC and BCL2 and/or BCL6 Rearrangements
- *MYC* rearranged 100%
- *BCL2* rearranged (*MYC/BCL2* double-hit lymphoma) 60% to 70%
- *BCL6* rearranged (*MYC/BCL6* double-hit lymphoma) 15% to 30%
- *BCL2* and *BCL6* rearranged (triple-hit lymphoma) 10%

High-Grade B-Cell Lymphoma, NOS
- *MYC* rearranged in some cases
- *BCL2* and/or *BCL6* may be rearranged, but not in the setting of a *MYC*
- Rearrangement

Treatment/Outcome
- Resistant to multi-agent chemotherapy

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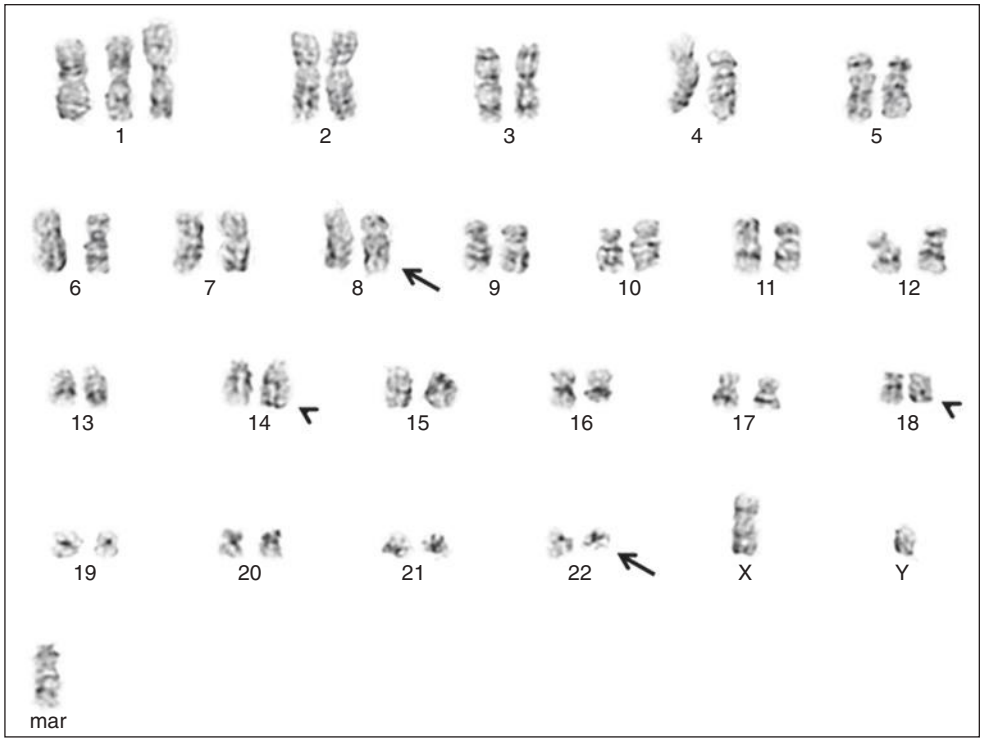


FIG. 8.18

"Double-hit" genetic abnormality. Complex karyotype with a "double-hit" abnormality including a t(8;22) *MYC* rearrangement *(arrows)* and t(14;18) *BCL2* rearrangement *(arrowheads)*.

Molecular Genetics

Lymphomas in this category show monoclonal rearrangement of the *IGH* gene. *MYC* rearrangement is common in this group of diseases: cases that lack an *MYC* rearrangement but are morphologically and phenotypically consistent with BL should be assessed for 11q aberrations before being classified as HGBCL (see previous discussion); DLBCL, NOS cases with an *MYC* rearrangement but lack *BCL2* or *BCL6* rearrangements should be diagnosed as DLBCL, NOS.

In HGBCL, *MYC* rearrangements differ from those seen in BL: they often involve non-Ig partner genes, are associated with complex karyotypes, and upward of 40% of cases co-occur with *BCL2* and/or *BCL6* gene rearrangements. Cases with the latter abnormality are referred to as double-hit or triple-hit lymphomas and are classified as high-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangement (Fig. 8.18). The majority (60% to 70%) of these lymphomas harbor *MYC*/*BCL2* rearrangements, with a minority (15% to 30%) harboring *MYC/BCL6* rearrangements and even fewer (10%) harboring rearrangements of all three genes (triple-hit lymphoma). Of note, the combination of *MYC* and *BCL2* rearrangement has been described in rare cases of B-cell lymphoblastic lymphoma and follicular lymphoma; however, according to the 2016 revision of the WHO classification of lymphoid neoplasms these entities are not included in the category of high-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangement. Cases that do not harbor a double- or triple-hit abnormality are classified as high-grade B-cell lymphoma, not otherwise specified. Given the diagnostic and therapeutic implications (see below) FISH testing for *MYC*, *BCL2*, and *BCL6* rearrangements is recommended for all cases of HGBCL.

HGBCL has also been shown to harbor recurrent mutations frequently seen in Burkitt lymphoma and diffuse large B-cell lymphoma. *ID3* is mutated in approximately 58% of HGBCL, including 22% of double-hit lymphomas. *MYC* mutations are enriched in cases with *MYC* translocations and are seen in upward of 45% of cases overall. *BCL2* mutations are present in 50% of cases, and *CREBBP/EP300* mutations are seen in 33% of cases, including 44% of double-hit lymphomas. *EZH2* mutations have also been reported.

Differential Diagnosis

The main differential diagnostic considerations are BL and DLBCL, NOS. Close attention to morphologic, immunophenotypic, and genetic features and application of the diagnostic criteria previously described in this chapter will help in making an accurate diagnosis. Cases with double or triple hits, as described previously, are, by definition, included in this HGBCL, with *MYC* and *BCL2* and/or *BCL6* category. In general, cases that do not fulfill the diagnostic criteria for either DLBLC, NOS or BL but have a combination of morphologic, phenotypic, and genetic features between the two of these two disease entities are appropriate for inclusion in the HGBCL category. In the future, better definitions based on gene expression or other genetic data may be forthcoming and refine or replace this category.

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Prognosis and Therapy

These are aggressive lymphomas. A standard approach to therapy has not yet been established for HGBCL, NOS, and they tend to be resistant to DLBCL, NOS- and BL-type treatment. Double-hit/triple-hit cases are especially aggressive, and DA-EPOCH-R with CNS prophylaxis is the treatment of choice where suitable. Despite this intense therapeutic regimen, patient survival is measured on the order of months. Interestingly, patients with *MYC/BCL6* achieve higher rates of complete response compared to *MYC/BCL2* patients but experience a shorter median overall survival compared to those *MYC/BCL2* patients who are able to achieve a complete response. Patients with *MYC/BCL2/BCL6* rearranged tumors appear to exhibit similar outcomes to patients with *MYC/BCL2* rearranged tumors; however, experience is still limited.

DOUBLE AND TRIPLE-HIT LYMPHOMA TREATED WITH DA-EPOCH-R + CNS PROPHYLAXIS—FACT SHEET

- Poor prognosis
- Median survival measured in months



The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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9

Peripheral T-Cell Lymphomas

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INTRODUCTION

Peripheral T-cell lymphomas (PTCLs) are much less common than B-cell lymphomas, and they constitute approximately 10% of non-Hodgkin lymphomas (NHLs) in the United States and Europe. Derived from post-thymic T cells, PTCLs generally arise in lymphoid tissues "peripheral" to the thymus, such as the lymph nodes, spleen, gastrointestinal tract, and skin. PTCLs have a mature T-cell phenotype and occur most frequently in adults. Because natural killer (NK) cells and T cells arise from a common progenitor cell and have some overlapping properties, NK-cell lymphomas are considered together with PTCLs in the World Health Organization (WHO) classification. The mature T- and NK-cell neoplasms can be grouped according to clinical presentation: leukemic, nodal, and extranodal. This chapter addresses the nodal and extranodal PTCLs and NK-cell lymphomas recognized in the WHO classification, other than adult T-cell leukemia–lymphoma, which is discussed in Chapter 13.

ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA AND OTHER NODAL LYMPHOMAS OF T FOLLICULAR HELPER CELL ORIGIN

Angioimmunoblastic T-cell lymphoma (AITL) is primarily a nodal type of PTCL. It is the second most common nodal PTCL and comprises approximately 20% of all noncutaneous T-cell lymphomas and 1% to 2% of NHLs. It is now generally accepted that these tumors arise from T follicular helper (TFH) cells.

Lesions characteristic of AITL were originally described in the early 1970s as non-neoplastic abnormal immune reactions, variously termed *angioimmunoblastic lymphadenopathy with dysproteinemia, immunoblastic lymphadenopathy, lymphogranulomatous X,* and *immunodysplastic disease*. The disease was characterized by generalized lymphadenopathy, fever, hypergammaglobulinemia, various autoimmune phenomena, and often a fatal outcome. Later, an immunoblastic lymphadenopathy–like malignant lymphoma was described and suggested that angioimmunoblastic lymphadenopathy with dysproteinemia was actually a type of PTCL. Subsequently, it was shown that most cases of angioimmunoblastic lymphadenopathy with dysproteinemia had clonal rearrangements of T-cell receptor (*TCR*) genes in addition to nonrandom chromosomal abnormalities. Therefore, lymphoproliferations with features of angioimmunoblastic lymphadenopathy with dysproteinemia were subsequently recognized as being de novo AITL in the WHO classification.

Clinical Features

Patients with AITL are typically middle-aged to elderly adults with a systemic illness characterized by generalized lymphadenopathy, fever, weight loss, hepatosplenomegaly, skin rash, and polyclonal hypergammaglobulinemia. There is a nearly equal incidence between genders. Most patients have stage III or IV disease at presentation. Autoimmune hemolytic anemia is seen in up to 45% of cases. AITL is moderately aggressive, with a median survival of 1 to 2 years.

Progression to a more aggressive tumor with immunoblastic morphology occasionally occurs. Such transformations are usually of the T-cell phenotype but occasionally may be of B-cell lineage.

Pathologic Features

Microscopic Findings

The nodal architecture is usually effaced by a diffuse lymphoproliferation that may extend beyond the capsule into the pericapsular soft tissue (Fig. 9.1). Follicular centers are generally absent and contain an abnormal proliferation of follicular dendritic cells (FDCs). One of the most striking features of AITL is the proliferation of branching high endothelial venules (HEVs) that are accentuated by periodic acid–Schiff (PAS) with hematoxylin staining. The lymphoid infiltrate may appear hypocellular and consists of a mixture

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ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA—FACT SHEET

Definition
- Nodal T-cell lymphoma characterized by clusters of T-cell immunoblasts, hypervascularity, polymorphic inflammatory cell infiltrate, and proliferated follicular dendritic cell (FDC) networks

Incidence and Location
- Uncommon (1% to 2% of all NHLs but 15% to 20% of peripheral T-cell lymphomas)
- Approximately 1000 new cases per year in the United States

Morbidity and Mortality
- Median survival: 1 to 2 years

Gender and Age Distribution
- Middle-aged to elderly adults
- No gender predilection

Clinical Features
- Generalized lymphadenopathy (stage III or IV disease), hepatosplenomegaly, and skin rash
- B symptoms
- Polyclonal hypergammaglobulinemia and autoimmune hemolytic anemia

Prognosis and Therapy
- Moderately aggressive disease with fair prognosis
- Chemotherapy

ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA—PATHOLOGIC FEATURES

Microscopic Findings
- Partially to completely effaced nodal architecture
- Diffuse lymphoproliferation with clusters of neoplastic T-cell immunoblasts, often in a perivascular distribution
- Proliferation of high endothelial venules
- Disrupted and proliferated FDC networks
- Polymorphic reactive cell background

Immunophenotypic Findings
- $CD4^+$ T-cell immunoblasts that often have aberrant phenotype
- T follicular helper (TFH) cell–associated antigens, such as CD10, CD279, BCL6, CXCL13, CXCR5, and ICOS frequently expressed
- CD21, CD23, CD35, and clusterin staining of disrupted and proliferated FDC networks

Molecular and Cytogenetic Findings
- Clonal T-cell receptor (*TCR*) gene rearrangements in 75% of cases
- Epstein-Barr virus (EBV)-positive B-cell immunoblasts in background
- No recurring chromosomal translocations, but trisomies 3 and 5, an additional X chromosome, and 1p alterations common
- TET2, DNMT3A, IDH2, CD28, and RHOA mutations detectable

Differential Diagnosis
- T-cell–rich large B-cell lymphoma variant of diffuse large B-cell lymphoma
- Mixed cellularity Hodgkin lymphoma
- Florid reactive processes

of small lymphocytes and immunoblasts, the latter having clear cytoplasm and clustering around vessels. Plasma cells, eosinophils, and histiocytes are often present in the background. Occasionally, clusters of epithelioid histiocytes impart a granulomatous appearance. This combination of morphologic features is most commonly seen in AITL and is regarded as pattern III. Pattern I has partially preserved architecture, hyperplastic follicles with poorly preserved mantles, and inconspicuous neoplastic cells distributed around the follicles. Pattern II has depleted or "burned-out" lymphoid follicles. FDCs are normal to only slightly increased in patterns I and II.

Immunophenotype

The neoplastic "clear cells" of AITL express the pan T-cell antigens CD2, CD3, CD5, and CD7 (Fig. 9.2); however, aberrant loss of one or more of these antigens is common. The tumor cells demonstrate a $CD4^+$ (T-helper cell) phenotype and should express two or more TFH cell–associated antigens, such as CD10, CD279 (programmed cell death–1 [PD1]), BCL6, CXC chemokine ligand 13 (CXCL13), CX chemokine receptor 5 (CXCR5), and inducible T-cell costimulator (ICOS). Disrupted and expanded FDC networks can be recognized using antibodies to CD21, CD23, CD35, and clusterin, a feature that helps to distinguish AITL from other PTCLs. Their coexpression of TFH cell–associated antigens and their association with expanded FDC networks are consistent with an origin from TFH cells. Most AITLs are derived from noncytotoxic αβ T cells.

Molecular and Cytogenetic Findings

Approximately 75% of cases of AITL will demonstrate clonal rearrangement of TCR genes. Many cases will also show an oligoclonal or small clonal population of B cells. Indeed, dominant B-cell clones in the absence of obvious diffuse large B-cell lymphoma (DLBCL) (see later discussion) have been reported in AITL, reflecting the immune dysregulation present in these patients. Trisomies 3 and 5, an additional chromosome X, and 1p alterations are the karyotypic abnormalities found most frequently; however, no single translocation is associated with most cases. Gene expression profiling has shown a strong contribution by non-neoplastic cell constituents with overexpression of B-cell–related and FDC-related genes and genes related to extracellular matrix and vascular biology. The tumor cell signature appears enriched in genes normally expressed by TFH cells, which has further established a TFH-cell derivation for AITL.

An increased number of Epstein-Barr virus (EBV)-positive B-cell immunoblasts is detected in many cases by in situ hybridization for EBV-encoded RNA (EBER) (see Fig. 9.2).

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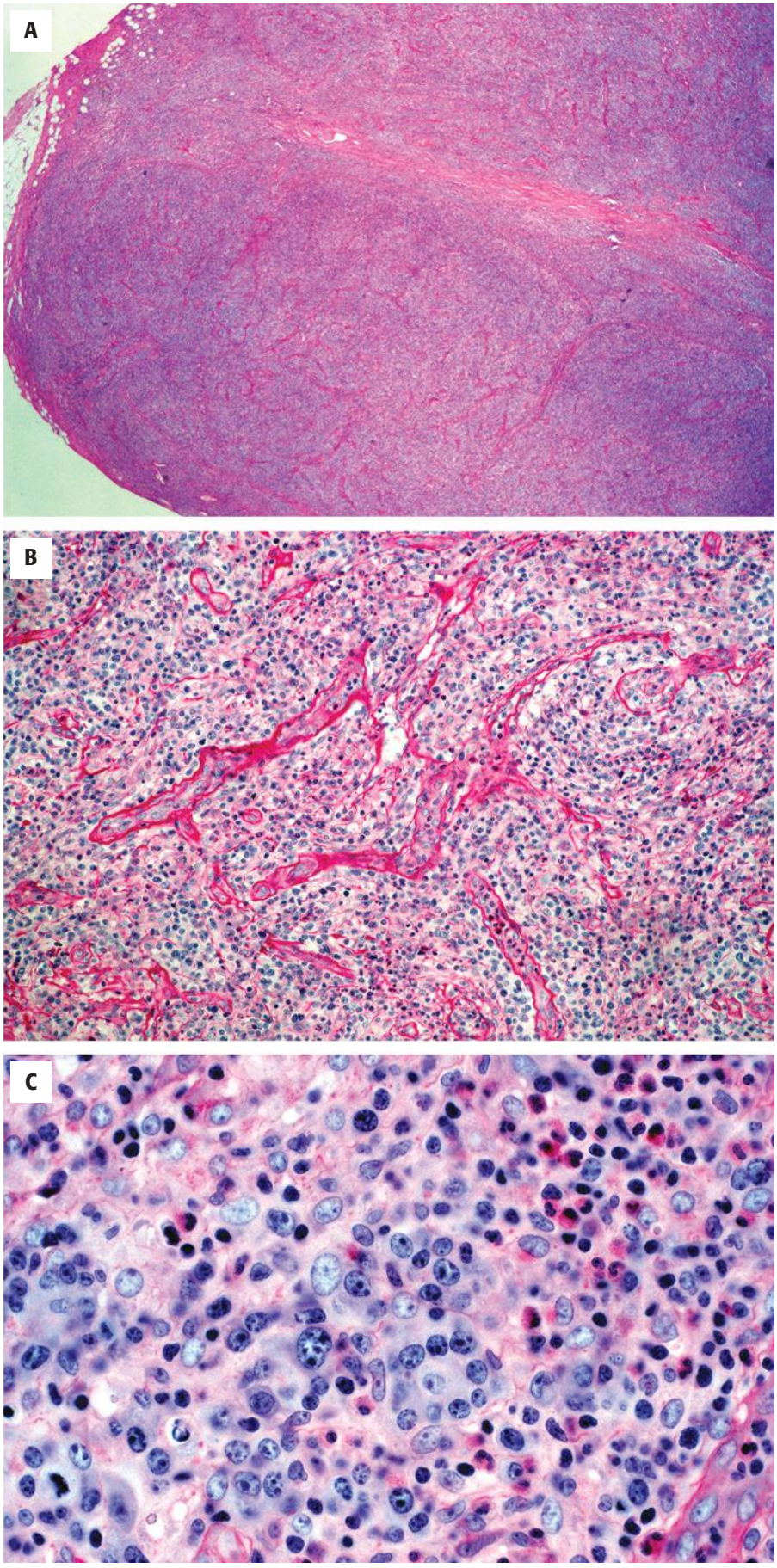


FIG. 9.1

Angioimmunoblastic T-cell lymphoma: microscopic features. A, At low magnification, the lymph node architecture is completely effaced by a diffuse lymphoproliferation that appears hypervascular in this periodic acid–Schiff (PAS) stain. B, At intermediate magnification, the branching pattern of the numerous high endothelial venules can be appreciated in this PAS stain. C, At high magnification, clusters of immunoblasts with clear cytoplasm are present within a polymorphic reactive cell background that includes frequent eosinophils.

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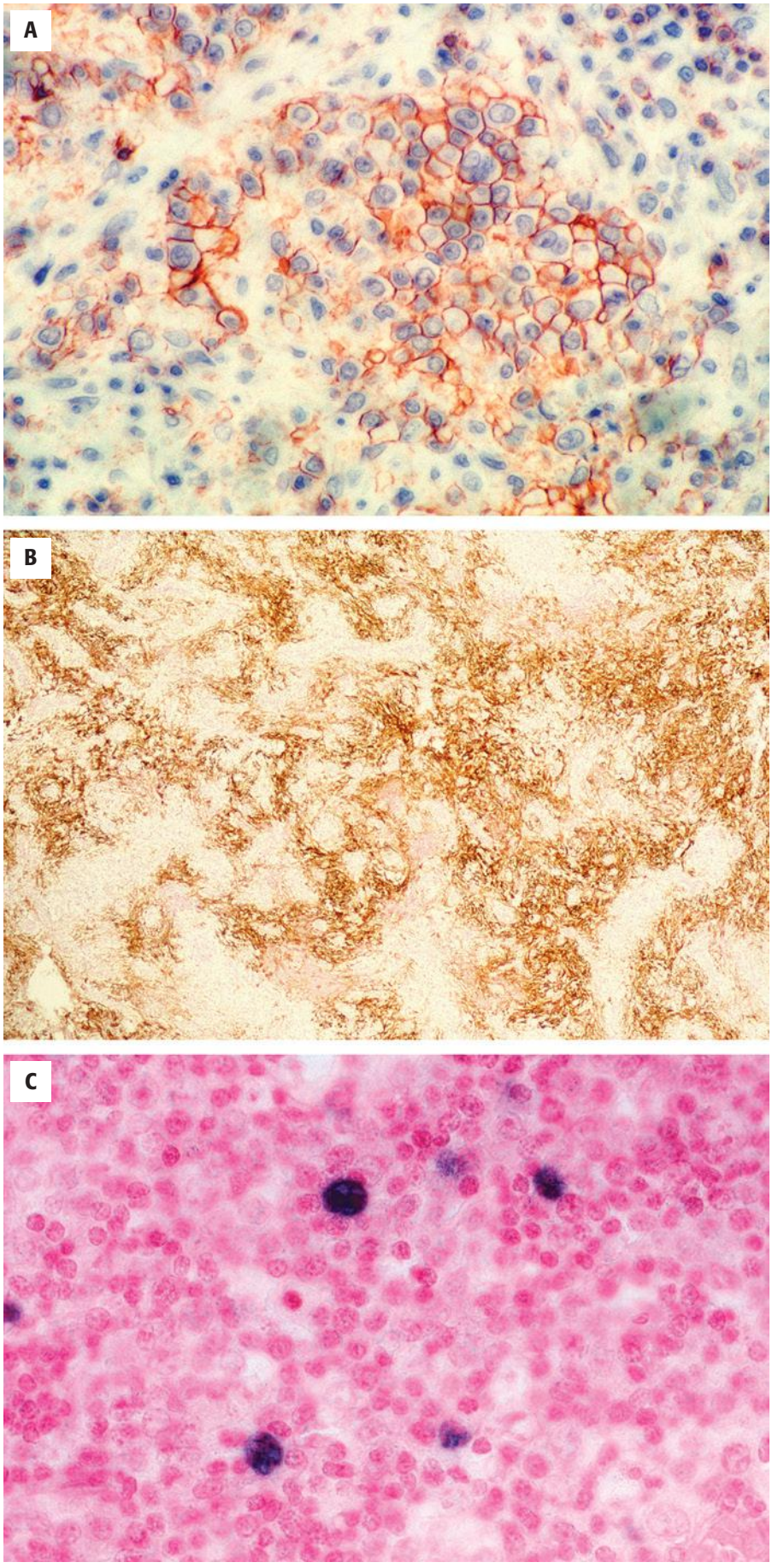


FIG. 9.2

Angioimmunoblastic T-cell lymphoma: immunophenotypic features and in situ hybridization. A, $CD3^+$ T-cell immunoblasts are easily identified in this paraffin immunoperoxidase stain. B, Disrupted and proliferated CD21-staining follicular dendritic cell networks stand out in this paraffin immunoperoxidase stain. C, A background of Epstein-Barr virus (EBV)-positive immunoblasts is detected by in situ hybridization using probes for EBV-encoded RNA.

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A specific role for EBV in the pathogenesis of AITL remains unproven.

Mutations in epigenetic regulators—*TET2, DNMT3A,* and *IDH2*—are common in AITL but are not specific for this disease. Mutations in the Ras homolog gene family, member A (*RHOA* G17V) appear to be more disease specific, occur in approximately 70% of AITLs, and may require a concurrent *TET2* mutation for the development of AITL. A highly recurrent novel missense mutation in *CD28* (*CD28* T195P) has been reported in approximately 10% of AITL cases and appears relatively specific for AITL. A *CTLA4-CD28* fusion also has been found in 58% of AITL but does not appear specific, being found in 23% of peripheral T-cell lymphomas, not otherwise specified (PTCL, NOS) and 29% of NK/T-cell lymphomas. Of note, within PTCL, NOS, those cases with a TFH phenotype more frequently had the fusion (56%) compared to those without a TFH phenotype (31%). Both the mutation and the fusion appear to result in activation of TCR signaling pathways (such as AKT, ERK1/2), and nuclear factor kappa B (NFκB) pathways, presumably providing a growth or survival advantage. These alterations in *CD28* remain to be independently confirmed.

Differential Diagnosis

AITL must be distinguished from the T-cell–histiocyte-rich large B-cell lymphoma variant of DLBCL (TCHRLBCL), mixed cellularity classical Hodgkin lymphoma, and some florid reactive lymph nodes. TCHRLBCLs do not have clusters of clear T-cell immunoblasts and may be immunoglobulin light-chain restricted. A diagnosis of TCHRLBCL may require confirmation by demonstrating clonal immunoglobulin gene rearrangements in the absence of T-cell clonality. EBV^+ immunoblasts are not seen in TCHRLBCL. Mixed cellularity classical Hodgkin lymphoma will generally show classic Reed-Sternberg cells or Reed-Sternberg–cell variants that are $CD45^-$, $CD15^+$, $CD30^+$, and fascin positive; the lack of proliferated FDC networks may also be a helpful distinguishing feature. Both florid reactive processes and AITL can show an expanded T zone, paracortical area, or vascular proliferation. Reactive processes generally show preserved germinal centers and no evidence of abnormal FDC networks. In contrast, AITL will usually show loss of or burned-out germinal centers plus abnormal FDC networks. Reactive processes generally lack clonal TCR gene rearrangements.

EBV^+ B-cell lymphomas, principally DLBCL but also rarely classical Hodgkin lymphoma, can complicate AITL; this can occur in up to 23% of cases. These cases are thought to arise as part of the overall immunosuppressed state of the patient; therefore when making a diagnosis of AITL, always search for evidence of a second lymphoma such as EBV^+ DLBCL. Diagnosis of DLBCL or Hodgkin lymphoma should be made only when clear histopathologic evidence, such as large sheets of large B cells in the former, is present.

OTHER NODAL LYMPHOMAS OF T FOLLICULAR HELPER CELL ORIGIN

The updated 2016 WHO classification introduced the concept of nodal T-cell lymphomas of TFH phenotype to include cases of AITL, follicular T-cell lymphomas (FTLs), and cases that would otherwise be classified as PTCL, NOS, but demonstrated a TFH phenotype (at least two to three markers associated with TFH phenotype). FTL is rare but is well described and is now included among the lymphomas of TFH cell origin because the tumor cells express CD10, CD279, BCL6, CXCL13, CXCR5, and/or ICOS similar to their expression in AITL. However, in FTL, neoplastic "clear cells" with a TFH-cell phenotype form intrafollicular or perifollicular clusters that do not efface the nodal architecture and are not associated with enlarged and disrupted FDC networks or with a proliferation of HEVs, as commonly observed in AITL. The follicular expansion may mimic follicular lymphoma or resemble nodular lymphocyte-predominant Hodgkin lymphoma when associated with progressive transformation of follicle centers. A subset of FTL has a t(5;9)(q33;q22) chromosomal abnormality, which is rarely detected in AITL and not at all in other PTCLs. This translocation results in a novel *ITK-SYK* fusion tyrosine kinase that mimics activated TCRs and has been shown to produce T-cell lymphoma in animal models. FTL, which is more likely than AITL to present with localized disease, may show subsequent development of AITL in serial biopsies, which suggests they are different morphologic patterns to a common biologic process.

Nodal peripheral T-cell lymphoma with TFH cell phenotype is a designation that should be reserved for lymphomas that have a $CD4^+$ and TFH cell phenotype and lack typical morphologic features of AITL or FTL. These lymphomas tend to have a diffuse growth pattern and do not have a polymorphic reactive cell background, expanded FDC networks, or hypervascularity. They may have similar genetic abnormalities as AITL.

ANAPLASTIC LARGE-CELL LYMPHOMA—ALK POSITIVE AND ALK NEGATIVE

Anaplastic large-cell lymphoma (ALCL) was first described in 1985 and is one of the three most common nodal PTCLs. It accounts for up to 3% of all adult NHLs and 10% to 30% of pediatric lymphomas. Prototypic features include a pleomorphic large-cell infiltrate with involvement of nodal sinuses, immunoreactivity with anti-CD30 antibodies, and the presence of a nonrandom chromosomal translocation, t(2;5)(p23;q35), in approximately 60% of cases. ALCL can be subdivided into systemic (nodal) and cutaneous forms, with the latter being discussed in the section on primary cutaneous $CD30^+$ T-cell lymphoproliferative disorders. The WHO classification further separates systemic ALCL into anaplastic lymphoma kinase (ALK)-positive and ALK-negative

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disease entities, both of which will be discussed together in this section.

Clinical Features

There is a bimodal age distribution for systemic ALCL, similar to that in Hodgkin lymphoma, with a large peak in children or young adults (mostly ALK positive) and a small peak in older adults (mostly ALK negative). Patients often have lymphadenopathy, extranodal masses, or both; B symptoms (e.g., fever, night sweats, and weight loss); and stage III or IV disease. Extranodal sites most commonly involved, in order of frequency, are skin, bone, soft tissues, lung, and liver.

There is a male predominance in younger patients and a slight female predominance in older patients.

Systemic ALCL is moderately aggressive, and prognosis is closely related to tumor cell genetics. Patients with ALK-positive tumors generally have a favorable response to treatment with up to 85% 5-year overall survival (OS) rate, whereas those with ALK-negative tumors, as a whole, have an approximately 50% 5-year OS rate. However, ALK-negative ALCLs are genetically heterogeneous, which affects patient outcome. ALK-negative ALCLs that have a *DUSP22(IRF4)* rearrangement at 6p25.3 have a 5-year OS rate (90%), similar to ALK-positive tumors. ALK-negative ALCLs that have a *TP63* rearrangement at 3q28 have a 17% 5-year OS rate. Triple-negative ALCLs, which lack rearrangement of *ALK, DUSP22*, or *TP63* have a 42% 5-year OS rate.

A provisional entity in the updated WHO classification of lymphoid neoplasms is breast implant–associated ALCL, which is a rare and indolent ALK-negative disorder that presents often with recurrent seromas around the breast implant.

ANAPLASTIC LARGE-CELL LYMPHOMA—FACT SHEET

Definition

- Systemic (nodal) T-cell lymphoma characterized by pleomorphic $CD30^+$ large T cells that often involve nodal sinuses

Incidence and Location

- Uncommon (3% of all adult NHLs but 10% to 30% of pediatric lymphomas)

Morbidity and Mortality

- ALK-positive ALCL: 85% 5-year overall survival rate
- ALK-negative ALCL: 50% 5-year overall survival rate
  - ALK-negative, *DUSP22* rearranged: 90% 5-year overall survival rate
  - ALK-negative, *TP63* rearranged: 17% 5-year overall survival rate
  - ALK-negative, *DUSP22*-negative, *TP63*-negative: 42% 5-year overall survival rate

Gender and Age Distribution

- Bimodal age distribution with large peak in childhood or young adults (mostly ALK-positive) and small peak in older adults (mostly ALK-negative)
- Male predominance in younger patients and slight female predominance in older patients

Clinical Features

- Lymphadenopathy or extranodal masses (stage III or IV disease), or both
- B symptoms

Prognosis and Therapy

- Moderately aggressive disease with prognosis related to tumor cell genetics
- Chemotherapy
- Anti-CD30 monoclonal antibody conjugated with monomethyl auristatin E (brentuximab vedotin)

Pathologic Features

Microscopic Findings

Histopathologic features are distinctive. The large cells, which may appear cohesive, extend from the subcapsular sinuses into the paracortical region, often sparing germinal centers (Fig. 9.3). The neoplastic cells are sometimes largely confined to the nodal sinuses, although more often complete architectural effacement is noted. The "hallmark" tumor cell is a large transformed cell with irregular nuclear contours, a prominent nucleolus, and abundant eosinophilic cytoplasm. Multinucleated tumor cells may have nuclei arranged in a circular pattern (i.e., wreath cells) or in a horseshoe shape. Several morphologic subtypes have been described, subdivided according to tumor cell size, nuclear pleomorphism, types of reactive cells, and degree of fibrosis. Histologic types that have been described for ALK-positive ALCL include the common type with pleomorphic or monomorphic large tumor cells (approximately 70% of ALCLs), lymphohistiocytic variant (10%), small-cell variant (5% to 10%), and Hodgkin-like variant (3%). In the lymphohistiocytic and small-cell variants, large tumor cells are a relatively minor population in a background of small, irregular lymphocytes. Occasional cases have abundant histiocytes, fibrosis, and increased neutrophils. In the small-cell variant, the large tumor cells can often be found around vessels. The Hodgkin-like variant has morphologic features resembling nodular sclerosis classical Hodgkin lymphoma. ALK-negative, *DUSP22* rearranged cases typically show sheets of "hallmark" cells, like the common type of ALK-positive ALCL, and few large pleomorphic cells, which tend to be more numerous in the ALK-negative, *DUSP22*-negative tumors.

Unlike other large-cell lymphomas, bone marrow involvement might not be obvious on routine hematoxylin and eosin–stained biopsy sections because of the propensity for single tumor–cell infiltration in ALCL. Studies have shown that with routine hematoxylin and eosin sections alone,

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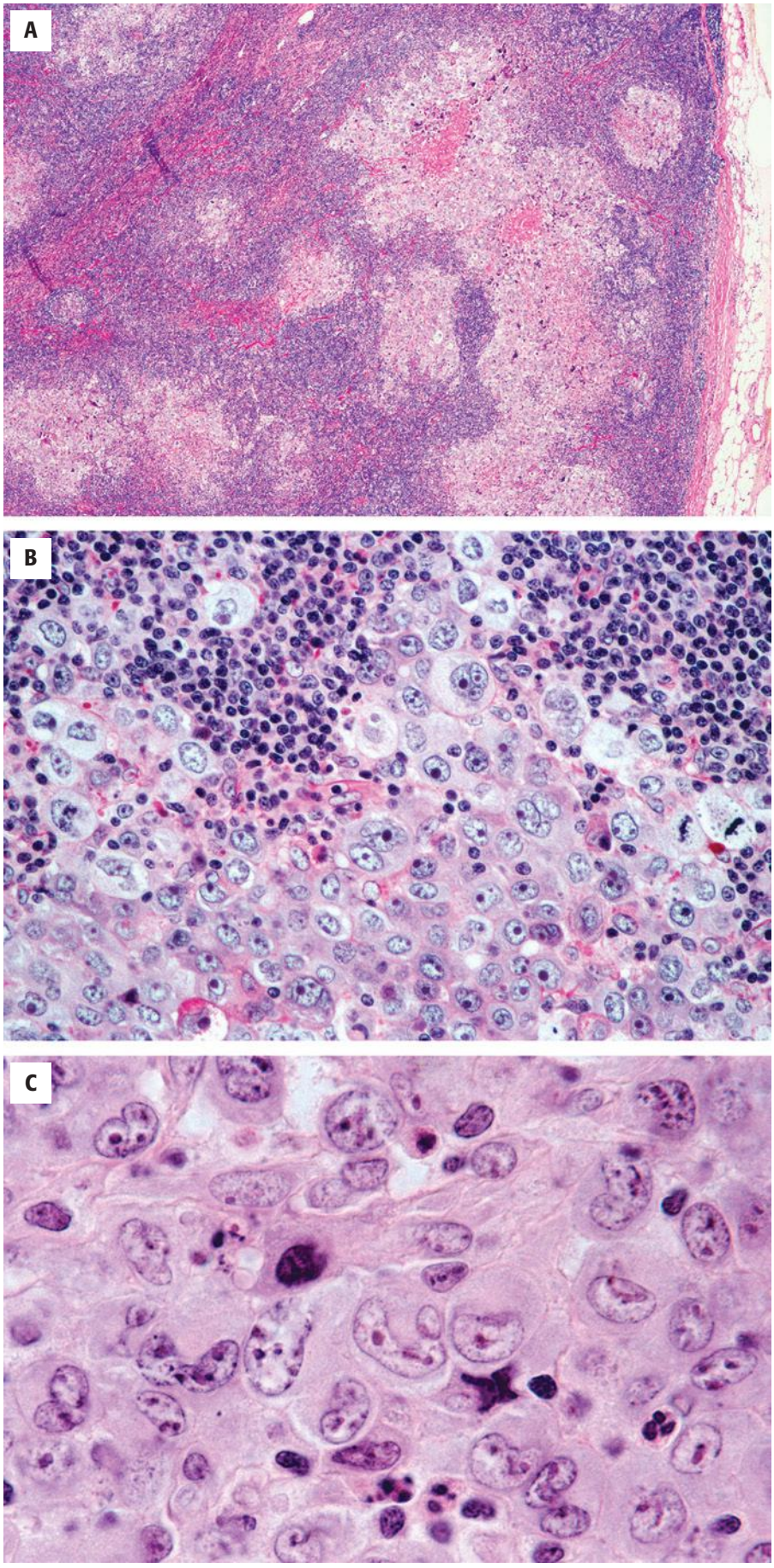


FIG. 9.3

Anaplastic large-cell lymphoma: microscopic features. A, At low magnification, neoplastic cells infiltrate and expand the lymph node sinuses. B, At intermediate magnification, large tumor cells appear cohesive. C, At high magnification, the large tumor cells show anaplastic nuclei and abundant eosinophilic cytoplasm. A "hallmark" cell with a horseshoe-shaped nucleus is located centrally.

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ANAPLASTIC LARGE-CELL LYMPHOMA—PATHOLOGIC FEATURES

Microscopic Findings
- Partially to completely effaced tissue architecture or confined to nodal sinuses
- Large tumor cells with circular or horseshoe-shaped nuclei, prominent nucleoli, and abundant eosinophilic cytoplasm ("hallmark" cells)
- Subtypes among ALK-positive tumors: common (70%), lymphohistiocytic (10%), small cell (5% to 10%), and Hodgkin-like (3%)

Immunophenotypic Findings
- $CD30^+$ tumor cells
- T-cell phenotype (80% to 90%); null cell phenotype (10% to 20%)
- ALK-positive (70% to 80%); ALK-negative (20% to 30%)

Molecular and Cytogenetic Findings
- Clonal TCR gene rearrangements in 90% of cases
- EBV-negative by EBER in situ hybridization
- t(2;5)(p23;q35) in 60% of cases (70% to 80% of ALK-positive cases)
- *DUSP22(IRF4)* or *TP63* rearrangement in some ALK-negative cases

Differential Diagnosis
- Metastatic carcinomas and melanomas
- Histiocytic sarcomas
- Classical Hodgkin lymphomas
- PTCL, NOS
- Reactive processes (lymphohistiocytic and small cell variants of ALCL)

approximately 10% of cases will show detectable involvement. However, if bone marrow evaluation is done in combination with staining for CD30 or ALK, or both, the percentage of cases with detectable involvement is closer to 30%.

Immunophenotype

The tumor cells of ALCL are always $CD30^+$, usually with both cytoplasmic membrane and Golgi region staining by immunohistochemistry (Fig. 9.4). The majority of ALCLs have a $CD4^+$ T-cell phenotype, but CD3 is absent in approximately 75% of cases. Approximately 10% to 20% of cases do not express T-cell antigens and are regarded as null cell type, but they usually can be shown to be of T-cell origin if T-cell gene rearrangement studies are performed. Although B-cell cases with an anaplastic morphology and CD30 positivity were accepted in the original definition of ALCL, they are now included among the diffuse large B-cell lymphoma category in the WHO classification. Most ALCLs are $CD45^+$; however, 20% to 40% of cases may lack or have only weak staining. Approximately 60% of cases are epithelial membrane antigen positive. Approximately 70% to 80% of ALCLs are ALK-positive, with the majority of these demonstrating both diffuse cytoplasmic and nuclear staining. Other ALK-positive ALCLs have cytoplasmic or, more rarely, membranous staining only. When this occurs, a variant *ALK* translocation is present other than *NPM1-ALK*. ALCLs are derived from cytotoxic T cells, as the tumor cells routinely stain for TIA-1, granzyme B, or perforin. ALK-negative, *DUSP22* rearranged ALCLs tend to lack cytotoxic proteins. A small subset (15% to 25%) of ALCL cases is also $CD15^+$.

Molecular and Cytogenetic Findings

Most ALCLs (90%) have clonal TCR gene rearrangements. Tumor cells are negative for the EBV by in situ hybridization for EBER. A t(2;5)(p23;q35) chromosomal abnormality is present in most ALCLs, which translocates a novel *ALK* gene on chromosome 2p23 next to the nucleolar organizing gene, nucleophosmin (*NPM*), on chromosome 5q35. This translocation results in a fusion protein that can be detected using antibodies that recognize ALK, which produces a cytoplasmic, nuclear, and nucleolar staining pattern. In addition, a number of variant translocations have been described that result in translocation of *ALK* to other gene partners, including some on chromosomes 1q21, 1q25, 3q21, 4q33 Xq11-12, 17q23, 17q25, 19p13.1, and 22q11.2 and in inversions of 2p23-2q35. These variant translocations can be seen in 20% or more of ALK-positive cases and are often associated with a cytoplasmic-only staining pattern with antibodies to ALK. These *ALK* translocations are seen mostly in young patients and in primary nodal cases. *DUSP22(IRF4)* rearrangement at 6p25.3 or *TP63* rearrangement at 3q28 can be detected in 30% and 8% of ALK-negative ALCLs, respectively.

Finally, gene-expression profiling can show molecular separation of ALK-positive ALCL from ALK-negative ALCL, PTCL, NOS, and adult T-cell leukemia–lymphoma. This finding supports the separate classification of these lymphomas.

Differential Diagnosis

Nodal ALCLs must be distinguished from metastatic carcinomas and melanomas and from histiocytic sarcomas because of their sinusoidal infiltrates. Other common-type ALCLs and the Hodgkin-like variant must be distinguished from classical Hodgkin lymphomas. The lymphohistiocytic and small-cell variants of ALCL need to be distinguished from reactive processes. Distinguishing among these different entities can generally be accomplished using appropriate immunohistochemical markers because Reed-Sternberg cells of Hodgkin lymphoma, in addition to CD30 expression, are almost always positive for PAX5, often express CD15, and lack T-cell markers. The small-cell variant of ALK^+ ALCL can present in leukemic phase, mimicking a mature T-cell leukemia. A high index of suspicion, expression of CD30 by flow cytometry or immunohistochemistry in tissue section (bone marrow biopsy or lymph node biopsy) with demonstration of an ALK translocation by molecular/cytogenetic

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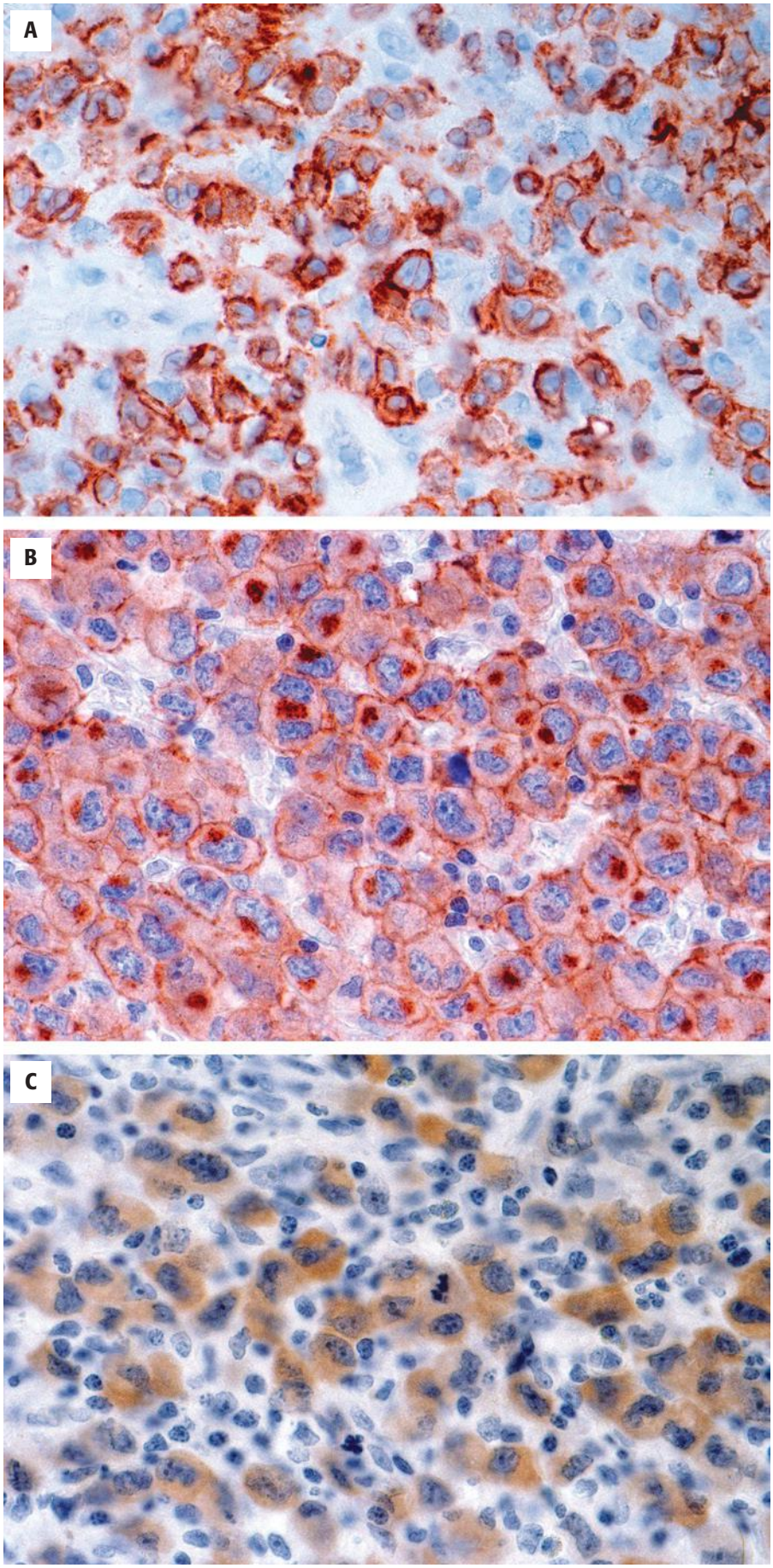


FIG. 9.4

Anaplastic large-cell lymphoma (ALCL): immunophenotypic features. A, The tumor cells are $CD3^+$ in this paraffin immunoperoxidase stain. B, Uniform strong cytoplasmic membrane and Golgi region CD30 positivity are seen in the tumor cells of most ALCLs. C, The tumor cells are ALK positive in this paraffin immunoperoxidase stain.

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methods or immunohistochemistry will result in the correct diagnosis.

ALK-negative ALCLs may be difficult to differentiate from CD30$^+$ PTCL, NOS. The presence of "hallmark" cells, growth patterns such as sinusoidal involvement, and a *DUSP22* or *TP63* rearrangement favor a diagnosis of ALK-negative ALCL. The separation is not entirely academic because some reports demonstrate a more favorable outcome for many ALK-negative ALCLs as compared with the generally poor outcome of PTCL, NOS. However, current first-line therapies for ALCL and PTCL, NOS are not yet based on histologic type.

The breast implant–associated ALCLs should be specifically recognized given the favorable and indolent nature of this lymphoproliferation. Although pathologically the cells have the cytologic and immunophenotypic features typical of ALK-negative ALCL, they occur in a fibrinous and serous background, usually without tissue infiltration. Tumors that are confined to the seroma and capsule typically resolve on removal of the implant. The few cases with overt tissue infiltration or nodal involvement may require treatment with systemic chemotherapy. Breast implant–associated ALCL can be identified readily based on the clinical context.

PERIPHERAL T-CELL LYMPHOMA, NOS—FACT SHEET

Definition
- Nodal or extranodal T-cell lymphoma, the features of which do not fit any of the defined T-cell lymphoma entities

Incidence and Location
- Uncommon, but the most frequently occurring nodal T-cell lymphoma

Morbidity and Mortality

30% 5-year overall survival rate
>70% neoplastic large cells in tumor associated with worse prognosis
Lymphoepithelioid lymphoma variant may have better prognosis than other PTCL, NOS

Gender and Age Distribution
- Adults
- No gender predilection

Clinical Features
- Generalized lymphadenopathy (stage III or IV disease)
- B symptoms

Prognosis and Therapy
- Aggressive disease with poor prognosis
- Chemotherapy

PERIPHERAL T-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED

PTCL, NOS, is the most common nodal PTCL, but it can occur in extranodal sites as well. It accounts for approximately 30% of all forms of PTCLs. This category is a catch-all for PTCLs that are yet to be well defined in terms of their classification into distinct entities.

Clinical Features

Most patients with PTCL, NOS, are adults with generalized lymphadenopathy and B symptoms. There is no gender predilection. These are aggressive lymphomas, and patients have an approximately 30% 5-year OS rate. A single morphologic variant that does not appear to be a distinct clinicopathologic entity has been recognized, lymphoepithelioid (Lennert) lymphoma, which may have a better prognosis than other PTCL, NOS.

Pathologic Features

Microscopic Findings

Tissue architecture is generally effaced by a diffuse lymphoproliferation that consists of tumor cells of varying size, often within a polymorphic reactive cell background. Large tumor cells may have hyperlobated nuclei or resemble Reed-Sternberg cells (Fig. 9.5). PTCLs, NOS, that have more than 70% neoplastic large cells tend to behave poorly.

Lymphoepithelioid lymphoma has a proclivity to involve Waldeyer's ring and cervical lymph nodes. Tissues are effaced, and there are numerous evenly dispersed clusters of epithelioid histiocytes between which are infiltrates of mostly small tumor cells (see Fig. 9.5).

Immunophenotype

Given that PTCL, NOS, is likely a heterogeneous category, there is no specific diagnostic immunophenotype. Most cases of PTCL, NOS, have an aberrant T-cell phenotype, with loss of CD3 or CD7 most frequently observed. Most PTCL, NOS, cases are CD4$^+$ αβ T-cell lymphomas, although some are CD8$^+$ and a few have γδ TCRs. Some of these lymphomas have a cytotoxic T-cell phenotype or express NK-cell–associated antigens such as CD56.

Molecular and Cytogenetic Findings

Most of these lymphomas have clonal TCR gene rearrangements, and a few may have EBV in the tumor cells or in non-neoplastic B-cell immunoblasts. No consistent specific cytogenetic abnormality has been identified for most PTCLs, NOS, but two cytotoxic PTCLs with skin and bone marrow involvement and a t(6;14)(p25;q11.2) involving *IRF4* and *TCRA* have been described. Gene expression profiling has identified

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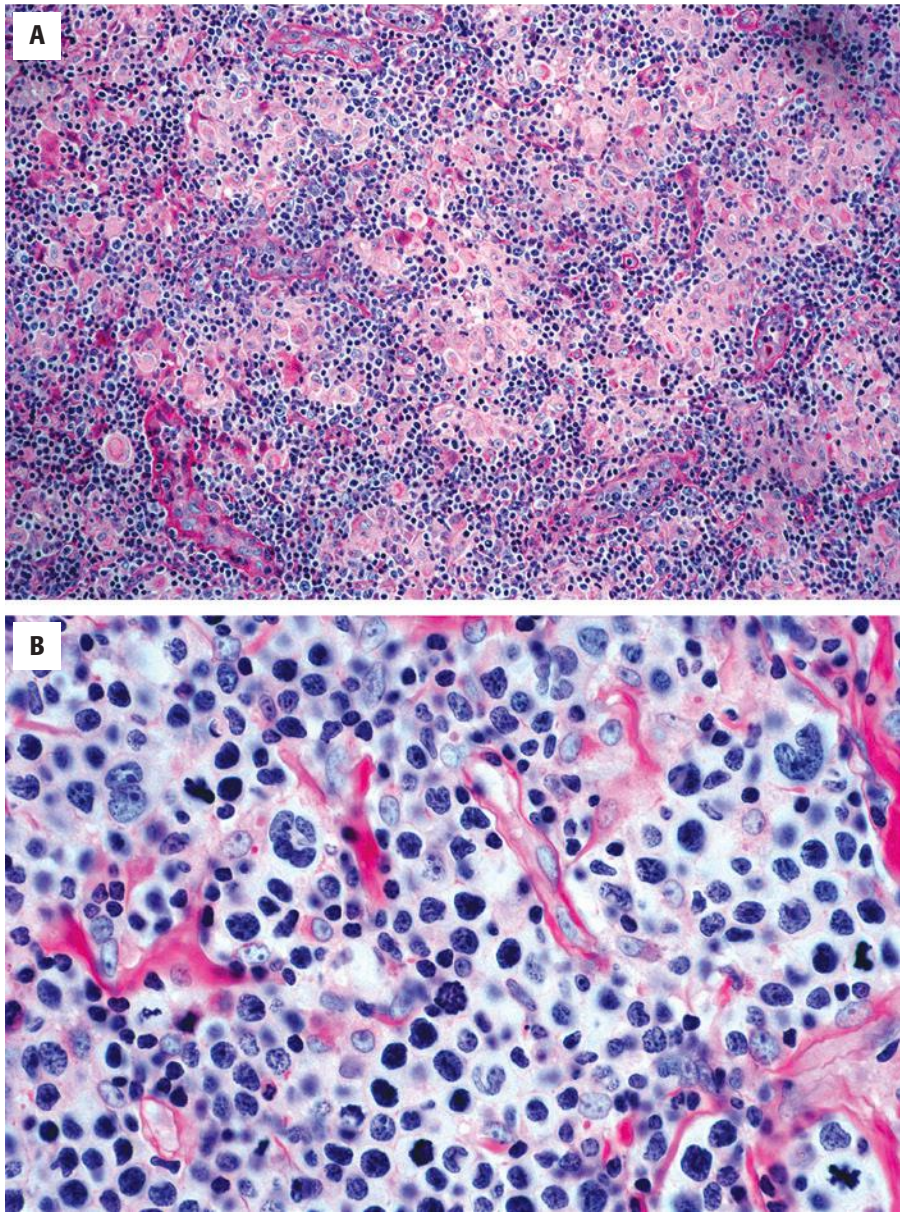


FIG. 9.5

Peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS): microscopic features. A, At low magnification, the lymph node architecture is effaced by a diffuse infiltrate of neoplastic T cells that lie between evenly dispersed clusters of epithelioid histiocytes in this lymphoepithelioid (Lennert) lymphoma variant of PTCL, NOS. B, At high magnification, pleomorphic tumor cells with clear cytoplasm, some resembling Reed-Sternberg cells, are present in another PTCL, NOS.

PERIPHERAL T-CELL LYMPHOMA, NOS—PATHOLOGIC FEATURES

Microscopic Findings

- Effaced architecture
- Diffuse lymphoproliferation with tumor cells of varying size, often with polymorphic reactive cell background
- Lymphoepithelioid variant: infiltrate of mostly small lymphocytes associated with numerous evenly dispersed epithelioid histiocytes

Immunophenotypic Findings

- Variable T-cell antigen expression that is often aberrant

Molecular and Cytogenetic Findings

- Clonal TCR gene rearrangements in most cases
- Tumor cells EBV-positive in few cases
- t(6;14)(p25;q11.2) in rare cytotoxic PTCL, NOS, involving skin and bone marrow
- *GATA3* and *TBX21* (*T-BET*) expression may represent clinically relevant molecular subgroups of PTCL, NOS

Differential Diagnosis

- B-cell lymphomas
- Classical Hodgkin lymphomas
- Adult T-cell leukemia–lymphoma
- Reactive processes (lymphoepithelioid lymphoma variant)

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two major molecular subgroups of PTCL, NOS, that express *GATA3* or *TBX21* (*T-BET*). *GATA3* expression is present in approximately 33% to 45% of PTCL, NOS, is associated with the production of Th2-associated cytokines, and has a poor prognosis (19% 5-year OS rate). *TBX21* expression can be detected in up to 49% of PTCL, NOS, is associated with the production of Th1-associated cytokines, often has tumor cells with a cytotoxic phenotype, and appears to have a better prognosis (38% 5-year OS rate) than the *GATA3* subgroup.

Mutations in *DNMT3A* (27%) and *TET2* (48%) appear to be relatively frequent and often occur together but are not specific for PTCL, NOS. Likewise, as noted in the section on AITL, *CTLA4-CD28* gene fusion has been seen in 23% of cases but is also not specific. *RHOA* G17V mutations may occur in PTCL, NOS, but appear to segregate with those cases that have a TFH phenotype.

Differential Diagnosis

PTCL, NOS, must be distinguished from B-cell lymphomas and occasionally from classical Hodgkin lymphomas. This can generally be accomplished by immunophenotyping, but gene rearrangement studies may also be helpful. It should be remembered that rare cases of PTCL, NOS, have been reported to express CD30 and CD15, mimicking Hodgkin lymphoma. However, there is often a monomorphic appearance of lymphoma cells or expression of multiple T-cell markers, or both, often including CD4 in such cases of PTCL. Some PTCLs, NOS, cannot be distinguished from adult T-cell leukemia–lymphoma without knowledge of the patient's human T-lymphotropic virus 1 (HTLV-1) status. Similarly, lymph node involvement by other T-cell leukemias or lymphomas is difficult to distinguish from PTCL, NOS, without clinical history. T-cell prolymphocytic leukemia (T-PLL) often involves lymph nodes, and without clinical history it might not be possible to make the appropriate diagnosis unless T-cell leukemia/lymphoma protein 1A (TCL-1A) staining is performed and shown to be positive. Lymph node involvement by mycosis fungoides (MF) also requires knowledge of the history of MF to most appropriately make the diagnosis. The lymphoepithelioid lymphoma variant must not be confused with reactive processes. It can be mistaken for a granulomatous process because of the prominent epithelioid histiocytic reaction, but attention should be focused on the accompanying lymphoproliferation that effaces the tissue architecture.

EXTRANODAL NATURAL KILLER/T-CELL LYMPHOMA, NASAL TYPE

As its name implies, extranodal NK/T-cell lymphoma, nasal type, is virtually always extranodal in presentation and most commonly occurs in the nasal cavity, nasopharynx, or palate. This tumor has also been called *polymorphic reticulosis, lethal midline granuloma*, and *angiocentric T-cell lymphoma*. It is most prevalent in eastern Asia and in indigenous people in Mexico and Central and South America. It is generally associated with EBV. Most cases are derived from NK cells, but some arise from cytotoxic T cells.

Clinical Features

Patients often have nasal obstruction, epistaxis, or midfacial destructive lesions with extension of the tumor into the paranasal sinuses and orbit. B symptoms may occur. The disease frequently disseminates to the skin or gastrointestinal tract. Other patients may exhibit cutaneous, gastrointestinal, or testicular tumors without apparent nasal involvement (i.e., extranasal NK/T-cell lymphomas). Lymph nodes are usually not involved. The clinical course is typically aggressive, but some of the lymphomas respond well to systemic chemotherapy. Patients with extranasal presentation tend to have a poorer overall survival compared to those with nasal disease.

Pathologic Features

Microscopic Findings

Regardless of site, the lymphoma will often have an angiocentric, angioinvasive, and angiodestructive infiltrate

EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE—FACT SHEET

Definition
- Extranodal lymphoma of NK or cytotoxic T cells, most frequently occurring in nasal cavity or nasopharynx

Incidence and Location
- Rare
- Most prevalent in east Asia, Mexico, and Central and South America

Morbidity and Mortality
- Variable

Gender and Age Distribution
- Adults
- Male predominance

Clinical Features
- Nasal obstruction, epistaxis, or midfacial destructive lesions
- B symptoms

Prognosis and Therapy
- Aggressive disease with variable prognosis
- Chemotherapy

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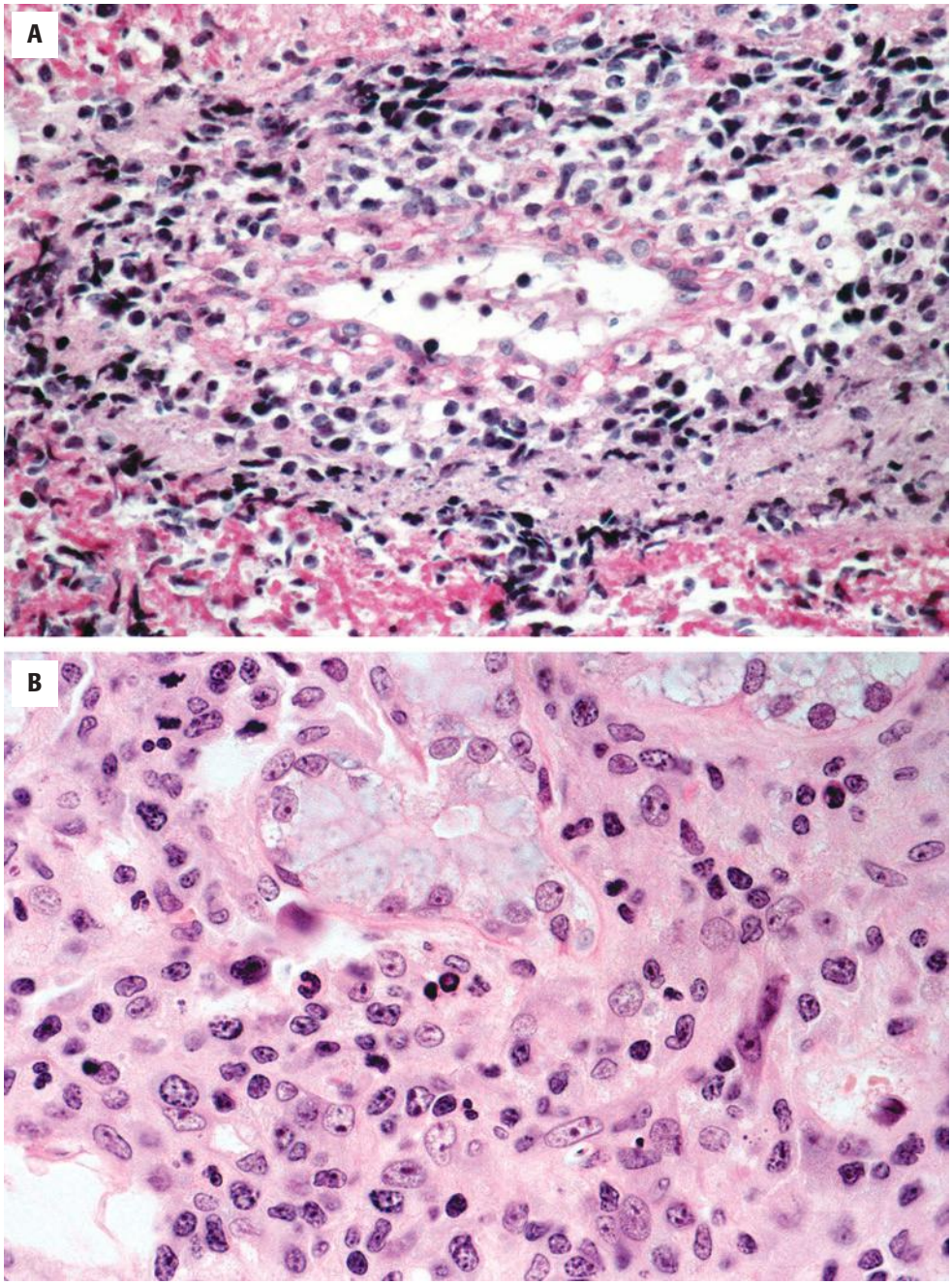


FIG. 9.6

Extranodal natural killer/T-cell lymphoma, nasal type: microscopic features. A, At intermediate magnification, an angiocentric infiltrate of pleomorphic lymphocytes in the nasal cavity is associated with necrosis at the upper and lower margins of the field of view. B, At high magnification, pleomorphic lymphocytes are present between intact glands in the nasal mucosa.

of cytologically atypical lymphocytes of varying size (Fig. 9.6). Many cases will have a diffuse growth of tumor cells. Necrosis is often present and may be extensive. Occasionally there is a polymorphic inflammatory cell background.

Immunophenotype

Tumors derived from NK cells typically have a $CD2^+$ and $CD56^+$ phenotype with expression of cytotoxic granule–associated proteins, TIA-1, granzyme B, and perforin (Fig. 9.7). The NK cells are surface $CD3^-$ and may have cytoplasmic CD3ε staining by immunohistochemistry but do not express TCR-αβ or TCR-γδ proteins. They may be $CD8^+$ but generally lack CD4 and CD5; CD7 is variably present. The few tumors that are T-cell derived will have a similar phenotype except for expression of surface CD3 and TCR proteins.

Molecular and Cytogenetic Findings

TCR genes have a germline configuration in all cases derived from NK cells, whereas those that arise from T cells often have clonal TCR gene rearrangements. The tumor cells are generally EBV^+ (see Fig. 9.7). The most common cytogenetic abnormality is del(6)(q21q25) or i(6)(p10). Gene expression profiling shows distinct clustering of extranodal NK/T-cell lymphoma, nasal type, from cytotoxic PTCL, NOS.

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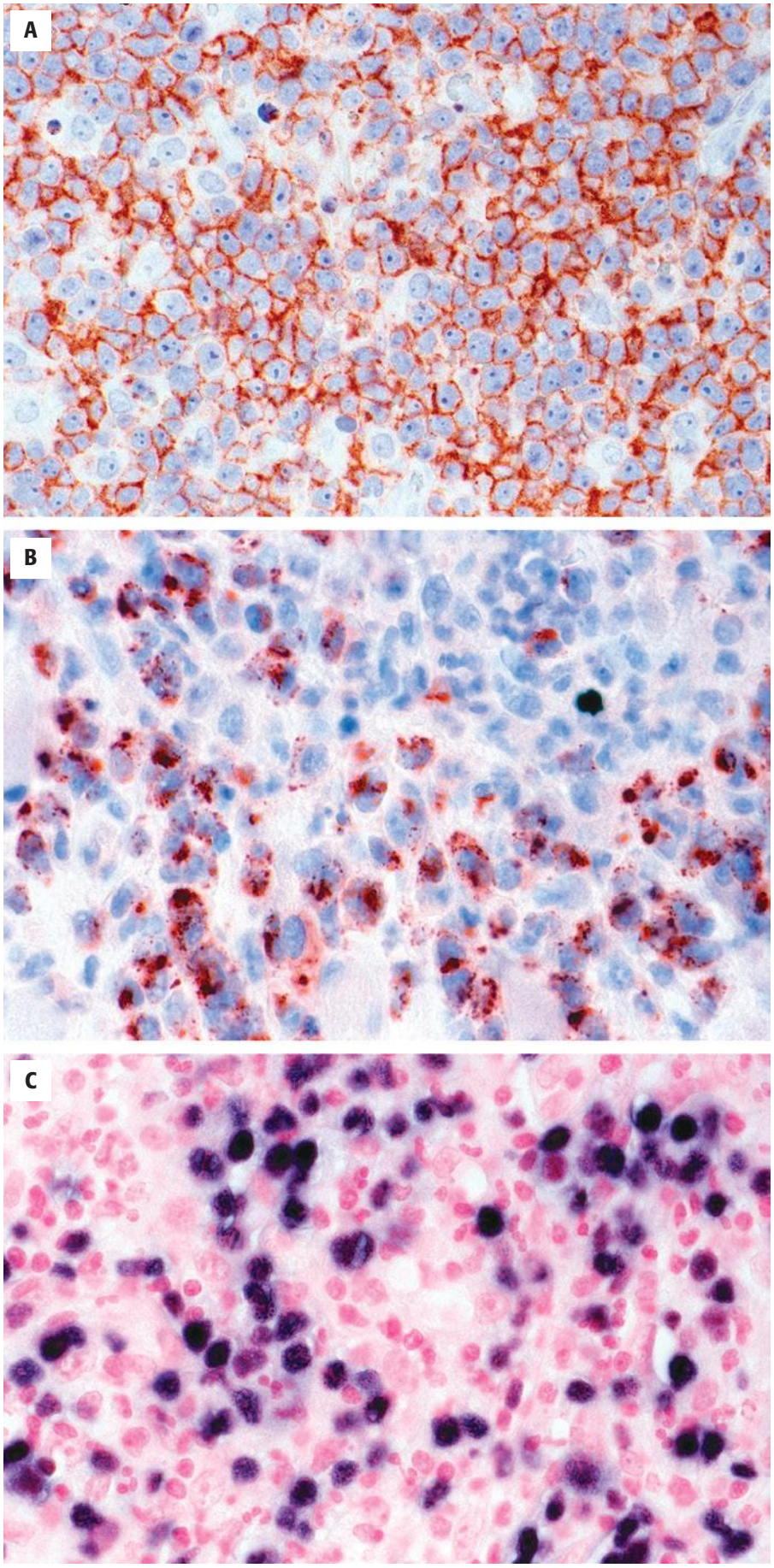


FIG. 9.7

Extranodal natural killer (NK)/T-cell lymphoma, nasal type: immunophenotypic features and in situ hybridization. A, Neoplastic NK cells, or sometimes T cells, generally express CD56 as in this paraffin immunoperoxidase stain. B, The tumor cells have a cytotoxic lymphocyte phenotype, as shown by the granzyme B positivity in this paraffin immunoperoxidase stain. C, Epstein-Barr virus (EBV) is generally detected in the tumor cell nuclei as in this in situ hybridization stain using probes for Epstein-Barr virus (EBV)-encoded RNA.

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EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE—PATHOLOGIC FEATURES

Microscopic Findings
- Angiocentric, angioinvasive, and angiodestructive infiltrate of cytologically atypical lymphocytes of varying size

Immunophenotypic Findings
- NK-cell tumors: $CD2^+$, surface $CD3^-$, $CD56^+$, TCR-αβ negative, and TCR-γδ negative, and expression of cytotoxic granule–associated proteins
- T-cell tumors: $CD2^+$, surface $CD3^{+/-}$, $CD56^{+/-}$, TCR-αβ positive or TCR-γδ positive, and expression of cytotoxic granule–associated proteins

Molecular and Cytogenetic Findings
- NK-cell tumors: no clonal TCR gene rearrangements
- T-cell tumors: clonal TCR gene rearrangements
- EBV detected by EBER in situ hybridization in neoplastic NK cells or in tumor cells of many T-cell cases
- No recurring chromosomal translocations, but del(6)(q21q25) or i(6)(p10) may be present

Differential Diagnosis
- Lymphomatoid granulomatosis
- Wegener granulomatosis
- Diffuse large B-cell lymphomas
- PTCL, NOS
- Subcutaneous panniculitis–like T-cell lymphomas (skin cases)
- NK-cell enteropathy (gastrointestinal cases)

Next-generation sequencing of extranodal NK/T-cell lymphoma, nasal type, samples has shown frequent Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway (55.9% of samples) and histone modification-related (38.2% of samples) gene alterations, and *STAT3, BCOR,* and *MLL2* were the most frequently mutated genes.

Differential Diagnosis

Extranodal NK/T-cell lymphoma, nasal type, has histologic features that are similar to those of lymphomatoid granulomatosis. However, lymphomatoid granulomatosis is recognized as a lymphoma of EBV-positive large B cells that are angiocentric, angioinvasive, and angiodestructive and have an accompanying reaction rich in T cells. Immunophenotyping should differentiate the two lymphomas, and lymphomatoid granulomatosis will likely have clonal immunoglobulin gene rearrangements not seen in extranodal NK/T-cell lymphoma, nasal type. Nasal lesions occasionally will need to be distinguished from Wegener granulomatosis, which can be accomplished with a complete immunophenotypic workup. Extranodal NK/T-cell lymphomas, nasal type, that have a diffuse growth pattern may resemble diffuse large B-cell lymphomas, but immunophenotyping will separate them. Nasal lymphomas that have a $CD3^+$, $CD56^-$ phenotype and are negative for both cytotoxic protein expression and EBV, should be classified as PTCL, NOS. Cutaneous extranodal NK/T-cell lymphomas, nasal type, may infiltrate subcutaneous tissue in a panniculitic pattern requiring distinction from subcutaneous panniculitis–like T-cell lymphoma (SPTCL). This can be accomplished by immunophenotyping and EBV studies, with SPTCL showing no EBV-positive cells.

NK/T-cell lymphoma in the gastrointestinal tract should also be distinguished from the recently described syndrome of *NK-cell enteropathy*. At present, patients with NK-cell enteropathy appear to complain of vague abdominal symptoms and demonstrate erythematous and ulcerative mucosal lesions composed of medium to large atypical cells with irregular nuclei, mature chromatin, and small nucleoli. The cells infiltrate the mucosa and displace glandular elements, but do not show epitheliotropism; they rarely involve the submucosa. However, extensive necrosis, angiocentricity, and angiodestruction are not seen as in nasal type extranodal NK/T-cell lymphoma. The immunophenotype of NK-cell enteropathy (cytoplasmic $CD3^+$, $CD7^+$, $CD56^+$, $CD5^-$, TIA1/GzB^+) may be similar to extranodal NK/T-cell lymphoma, but EBV is absent. Furthermore, the Ki67 proliferative index is usually low (approximately 25%). T-cell clonality studies are polyclonal, and patients appear to have an indolent, nonprogressive course.

SUBCUTANEOUS PANNICULITIS–LIKE T-CELL LYMPHOMA

SPTCL is a rare form of PTCL that produces subcutaneous nodular lesions and is composed of cytotoxic αβ T cells. In prior classifications, similarly appearing lymphomas derived from γδ T cells were included as SPTCL, but these have since been separated and classified as primary cutaneous γδ T-cell lymphoma because of their different clinical behavior.

Clinical Features

SPTCLs usually manifest with multiple, nontender, small to large (0.5 to 12 cm) erythematous subcutaneous nodules or plaques on the extremities, trunk, or both. The face and head are occasionally involved. Most patients are younger adults (median age, 43 years; range, 5 months to 84 years), with a slight female predominance. B symptoms are common. Most patients have stage I disease localized to the skin without dissemination to lymph nodes, liver, spleen, or bone marrow.

Exceptional cases with terminal bone marrow involvement and overt leukemia have been reported. Patients generally follow one of two clinical courses: most have a prolonged indolent period with regressing and recurring lesions that eventually progress to aggressive disease, whereas others have aggressive disease from the outset. Clinically aggressive disease appears to correlate with the presence and severity

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SUBCUTANEOUS PANNICULITIS–LIKE T-CELL LYMPHOMA—FACT SHEET

Definition
- Lymphoma of cytotoxic T cells that produces subcutaneous nodular lesions

Incidence and Location
- Rare

Morbidity and Mortality
- 80% 5-year overall survival rate
- Poor prognosis upon development of hemophagocytic syndrome

Gender, Race, or Age Distribution
- Younger adults
- Slight female predominance

Clinical Features
- Nontender, erythematous subcutaneous nodules usually on extremities or trunk, or both (stage I disease)
- B symptoms
- Hemophagocytic syndrome

Prognosis and Therapy
- Variably indolent to aggressive disease
- Chemotherapy

SUBCUTANEOUS PANNICULITIS–LIKE T-CELL LYMPHOMA—PATHOLOGIC FEATURES

Microscopic Findings
- Lobular panniculitic infiltrate of pleomorphic lymphocytes of varying size
- Neoplastic cells often rim fat spaces
- Karyorrhexis and fat necrosis always present

Immunophenotypic Findings
- $CD8^+$, TCR-αβ positive, and expression of cytotoxic granule–associated proteins

Molecular and Cytogenetic Findings
- Clonal TCR gene rearrangements in most cases
- EBV-negative by EBER in situ hybridization
- No recurring chromosomal translocations

Differential Diagnosis
- Reactive panniculitis
- Cutaneous γδ T-cell lymphomas
- Extranodal NK/T-cell lymphoma, nasal type
- Cutaneous dissemination of enteropathy-associated T-cell lymphomas
- Diffuse large B-cell lymphomas

of hemophagocytosis rather than systemic dissemination of lymphoma. The hemophagocytic syndrome (hepatosplenomegaly, peripheral blood cytopenias, and hypertriglyceridemia) develops in most patients at some time during their course but may not occur for up to 5 years after the initial diagnosis. Patients with clinically aggressive disease and hemophagocytic syndrome usually die of their disease despite aggressive chemotherapy. The 5-year OS rate is 80%. Death is usually caused by hemorrhagic and infectious complications of pancytopenia and coagulation abnormalities.

Pathologic Features

Microscopic Findings

SPTCLs have a lobular panniculitic infiltrate of pleomorphic small, medium, and large lymphocytes with highly irregular hyperchromatic nuclei (Fig. 9.8). The tumor is predominantly localized to the subcutis, where there is infiltration between fat cells, often with rimming of the neoplastic cells around fat spaces. Infiltration of interlobular septae may occur. The deep reticular dermis may also be involved in some cases, but the upper reticular dermis, papillary dermis, and epidermis are spared. Vascular invasion is common, but angiodestructive growth with fibrinoid necrosis is not seen. Karyorrhexis and fat necrosis are always present to various degrees. Benign histiocytes with voluminous foamy cytoplasm and bland, eccentric nuclei are usually present and often exhibit erythrophagocytosis or phagocytosis of nuclear fragments (cytophagocytosis). Occasional cases show striking sarcoid-like granulomatous reactions with giant cells mimicking granulomatous panniculitis. Scattered plasma cells are often seen, but eosinophils and neutrophils are absent or rare.

Immunophenotype

The tumor cells of SPTCLs always express a cytotoxic T-cell phenotype. The typical profile is $CD2^+$, $CD3^+$, $CD5^+$, $CD7^+$, $CD8^+$, TIA-1 positive, and granzyme B positive. Similar to other PTCLs, loss or diminished expression of pan–T-cell antigens may be seen, particularly with lack of CD5 or CD7. SPTCLs are derived from αβ T cells ($\beta F1^+$). PTCLs resembling SPTCL but composed of γδ T cells are segregated from SPTCLs in the category of primary cutaneous PTCLs.

Molecular and Cytogenetic Findings

Clonal rearrangement of TCRs has been demonstrated in most cases. SPTCLs are EBV^-. No recurring cytogenetic abnormalities have been reported.

Differential Diagnosis

The differential diagnosis for SPTCLs includes benign panniculitis such as lupus profundus and other cutaneous and subcutaneous lymphomas, particularly cutaneous γδ T-cell

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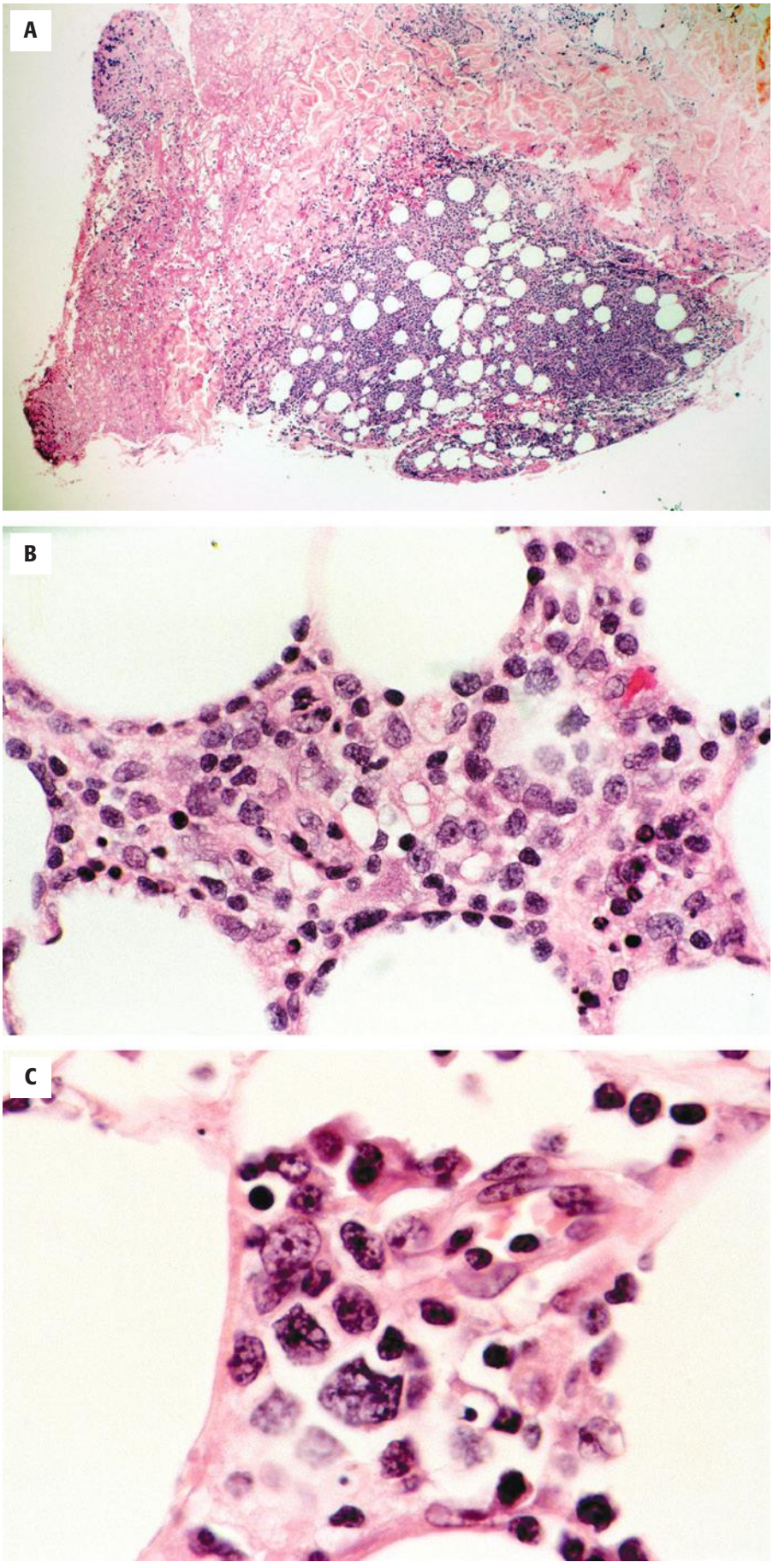


FIG. 9.8

Subcutaneous panniculitis–like T-cell lymphoma: microscopic features. A, At low magnification, there is a lobular panniculitis-like lymphoid infiltrate in this skin excision. B, At intermediate magnification, tumor cells are seen in the inter-fatty spaces, and some show tight rimming around the adipocytes. C, At high magnification, the cytologic pleomorphism of the tumor cells is appreciated.

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lymphoma and extranodal NK/T-cell lymphoma, nasal type, as discussed previously. Features that favor lupus profundus include involvement of the epidermis and presence of numerous plasma cells, reactive germinal centers, and B cells. Most reports show polyclonal gene rearrangement. Concomitant clinical features or existing diagnosis of lupus erythematosus also favor lupus profundus over lymphoma. In difficult cases, repeated biopsies may be required. Subcutaneous dissemination by other PTCLs, such as enteropathy-associated T-cell lymphoma, and by some diffuse large B-cell lymphomas, must be considered and can usually be differentiated by immunohistochemistry or with detailed clinical information.

HEPATOSPLENIC T-CELL LYMPHOMA

T cells can be subdivided based on the nature of their surface TCRs. Normally the majority (95%) of T cells are of the αβ type, and a minority (5% or less) are of the γδ type. γδ T cells are preferentially found in the small bowel epithelium, skin, and splenic red pulp. These normal γδ T-cell subpopulations are thought to be the normal counterparts for several subtypes of γδ T-cell lymphomas, including some intestinal T-cell lymphomas, primary cutaneous γδ T-cell lymphomas, and most hepatosplenic T-cell lymphomas (HSTCLs). Subsequent to the initial descriptions identifying hepatosplenic γδ T-cell lymphomas, clinically identical cases of hepatosplenic αβ T-cell lymphoma have been described. Therefore the WHO classification uses the term *HSTCL* rather than the originally proposed term *hepatosplenic γδ T-cell lymphoma*.

HEPATOSPLENIC T-CELL LYMPHOMA—FACT SHEET

Definition
- Extranodal lymphoma of nonactivated cytotoxic T cells that produce massive hepatosplenomegaly and no lymphadenopathy

Incidence and Location
- Rare

Morbidity and Mortality
- Median survival: <2 years
- May be associated with chronic immunosuppressive therapy using thiopurine

Gender and Age Distribution
- Young adult males predominate in γδ type
- Females predominate in αβ type

Clinical Features
- Massive hepatosplenomegaly and no lymphadenopathy
- B symptoms
- Moderate anemia and marked thrombocytopenia common

Prognosis and Therapy
- Aggressive disease with poor prognosis

Clinical Features

Most patients with HSTCLs exhibit B symptoms, hepatosplenomegaly (often massive), no lymphadenopathy, moderate anemia, and marked thrombocytopenia. Leukocytosis may be present in those with peripheral blood involvement. The γδ type is most commonly seen in young adult males, whereas the αβ type tends to occur more frequently in females. Approximately 20% of HSTCLs occur in the setting of chronic immunosuppressive therapy with thiopurine following solid organ transplantation or with infliximab in patients with Crohn disease. HSTCL is rare and aggressive. Most patients die within 2 years of diagnosis, even if an initial remission is achieved with therapy.

Pathologic Features

Microscopic Findings

This lymphoma preferentially infiltrates the cords and sinuses of the splenic red pulp, hepatic sinusoids, and marrow sinuses (Fig. 9.9). Tumor cells are generally small to intermediate in size, but some cases may have a predominance of large cells. There may be an associated hemophagocytosis by benign histiocytes. A leukemic phase may develop as the disease progresses. Circulating tumor cells are generally agranular, but cytoplasmic granules have been detected by electron microscopy in some cases.

Immunophenotype

The characteristic phenotype is $CD2^+$, $CD3^+$, $CD4^-$, $CD5^-$, $CD7^+$, and $CD8^-$ (see Fig. 9.9). The majority of HSTCLs are TCR-γδ positive, but a smaller number of cases are TCR-αβ positive. NK-cell–associated antigens, such as CD16 and CD56, are often expressed. Furthermore, these lymphomas have a nonactivated cytotoxic T-cell phenotype, with expression of the cytolytic granule–associated proteins TIA-1 and granzyme M but not granzyme B or perforin. HSTCLs often have aberrant coexpression of multiple killer-cell immunoglobulin–like receptor isoforms along with dim or absent CD94. Most γδ HSTCLs express the Vδ1 variable region, indicating that they are likely derived from the subset of γδ T cells that normally is present in the splenic red pulp.

Molecular and Cytogenetic Findings

Rearrangements of TCR-γ and TCR-δ genes can be seen in most cases; a few may have TCR-β–chain gene rearrangements, particularly those of the αβ T-cell type. Karyotypic studies often show isochromosome 7q, which may be

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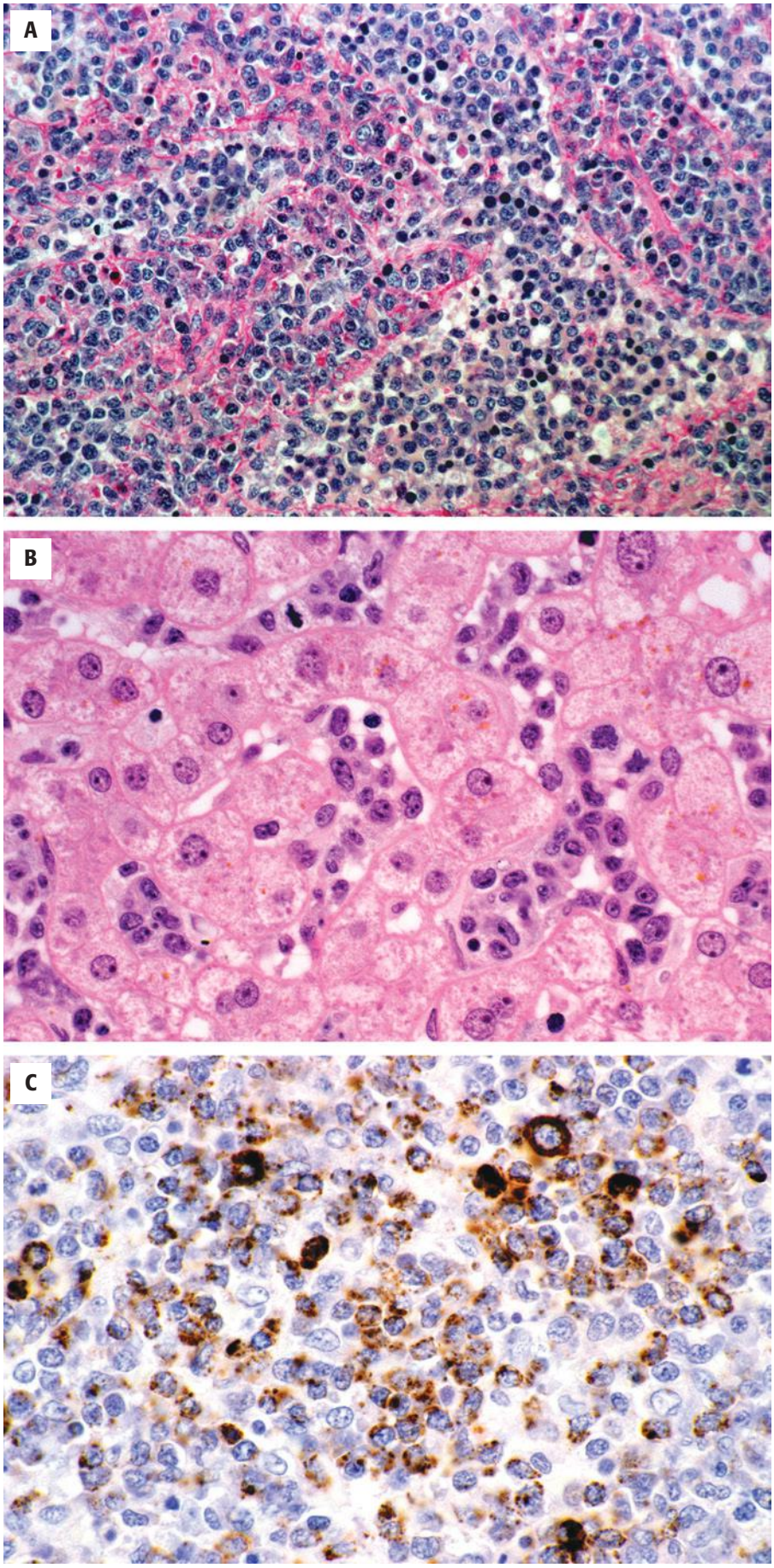


FIG. 9.9

Hepatosplenic T-cell lymphoma: microscopic and immunophenotypic features. A, At intermediate magnification, the neoplastic T cells are distributed in the splenic red pulp, where they expand and fill sinuses. B, At high magnification, intermediate-sized tumor cells are prominently displayed in hepatic sinusoids. C, The neoplastic T cells are T-cell intracellular antigen 1 (TIA-1) positive, indicating their cytotoxic lymphocyte phenotype in this paraffin immunoperoxidase stain.

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HEPATOSPLENIC T-CELL LYMPHOMA—PATHOLOGIC FEATURES

Microscopic Findings

- Infiltration of splenic red pulp cords and sinuses, hepatic sinusoids, and bone marrow sinuses by small- to intermediate-sized lymphocytes

Immunophenotypic Findings

- $CD4^-$, $CD5^-$, and $CD8^-$ γδ (majority) or αβ (minority) T cells with a nonactivated (TIA-1–positive, granzyme B–negative, and perforin-negative) cytotoxic T-cell phenotype
- May coexpress multiple killer-cell immunoglobulin-like receptor isoforms

Molecular and Cytogenetic Findings

- Clonal TCR gene rearrangements in most cases
- EBV negative by EBER in situ hybridization
- Isochromosome 7q often present along with trisomy 8 and loss of a sex chromosome

Differential Diagnosis

- Histiocytic sarcoma

accompanied by trisomy 8 and loss of a sex chromosome. Next-generation sequencing of HSTCL cases has begun to elucidate the mutational landscape of this lymphoma. Chromatin-modifying genes *SETD2, INO80, ARID1B, TET3, SMARCA2,* and *DNMT3A* are recurrently mutated, as are the signaling pathway genes *STAT5B, STAT3*, and *PIK3CD*. Other driver genes that recurrently mutate include *TP53, UBB5*, and *IDH2*. *SETD2, INO80*, and *STAT5B* appear to be mutated in approximately 20% to 30% of cases. The *SETD2* mutations are a mix of stop gain, frameshift, and missense mutations. The signaling pathway mutations are largely missense mutations. Functional studies suggest that the *SETD2* mutations provide survival/proliferation advantage to lymphoma cells and that the signaling molecule mutations are activating mutations. These provide insight into the biology of this lymphoma as well as possible therapeutic targets.

Differential Diagnosis

Few disorders exhibit the clinicopathologic features of HSTCL. Some cases of histiocytic sarcoma may have a similar clinical course, disease distribution, and presence of hemophagocytosis. However, histiocytic sarcoma can usually be ruled out by immunophenotypic studies.

INTESTINAL T-CELL LYMPHOMA

The gastrointestinal tract is the most common site of primary extranodal NHLs. Most of these are B-cell lymphomas that presumably arise from mucosa-associated lymphoid tissue (MALT). Primary intestinal T-cell lymphomas are rare, but their clinicopathologic features are sufficiently distinct that they are recognized as a separate entity in the WHO classification. These lymphomas often arise from small intestinal mucosal T cells that have cytotoxic function.

These lymphomas are regarded as being enteropathy associated if there is clinical evidence of malabsorption or if there are morphologic features of malabsorption in the adjacent small bowel mucosa (villous atrophy of the mucosa, crypt hyperplasia, and increased number of intraepithelial lymphocytes). Enteropathy-associated T-cell lymphoma (EATL) is the current designation for cases previously called type I EATL. It occurs with increased frequency in populations prone to celiac disease, such as in those of northern European ancestry. A genetic predisposition may be present because some patients with EATL appear to have the celiac disease–associated alleles, HLA-DQ2 or HLA-DQ8.

Intestinal T-cell lymphomas that are not linked with celiac disease and do not have a malabsorption pattern on tissue sections are now classified as monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL) rather than the previous term, type II EATL. MEITL has a higher incidence in Asian and Hispanic populations.

Intestinal T-cell lymphoma, NOS, is the designation that should be used for cases that appear to arise in the intestine but do not fit the features of EATL or MEITL. It is not a specific disease entity, and many are detected in the colon rather than having a small bowel presentation characteristic of the preceding two lymphomas. This designation can also be used for inadequate small bowel biopsies in which the intestinal mucosa cannot be evaluated.

Clinical Features

Most patients with EATL and MEITL are middle aged to elderly. It is unusual for these lymphomas to appear before 40 years of age. There is a slight male preponderance in both types of lymphomas. The most common presenting symptoms are abdominal pain, diarrhea, and weight loss. There may be signs of acute obstruction or spontaneous perforation. The tumors occur most commonly in the jejunum or ileum, although rare reported cases have been documented in the duodenum, stomach, and colon. Hematologic findings can include anemia and leukocytosis without lymphocytosis. Patients with EATL typically have a history of celiac disease or other malabsorptive problems, which are not associated with MEITL. In particular, patients with EATL may have a history of refractory celiac disease or celiac disease with development of intestinal ulceration (ulcerative jejunitis). These T-cell lymphomas are aggressive, and most patients die of their disease within 2 years of diagnosis.

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INTESTINAL T-CELL LYMPHOMA—FACT SHEET

Definition
- EATL and MEITL: extranodal lymphomas of cytotoxic T cells that produce small bowel lesions and masses

Incidence and Location
- Rare

Morbidity and Mortality
- Median survival: <2 years
- EATL often associated with celiac disease unlike MEITL

Gender and Age Distribution
- Adults
- Slight male predominance

Prognosis and Therapy
- Aggressive disease with poor prognosis

INTESTINAL T-CELL LYMPHOMA—PATHOLOGIC FEATURES

Microscopic Findings
- EATL: infiltrate of medium to large tumor cells with oval to pleomorphic nuclei that are frequently associated with inflammatory cells and necrosis
- MEITL: infiltrate of uniform small to medium tumor cells with round nuclei and little to no association with inflammatory cells and necrosis
- Villous atrophy, crypt hyperplasia, and increased number of intraepithelial lymphocytes consistent with malabsorptive disease often present in mucosa adjacent to EATL but not MEITL
- Epitheliotropism common to both EATL and MEITL

Immunophenotypic Findings
- EATL: $CD3^+$, $CD4^-$, $CD5^-$, $CD8^{-/+}$, $CD30^{-/+}$, $CD56^-$, $CD103^+$, TCR-αβ positive > TCR-γδ positive, and expression of cytotoxic granule–associated proteins
- MEITL: $CD3^+$, $CD4^-$, $CD5^-$, $CD8^+$, $CD30^{-/+}$, $CD56^+$, $MATK^+$, TCR-γδ positive > TCR-αβ positive, and expression of cytotoxic granule-associated proteins

Molecular and Cytogenetic Findings
- Clonal TCR gene rearrangements in most cases
- EBV by EBER in situ hybridization generally negative
- No recurring chromosomal translocations; gains at chromosomes 1q and 5q for EATL and *MYC* amplification for MEITL
- EATL and MEITL have some similar pathogenetic features with *SETD2* gene silencing (32%) and activating JAK-STAT pathway mutations (16% to 29%)

Differential Diagnosis
- Celiac disease
- Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
- Diffuse large B-cell lymphomas
- PTCL, NOS
- ALCLs (for $CD30^+$ cases)
- Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract
- NK-cell enteropathy
- Histiocytic sarcoma

Pathologic Features

Microscopic Findings

EATL and MEITL may have single or multifocal small bowel lesions, and the neoplastic cells always involve the mucosa (Fig. 9.10). There is often ulceration, and EATL may be associated with ulcerative jejunitis. In addition, lymphoma cells may infiltrate residual glandular structures, producing lymphoepithelial lesions, such as in extranodal marginal zone B-cell lymphomas of the MALT type. This epitheliotropism is also reminiscent of that seen in the skin in patients with MF. The lymphoma may extend through the bowel wall, resulting in perforation. Regional lymph nodes are often involved.

The tumor cells of EATL are usually medium to large with oval to pleomorphic nuclei, are admixed with inflammatory cells, and may be associated with necrosis. Intact adjacent small bowel mucosa shows villous atrophy, crypt hyperplasia, and an increased number of intraepithelial lymphocytes.

MEITL has monomorphic small to medium-sized tumor cells with round nuclei, and there is no significant associated inflammatory cell background and little, if any, necrosis. There is generally abundant clear to pale eosinophilic cytoplasm, and mitotic activity is usually brisk. Tumor cells often infiltrate throughout the adjacent mucosa.

Immunophenotype

The phenotype is variable, and EATL is often $CD2^+$, $CD3^+$, $CD4^-$, $CD5^-$, $CD7^+$, $CD8^{-/+}$, $CD56^-$, $CD103^+$, and TCR-αβ positive (see Fig. 9.10). MEITL is often $CD8^+$, $CD56^+$, megakaryocyte-associated tyrosine kinase $(MATK)^+$, and TCR-γδ positive. The presence of TIA-1 and variable marking for granzyme B in the tumor cells indicates that these lymphomas arise from cytotoxic T cells. Some cases may be $CD30^+$ and must be distinguished from ALCLs.

Molecular and Cytogenetic Findings

Many cases demonstrate clonal TCR-β–chain gene rearrangements. EBV is usually absent. Karyotypic abnormalities may be present, but a specific pattern has not been identified. Recent studies using comparative genomic hybridization, however, have shown gains involving chromosomes 9q (58% of cases), 7q (24%), 5q (18%), and 1q (16%), with losses at chromosomes 8p (24%), 13q (24%), and 9p (18%). EATL, which is pathogenetically linked to celiac disease, has a higher frequency of chromosomal gains of 1q and 5q. MEITL, which is not closely linked to celiac disease, has *MYC* oncogene locus gain. Although EATL and MEITL have different phenotypes, they appear to have shared genetic mechanisms

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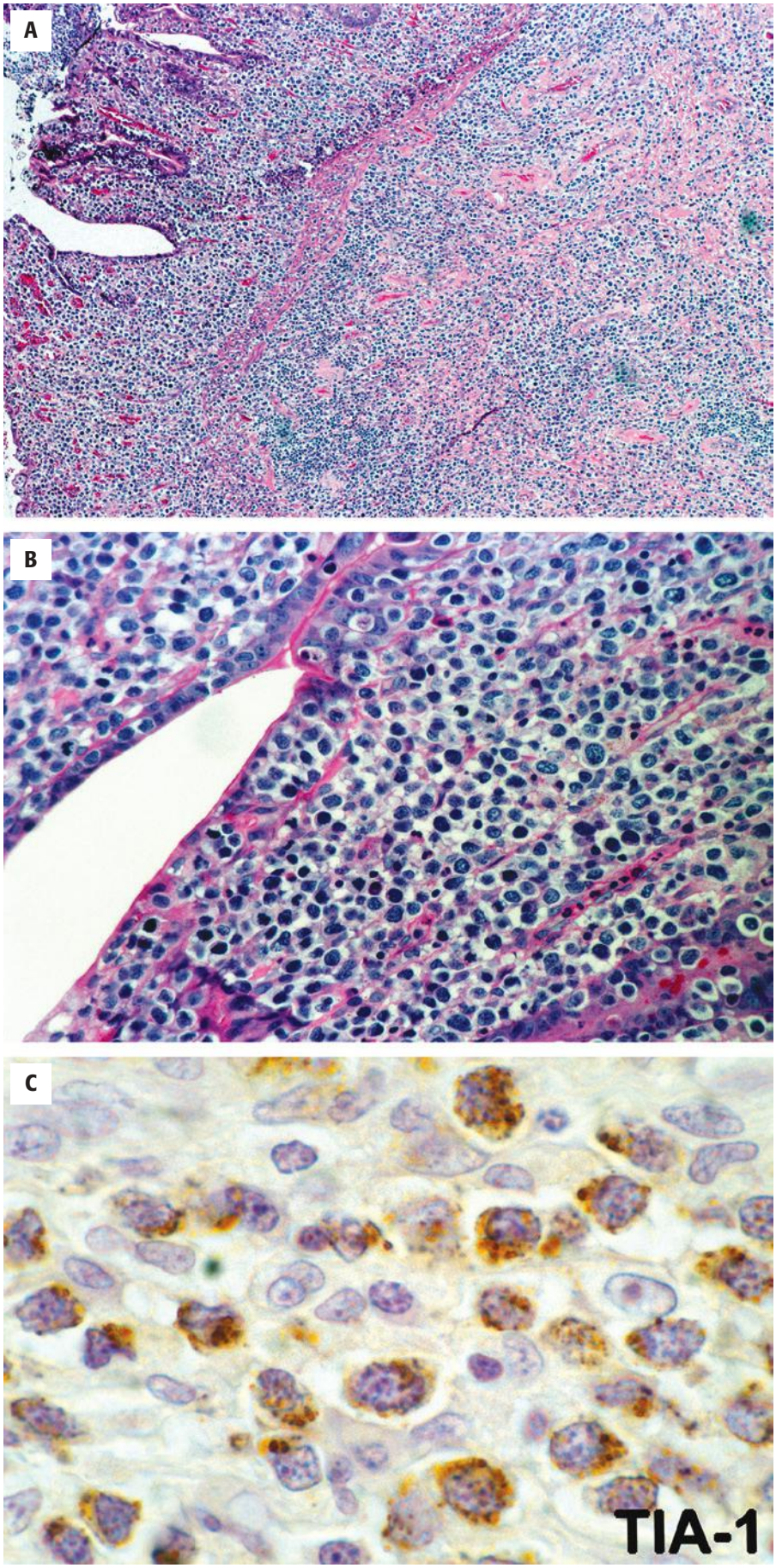


FIG. 9.10

Enteropathy-associated T-cell lymphoma: microscopic features and immunophenotypic features. A, At low magnification, the small bowel surface shows villous blunting, and there is a dense, diffuse lymphoproliferation throughout the mucosa and submucosa. B, At intermediate magnification, neoplastic T cells with clear cytoplasm destroy most of the glandular pattern in the mucosa, but a residual gland at the lower right is infiltrated by occasional tumor cells. C, The pleomorphic T cells are TIA-1 positive, indicating their cytotoxic lymphocyte phenotype in this paraffin immunoperoxidase stain.

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in their pathogenesis. *SETD2* is the most frequently silenced gene (32%), and the JAK-STAT pathway (*STAT5B, JAK1, JAK3*, and *STAT3* in 16% to 29% of cases) is the most frequently mutated pathway in both.

Differential Diagnosis

Celiac disease is a consideration because 5% to 10% of these patients will develop an EATL, and there may be similar histologic features between celiac disease and EATL, such as villous atrophy and crypt hyperplasia. However, the lymphoma will have a much more extensive lymphoid infiltrate that also frequently exhibits too much nuclear pleomorphism to be a reactive process. To exclude EATL, biopsy specimens from patients with ulcerative jejunitis or refractory celiac disease should be searched carefully for atypical T cells within the ulcer bed and evidence of abnormal T-cell phenotype or T-cell clonality. In fact, some consider ulcerative jejunitis and refractory celiac disease as forms of lymphoproliferative disorders/lymphoma occurring in patients with celiac disease.

Extranodal marginal zone B-cell lymphomas of the MALT type are well known to have lymphoepithelial lesions that also may be seen in EATLs and MEITLs. However, EATL generally has more pleomorphism, and both EATL and MEITL have greater mitotic activity and no association with lymphoid follicles. Furthermore, immunophenotyping readily distinguishes the B-cell lymphoma from the T-cell processes. Immunoproliferative small intestinal disease, or Mediterranean lymphoma, is a special type of MALT lymphoma that involves the jejunum predominantly and is associated with malabsorption and α heavy chain disease.

EATLs composed of neoplastic large cells must be distinguished from diffuse large B-cell lymphomas, PTCL, NOS, and ALCLs for $CD30^+$ cases; this can be done largely with complete immunohistochemical staining. Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract has uniform small $CD8^+$ cytotoxic lymphocytes without admixed inflammatory cells that can mimic MEITL, but mucosal glands are not destroyed and epitheliotropism is unusual. NK-cell enteropathy can mimic EATL, particularly when ulceration is present; however, there is no epitheliotropism and cells are NK type and lack surface CD3. Histiocytic sarcoma enters the differential diagnosis because of the cytologic appearance of the tumor cells. In fact, most cases of EATL were incorrectly classified as malignant histiocytosis until adequate phenotyping studies were performed.

MYCOSIS FUNGOIDES AND SÉZARY SYNDROME

Cutaneous T-cell lymphomas (CTCLs) are a group of T-cell lymphomas that are primarily localized to the skin and presumably derived from T cells of the skin-associated lymphoid tissue. MF, although rare, is the most common form of CTCL and comprises approximately 50% of all primary CTCLs. MF and its leukemic counterpart, Sézary syndrome (SS), are specific clinicopathologic subtypes of CTCL and represent the majority of CTCLs in Western countries.

Clinical Features

MF occurs most frequently in middle-aged to elderly adults, and approximately two thirds of cases are in males. The disease is generally protracted and is typically preceded by years of nonspecific scaly rash that is often localized and on the trunk. MF has four clinical appearances in the skin: patches, plaques, tumors, and generalized erythroderma. The erythematous patches and thickened plaques can persist for years, become more generalized in distribution, and eventually progress to nodular tumors. Extracutaneous dissemination to lymph nodes and visceral organs occurs late in the disease course. Bone marrow involvement is uncommon. Patients with SS have generalized erythroderma, lymphadenopathy, and peripheral blood involvement.

Survival is dependent on stage. Patients with limited patch- or plaque-stage MF have survival rates equal to that of age-matched controls. Ten-year disease-specific survival rate declines to 42% for tumor-stage MF and 20% for those with histologically proven lymph node involvement. Modern staging systems include skin, lymph node, visceral, and blood assessment. Enumeration of percent (≤5%, >5%, and absolute count >1000/μL) Sézary cells separates patients with absence of significant, low, and high tumor burden, respectively. High CD4:CD8 ratio (≥10) or abnormal immunophenotype by flow cytometry and detection of monoclonal TCR gene rearrangement in the blood also influences staging; therefore pathologists may be asked to perform this type of analysis in patients with MF. Treatment is usually conservative (i.e., skin targeted), with systemic chemotherapy reserved for advanced disease. Transformation to large-cell lymphoma portends an aggressive clinical course.

Clinical variants of MF include pagetoid reticulosis, granulomatous slack skin, and folliculotropic. Pagetoid reticulosis presents as localized patches or plaques. Granulomatous slack skin is extremely rare, has an indolent clinical course, and appears with pendulous folds of skin, usually in the axilla or groin. Folliculotropic MF is a variant that preferentially involves hair follicles and manifests as grouped acneiform papules or plaques on the head and neck. Alopecia is common and mucinorrhea may be present. This variant tends to have a worse prognosis than classic MF.

Pathologic Features

Microscopic Findings

The histologic appearance of MF varies with the type of clinical lesion. The classic histologic pattern of MF is

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MYCOSIS FUNGOIDES AND SÉZARY SYNDROME—FACT SHEET

Definition
- Mycosis fungoides (MF): a specific type of CTCL derived from $CD4^+$ T cells of skin-associated lymphoid tissue
- Sézary syndrome (SS): leukemic counterpart of MF accompanied by generalized erythroderma and lymphadenopathy

Incidence and Location
- Rare, but the most common form of CTCL

Morbidity and Mortality
- Survival equal to age-matched controls for limited patch or plaque stage
- 42% 10-year disease-specific survival for tumor stage
- 20% 10-year disease-specific survival with proven lymph node involvement

Gender and Age Distribution
- Middle-aged to elderly adults
- Males predominate 2 : 1

Clinical Features
- Persistent skin rash (patches, plaques, tumors, and generalized erythroderma) often localized and on trunk
- Clinical variants: pagetoid reticulosis, granulomatous slack skin, and folliculotropic

Prognosis and Therapy
- Generally indolent disease with survival dependent on stage
- Skin-targeted therapy for limited-stage disease
- Chemotherapy for advanced-stage disease

MYCOSIS FUNGOIDES AND SÉZARY SYNDROME—PATHOLOGIC FEATURES

Microscopic Findings
- Patch stage: slight superficial dermal lymphocytic infiltrate with medium to large cerebriform cells in the epidermis, often as single cells aligned along the basement membrane or in dermal clusters
- Plaque stage: dense band-like superficial dermal infiltrate of cytologically atypical, hyperchromatic, cerebriform lymphocytes exhibiting epidermotropism and possibly forming Pautrier microabscesses
- Tumor stage: top-heavy dermal infiltrate of medium to large pleomorphic cerebriform lymphocytes that often lack epidermotropism
- Large cell transformation defined by presence of >25% large lymphoid cells in the dermis
- Erythroderma: mild superficial dermal infiltrate of cerebriform lymphocytes that have limited epidermotropism but are frequently in the peripheral blood

Immunophenotypic Findings
- $CD4^+$ T cells that are often $CD7^-$
- $CD30^+$ tumor cells in large cell transformation

Molecular and Cytogenetic Findings
- Clonal TCR gene rearrangements in most cases
- No recurring chromosomal translocations

Differential Diagnosis
- Reactive T-cell infiltrates (parapsoriasis, drug eruptions, contact dermatitis, benign erythroderma, and follicular mucinosis)
- Skin involvement by other peripheral T-cell lymphomas, including adult T-cell leukemia–lymphoma

associated with plaque-stage lesions and is characterized by a dense bandlike lymphocytic infiltrate in the superficial papillary dermis, epidermal infiltration (epidermotropism) with formation of Pautrier microabscesses, and cytologically atypical, hyperchromatic cerebriform lymphocytes (Fig. 9.11).

In patch-stage or early plaque-stage lesions, the infiltrate is less dense and less cytologically atypical and may not have Pautrier microabscesses. In these early lesions, the most reliable histologic features are the presence of medium to large (7- to 9-μm nuclear diameter [approximately the size of basal keratinocyte nuclear diameter]) cerebriform cells in the epidermis or in clusters in the dermis. Epidermotropism as single cells align along basal keratinocytes of the epidermal–dermal junction, absence of significant papillary dermal fibrosis, and absence of significant numbers of blast-like cells are other useful histologic features of early MF. However, the efficacy of single histologic features in the diagnosis of early MF is generally poor. Pautrier microabscesses strongly suggest MF when present, but they are often absent in early lesions and are not entirely specific. The level of certainty in the diagnosis of MF increases as the percentage, size, and degree of nuclear contour irregularities increase. In equivocal cases, it is best to call the biopsy specimen suspicious but not diagnostic and to recommend a repeat biopsy and clinical follow-up.

In tumor-stage MF, the diagnosis of lymphoma is usually not an issue. Instead, the problem is differentiating MF from cutaneous involvement by other PTCLs. Tumor-stage MF often becomes nonepidermotropic but still maintains a top-heavy pattern with the broadest part of the infiltrate in the superficial dermis. Tumor-stage MF usually extends deep into the reticular dermis and often involves the superficial subcutaneous tissue. Cytologic atypia is usually prominent in tumor-stage MF, with an increased percentage of medium to large pleomorphic cerebriform cells. Large-cell transformation, defined by the presence of more than 25% large lymphoid cells in the dermis, is seen in up to 50% of tumor-stage MF cases.

Erythrodermic MF in patients with SS can be difficult to diagnose. The cutaneous infiltrates in SS tend to show less epidermotropism and are more frequently nondiagnostic than patch-stage or plaque-stage MF. Pautrier microabscesses are present in approximately 50% of erythrodermic SS skin biopsy specimens. Multiple or repeat biopsies may be necessary for a definitive diagnosis of MF in the skin in patients with SS. However, the diagnosis can often be confirmed by morphologic evaluation of the peripheral blood for circulating Sézary cells in combination with flow cytometry to detect restricted TCR

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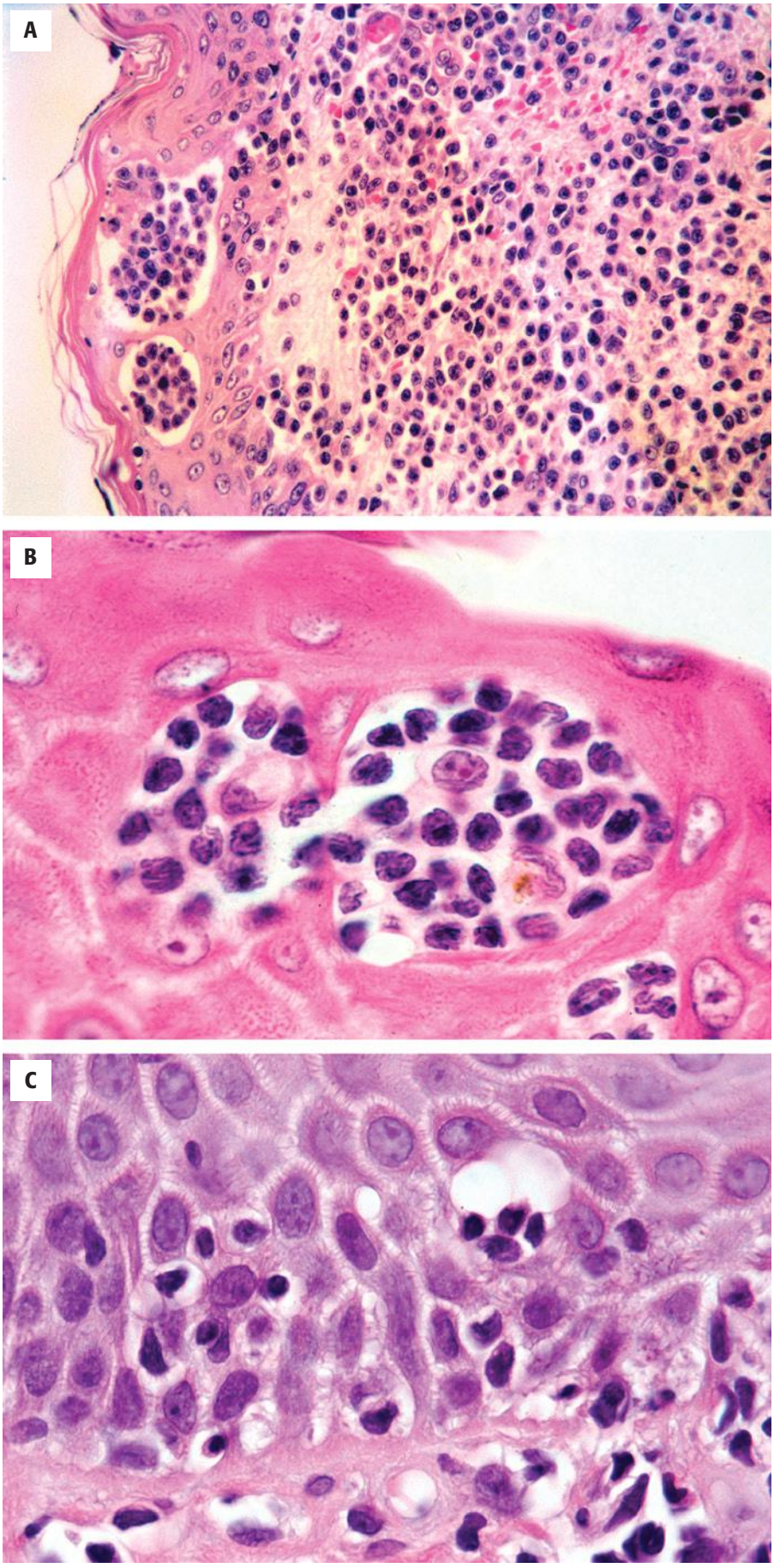


FIG. 9.11

Mycosis fungoides (MF): microscopic features. A, At low magnification, this skin biopsy specimen contains a dense lichenoid lymphocytic infiltrate in the superficial dermis that is epidermotropic, including formation of Pautrier microabscesses. B, At high magnification, the Pautrier microabscess contains numerous hyperchromatic cerebriform lymphocytes. C, At high magnification, hyperchromatic, medium to large cerebriform lymphocytes are singly distributed or in a focal small cluster in the epidermis in this early lesion of MF.

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Vβ chain use and with TCR gene rearrangement studies. Sézary cells are enlarged lymphocytes with complex nuclear folds and scant cytoplasm. Nuclear chromatin in large Sézary cells is more finely dispersed than in normal peripheral blood lymphocytes. Small Sézary cells can be difficult to recognize in peripheral blood smears. There is currently no consensus on the number of Sézary cells required to diagnosis SS. The minimum criterion is more than five Sézary cells per 100 lymphocytes. More stringent criteria require that Sézary cells constitute at least 10% to 20% of peripheral blood lymphocytes or that there is an absolute Sézary cell count of more than 1000/μL.

Regarding the variants, pagetoid reticulosis manifests as a localized epidermotropic atypical lymphoid infiltrate limited to the epidermis. No dermal component should be present. Pagetoid reticulosis should not be considered when lesions are generalized. Granulomatous slack skin shows a dense and destructive dermal atypical lymphoid infiltrate with cerebriform nuclei. Macrophages are present and produce a prominent granulomatous component with multinucleated giant cells. Folliculotropic MF has an atypical perivascular and periadnexal lymphoid infiltrate that demonstrates folliculotropism. Mucinous degeneration is often present within the involved follicles. Uncommonly, there may be involvement of the eccrine glands (so-called syringotropic MF).

Extracutaneous dissemination of MF most commonly involves peripheral lymph nodes draining the involved skin, but it can also involve the liver, spleen, lungs, and other sites. Extracutaneous MF can be recognized histologically by infiltrates of pleomorphic cerebriform cells. Large-cell transformation of MF can occur initially in extracutaneous sites, giving the appearance of diffuse large-cell lymphoma or ALCL. Bone marrow involvement by MF is often subtle and rarely extensive or hematologically significant. MF in the bone marrow forms lymphoid aggregates with atypical cerebriform cells, but in SS it often shows subtle interstitial infiltrates with small clusters of Sézary cells.

Immunophenotype

MF is a neoplasm of $CD4^+$ memory, helper/inducer T cells, but rare $CD8^+$ suppressor/cytotoxic cases are reported. In addition to CD4, these lymphomas express most T-cell antigens expressed by normal peripheral blood T cells (CD2, CD3, CD5, and TCR-αβ), but they are often negative for CD7. Absent or diminished expression of T-cell antigens other than CD7 supports a diagnosis of MF over a reactive T-cell proliferation; however, these aberrant T-cell phenotypes generally do not occur in early MF but rather in advanced plaque or tumor stage lesions. Loss of CD26 on neoplastic $CD4^+$ T cells is characteristically seen in Sézary syndrome. CD30 can be expressed in transformed cases and does not have the association of a good prognosis that is seen in de novo $CD30^+$ cutaneous lymphoproliferative disorders. Although MF is extremely uncommon in children, the $CD8^+$ phenotype is known to occur more frequently in pediatric MF and is associated with a hypopigmented clinical presentation.

Molecular and Cytogenetic Findings

Clonal rearrangement of TCR genes (TCR-β and TCR-γ) can usually be demonstrated. Characteristic cytogenetic abnormalities for MF have not been identified, although there may be an association with lp22-1p36 abnormalities.

Differential Diagnosis

The differential diagnosis for MF-SS includes reactive conditions such as parapsoriasis, drug eruptions, contact dermatitis, benign erythroderma, and follicular mucinosis; cutaneous involvement by other PTCLs are also included in the differential diagnosis. Immunophenotyping may be of limited usefulness in distinguishing early MF from benign dermatitis because a lack of CD7 can be seen in both, and aberrant T-cell antigen expression is rarely seen in early MF. A CD4-to-CD8 ratio of more than 10 : 1 favors MF, but this can also occur in benign dermatitis. Furthermore, clonal TCR gene rearrangements can be detected in some dermatitides. Therefore, multiple skin biopsies and clinical correlation are often necessary to make the correct diagnosis. The demonstration of the same TCR rearrangement amplicon in two separate biopsy specimens strongly supports a diagnosis of lymphoma. The differential diagnosis with adult T-cell leukemia–lymphoma has already been discussed.

PRIMARY CUTANEOUS $CD30^+$ T-CELL LYMPHOPROLIFERATIVE DISORDERS

The primary cutaneous $CD30^+$ T-cell lymphoproliferative disorders are the second most common group of CTCLs (approximately 30% of all primary CTCLs) and consist of a spectrum of skin lesions composed of atypical large $CD30^+$ T cells. Primary cutaneous $CD30^+$ T-cell lymphoproliferative disorders include primary cutaneous ALCL (C-ALCL), lymphomatoid papulosis (LyP), and borderline lesions. The latter is a term used when the clinicopathologic features of the lesion are not distinguishable for C-ALCL or LyP.

Clinical Features

Primary cutaneous $CD30^+$ T-cell lymphoproliferative disorders occur mainly in older adults and in males more frequently than in females. Primary C-ALCL typically manifests as a primary disease of the skin with solitary or localized nodules. The lesions may spontaneously regress but generally recur. The disease often responds to local excision and radiation therapy, if isolated in location. The overall 5-year survival in primary C-ALCL approaches 90% with current therapy. Dissemination to regional lymph nodes may occur, but visceral

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PRIMARY CUTANEOUS CD30⁺ T-CELL LYMPHOPROLIFERATIVE DISORDERS—FACT SHEET

Definition
- A spectrum of skin disorders containing atypical large CD30⁺ T cells

Incidence and Location
- Rare, but the second most common form of CTCL

Morbidity and Mortality
- Primary cutaneous anaplastic large cell lymphoma: 90% 5-year overall survival
- Lymphomatoid papulosis: benign clinical course of long duration

Gender and Age Distribution
- Older adults
- Males predominate

Clinical Features
- Primary cutaneous anaplastic large cell lymphoma: solitary or localized skin nodules that may spontaneously regress but generally recur
- Lymphomatoid papulosis: spontaneously regressing papules typically isolated on extremities

Prognosis and Therapy
- Primary cutaneous anaplastic large cell lymphoma: skin-targeted therapy for limited stage disease and chemotherapy for extracutaneous disease
- Lymphomatoid papulosis: low-dose methotrexate and psoralen with ultraviolet A

PRIMARY CUTANEOUS CD30⁺ T-CELL LYMPHOPROLIFERATIVE DISORDERS—PATHOLOGIC FEATURES

Microscopic Findings
- Primary cutaneous ALCL: diffuse dermal infiltrate of numerous atypical large cells that extends to subcutaneous tissue but has little epidermotropism
- Lymphomatoid papulosis: wedge-shaped dermal infiltrate of variable numbers of atypical large cells admixed with polymorphic reactive cell background
- Type A: Reed-Sternberg–like cells with numerous inflammatory cells
- Type B: Cerebriform lymphocytes and modest inflammatory cell infiltrate
- Type C: Confluent sheets of atypical large cells morphologically but regressing papules clinically
- Type D: Extensive epidermotropic infiltrates of pleomorphic small- to medium-sized lymphocytes
- Type E: Angiocentric, angiodestructive, and angioinvasive lesions produced by pleomorphic small- to medium-sized lymphocytes
- *DUSP22/IRF4* rearranged: biphasic morphology with small cerebriform lymphocytes in the epidermis and large lymphoid cells in the dermis

Immunophenotypic Findings
- CD30⁺ atypical large cells that usually express CD4, have a cytotoxic lymphocyte phenotype, and are ALK negative
- LyP, type D, has CD8⁺ tumor cells and LyP, type E, usually does as well
- *DUSP22/IRF4* rearranged cases have weak CD30⁺ epidermal lymphocytes and strong CD30⁺ dermal cells

Molecular and Cytogenetic Findings
- Clonal TCR gene rearrangements in most primary C-ALCLs and LyP
- *DUSP22/IRF4* (6p25.3) rearrangement detected in all cases of one type of LyP, present in approximately 30% of primary C-ALCLs, and rare in LyP, types A–E, and transformed MF

Differential Diagnosis
- Secondary cutaneous involvement by systemic ALCL
- Large cell transformation of MF

involvement is rare. LyP is a chronic, relapsing condition with a benign clinical course. It appears as spontaneously regressing papules typically isolated to the extremities. Localized therapy may reduce the number of lesions and relapses; however, the disease will continue its natural course upon discontinuation of therapy. Approximately 10% to 20% of cases will progress to lymphoma. Extracutaneous dissemination occurs only in those cases with lymphomatous progression.

Pathologic Features

Microscopic Findings

The infiltrates of primary C-ALCL are diffuse and extend from the papillary dermis to the subcutaneous tissue (Fig. 9.12). There is often ulceration but little epidermotropism. The cytologic features of the tumor cells are similar to those in systemic ALCL. An inflammatory cell background may be present but is not prominent.

LyP has several histologic appearances, but the lesions are generally wedge-shaped dermal infiltrates of cytologically atypical lymphocytes admixed with a polymorphic inflammatory cell reaction. LyP, type A, is the most common type of LyP (>80% of all LyP) and contains Reed-Sternberg–like cells with numerous inflammatory cells, producing a resemblance to classical Hodgkin lymphoma. LyP, type B, is uncommon and has a predominance of cerebriform lymphocytes and a less pronounced inflammatory cell infiltrate. LyP, type C, has confluent sheets of large tumor cells characteristic of primary C-ALCL but regressing papules clinically. LyP, type D, has extensive epidermotropic infiltrates of pleomorphic small to medium-sized lymphocytes. LyP, type E, has angiocentric, angiodestructive, and angioinvasive lesions produced by pleomorphic small to medium-sized lymphocytes. Ulceration, hemorrhage, and extensive necrosis may be present. Another form of LyP has biphasic morphology with small cerebriform lymphocytes in the epidermis and large lymphoid cells in the dermis. These latter cases have *DUSP22/IRF4* rearrangements on 6p25.3.

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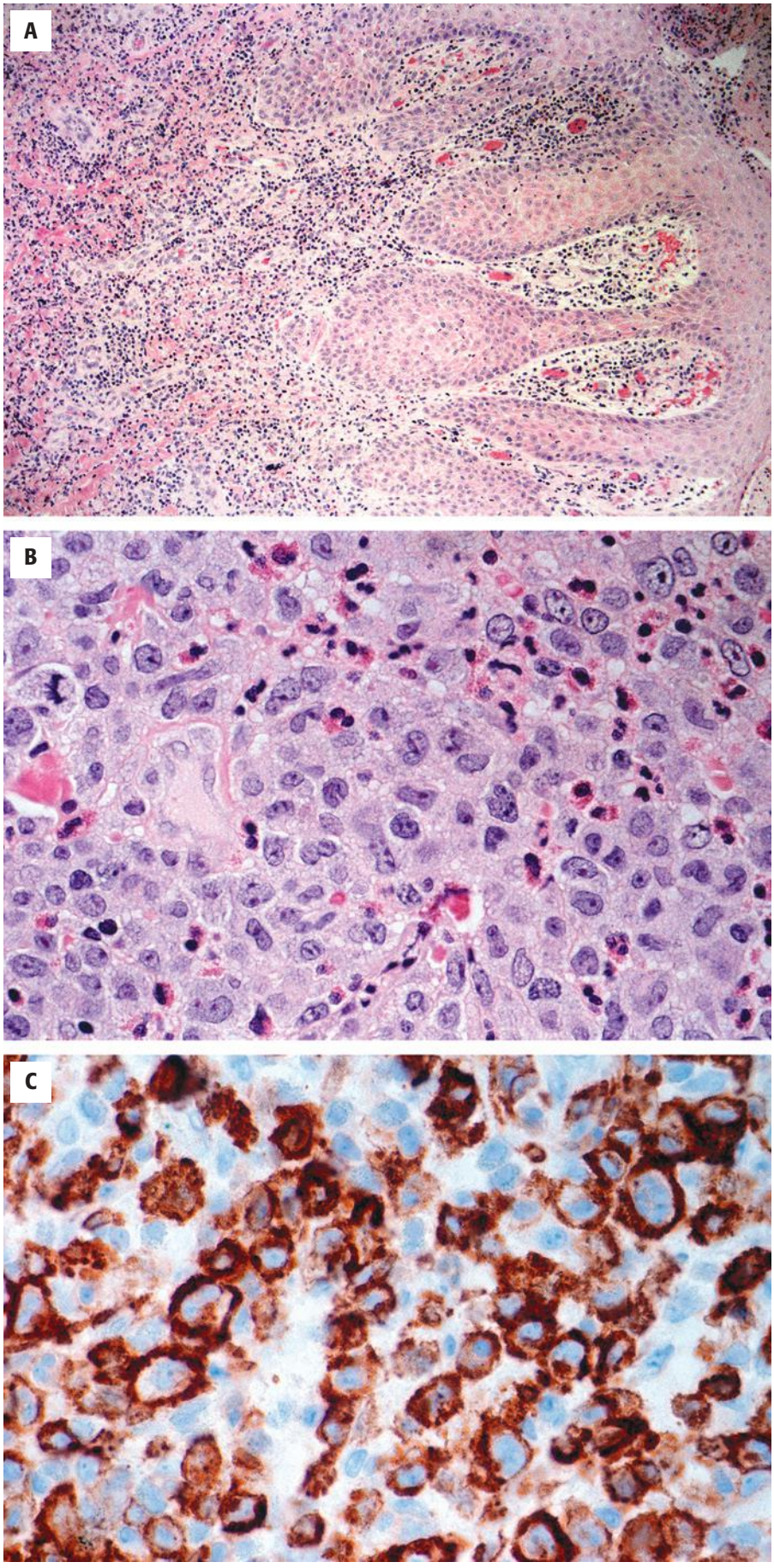


FIG. 9.12

Primary cutaneous CD30⁺ T-cell lymphoproliferative disorders: microscopic features and immunophenotypic features. A, At low magnification, this skin excision exhibits pseudoepitheliomatous hyperplasia associated with a dermal lymphocytic infiltrate. B, At high magnification, there is a mixed lymphoeosinophilic infiltrate in which most of the lymphocytes are large. C, The large lymphocytes are CD30⁺ in this paraffin immunoperoxidase stain.

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Immunophenotype

More than 75% of the atypical large cells are $CD30^+$ in primary C-ALCL and in most LyP (see Fig. 9.12C). The cerebriform lymphocytes in LyP, type B, are typically negative for CD30. The atypical T cells in primary C-ALCL and LyP, types A–C, are usually $CD4^+$ and often have a cytotoxic lymphocyte phenotype with expression of TIA-1, granzyme B, and perforin. LyP, type D, is composed of $CD8^+$ T cells, which is usually the case for LyP, type E. *DUSP22/IRF4* rearranged cases are variable in their expression of CD4 and CD8. In addition to their biphasic morphologic pattern, they have biphasic expression of CD30 because the epidermal small cerebriform cells have weak expression of that antigen and the dermal large lymphoid cells have strong staining. An aberrant T-cell phenotype is common to all types of primary cutaneous $CD30^+$ T-cell lymphoproliferative disorders, and the atypical cells are consistently ALK negative.

Molecular and Cytogenetic Findings

Clonal TCR gene rearrangements are found in most primary C-ALCLs and in approximately 60% of LyP. Translocations involving *ALK* are not seen. However, 6p25.3 rearrangements involving the region of *IRF4* and *DUSP22* help define one type of LyP, can be seen in nearly 30% of primary C-ALCL, and are rarely observed in LyP, types A–E and in transformed MF. This translocation may be present in systemic ALCL but not in other PTCLs studied. Gene expression profiling shows marked differences in expression patterns of chemokine receptors and apoptosis regulators between primary C-ALCL and primary cutaneous PTCL, NOS.

Differential Diagnosis

Primary cutaneous ALCL must be distinguished from secondary cutaneous involvement by systemic ALCL, from large-cell transformation of MF, and from LyP. Microscopic appearance alone may be misleading. For example, type B LyP resembles MF, type C LyP resembles primary cutaneous or secondary ALCL, and type D LyP may resemble primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma, but clinically they present as LyP. It is therefore often critical in skin lesions to correlate the histopathology and immunostain findings with clinical or clonality studies, or both, to classify these lesions accurately in a specific patient.

PRIMARY CUTANEOUS PERIPHERAL T-CELL LYMPHOMAS: RARE SUBTYPES

CTCLs also include four rare types of primary cutaneous PTCLs: primary cutaneous γδ T-cell lymphoma, primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma, primary cutaneous acral $CD8^+$ T-cell lymphoma, and primary cutaneous $CD4^+$ small/medium T-cell lymphoproliferative disorder. All these types must be distinguished from MF or one of its variants by their clinicopathologic features.

Clinical Features

Primary cutaneous γδ T-cell lymphomas occur mainly in adults who have B symptoms. There is no gender predilection. Skin lesions are often generalized, mostly on the extremities. The lesions vary from papules, patches, and plaques to deep dermal or subcutaneous nodules that may be ulcerated. The tumor frequently disseminates to mucosal and other extranodal sites, but lymph nodes, spleen, and bone marrow are usually spared. A hemophagocytic syndrome may develop. Median survival is less than 2 years.

Primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma occurs mainly in adults with generalized skin lesions that vary from superficial hyperkeratotic patches and plaques to eruptive papules and centrally ulcerated nodules. The disease may disseminate to visceral sites, but lymph nodes are usually spared. Median survival is less than 3 years.

Primary cutaneous acral $CD8^+$ T-cell lymphoma occurs in adults as isolated slow-growing reddish papules or nodules most commonly on the ear helix or conch (rarely the lobe), nose, or foot. This disease has a very good prognosis, and complete remission is generally achieved with surgical excision or local irradiation. There may be local recurrences.

Primary cutaneous $CD4^+$ small/medium T-cell lymphoproliferative disorder occurs mainly in asymptomatic adults as solitary plaques or nodules preferentially in the head and neck region or sometimes in the upper extremities or trunk. The disease is indolent, and surgical excision or local irradiation is usually the treatment of choice. There is a 5-year survival rate of approximately 80% or better. Because of uncertainty as to the true malignant potential of this proliferation including instances of regression, the WHO classification refers to these cases as lymphoproliferative disorders rather than lymphoma.

Pathologic Features

Microscopic Findings

The infiltrates of primary cutaneous γδ T-cell lymphomas have several histologic appearances that may be present within the same lesion or among different lesions. Epidermotropism varies from focally mild to overtly pagetoid. Subcutaneous lesions resemble SPTCL but are often accompanied by a dermal component not seen in SPTCL. Tumor cells are typically

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PRIMARY CUTANEOUS PERIPHERAL T-CELL LYMPHOMA, RARE SUBTYPES—FACT SHEET

Definition
- Four subtypes of primary cutaneous PTCLs that are distinguishable from the more common MF and primary cutaneous CD30$^+$ T-cell lymphoproliferative disorders: primary cutaneous γδ T-cell lymphoma, primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic T-cell lymphoma, primary cutaneous acral CD8$^+$ T-cell lymphoma, and primary cutaneous CD4$^+$ small/medium T-cell lymphoproliferative disorder

Incidence and Location
- Extremely rare

Morbidity and Mortality
- Primary cutaneous γδ T-cell lymphoma and primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic T-cell lymphoma: both subtypes are aggressive, with median survival less than 3 years
- Primary cutaneous acral CD8$^+$ T-cell lymphoma: very good prognosis
- Primary cutaneous CD4$^+$ small/medium T-cell lymphoproliferative disorder: indolent with 5-year survival of approximately 80%

Gender and Age Distribution
- Adults
- No gender predilection

Clinical Features
- Primary cutaneous γδ T-cell lymphoma: generalized papules, patches, and plaques to deep dermal or subcutaneous nodules that may be ulcerated, followed by dissemination to mucosal and other extranodal sites
- Primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic T-cell lymphoma: generalized superficial hyperkeratotic patches and plaques to eruptive papules and centrally ulcerated nodules followed by extranodal dissemination
- Primary cutaneous acral CD8$^+$ T-cell lymphoma: isolated slow-growing reddish papules or nodules most commonly on the ear, nose, or foot
- Primary cutaneous CD4$^+$ small/medium T-cell lymphoproliferative disorder: solitary plaques or nodules preferentially in head and neck region but sometimes in the upper extremities or trunk

Prognosis and Therapy
- Primary cutaneous γδ T-cell lymphoma and primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic T-cell lymphoma: chemotherapy but poor prognosis for both
- Primary cutaneous acral CD8$^+$ T-cell lymphoma: very good prognosis following excision or local irradiation
- Primary cutaneous CD4$^+$ small/medium T-cell lymphoproliferative disorder: good prognosis with surgical excision or local irradiation

PRIMARY CUTANEOUS PERIPHERAL T-CELL LYMPHOMA, RARE SUBTYPES—PATHOLOGIC FEATURES

Microscopic Findings
- Primary cutaneous γδ T-cell lymphoma: variable with focal mild to overtly pagetoid epidermotropism, dermal, and subcutaneous involvement by medium to large tumor cells associated with karyorrhexis and necrosis
- Primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic T-cell lymphoma: marked pagetoid epidermotropism by pleomorphic small–medium to medium–large tumor cells, possibly associated with lichenoid pattern and destruction of adnexal structures
- Primary cutaneous acral CD8$^+$ T-cell lymphoma: dermal infiltrate of uniform medium-sized lymphocytes with irregular nuclei that spare the epidermis and skin appendages and are not associated with necrosis
- Primary cutaneous CD4$^+$ small/medium T-cell lymphoproliferative disorder: dense nodular dermal infiltrates of pleomorphic small–medium tumor cells that may extend into subcutis but have no conspicuous epidermotropism or significant large cell component

Immunophenotypic Findings
- Primary cutaneous γδ T-cell lymphoma: CD3$^+$, CD4$^-$, CD5$^-$, CD7$^{+/-}$, CD8$^-$, CD56$^+$, TCR-αβ$^-$, TCR-γδ$^+$, TIA-1$^+$, and granzyme B$^+$
- Primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic T-cell lymphoma: CD3$^+$, CD4$^-$, CD5$^-$, CD7$^{+/-}$, CD8$^+$, CD30$^-$, CD56$^-$, TCR-αβ$^+$, TCR-γδ$^-$, TIA-1$^+$, and granzyme B$^+$
- Primary cutaneous acral CD8$^+$ T-cell lymphoma: CD3$^+$, CD4$^-$, CD5$^+$, CD7$^{+/-}$, CD8$^+$, CD68$^+$, TCR-αβ$^+$, TCR-γδ$^-$, TIA-1$^+$, and granzyme B$^-$
- Primary cutaneous CD4-positive small/medium T-cell lymphoproliferative disorder: CD3$^+$, CD4$^+$, CD8$^-$, CD30$^-$, PD1$^+$, TCR-αβ$^+$, TCR-γδ$^-$, TIA-1$^-$, and granzyme B$^-$

Molecular and Cytogenetic Findings
- Clonal TCR gene rearrangements in all four subtypes
- No association with EBV
- No specific genetic abnormalities

Differential Diagnosis
- MF or its variants for all four subtypes
- SPTCL for primary cutaneous γδ T-cell lymphomas having subcutaneous predominance
- Cutaneous lymphoid hyperplasia for primary cutaneous CD4$^+$ small–medium T-cell lymphoproliferative disorder
- Cutaneous B-cell lymphomas for primary cutaneous CD4$^+$ small–medium T-cell lymphoproliferative disorder with numerous non-neoplastic B cells

medium to large with clumped chromatin. Karyorrhexis and necrosis are common, and angioinvasion may be seen.

Primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic T-cell lymphomas have marked pagetoid epidermotropism, possibly associated with a lichenoid pattern (Fig. 9.13). Invasion and destruction of adnexal structures are common, and angioinvasion may be present. Tumor cells vary from small to medium to medium to large and are pleomorphic.

Primary cutaneous acral CD8$^+$ T-cell lymphoma is composed of uniform medium-sized lymphocytes with irregular nuclei that are in the dermis, possibly extending into the subcutis, with sparing of the epidermis. Skin appendages are also spared. There is no angiotropism, angiodestruction, or necrosis. Lymphoid follicles may be present.

Primary cutaneous CD4$^+$ small/medium T-cell lymphoproliferative disorders show dense nodular dermal infiltrates that may extend into the subcutis (Fig. 9.14). Epidermotropism, if present, is not conspicuous. Tumor cells are typically

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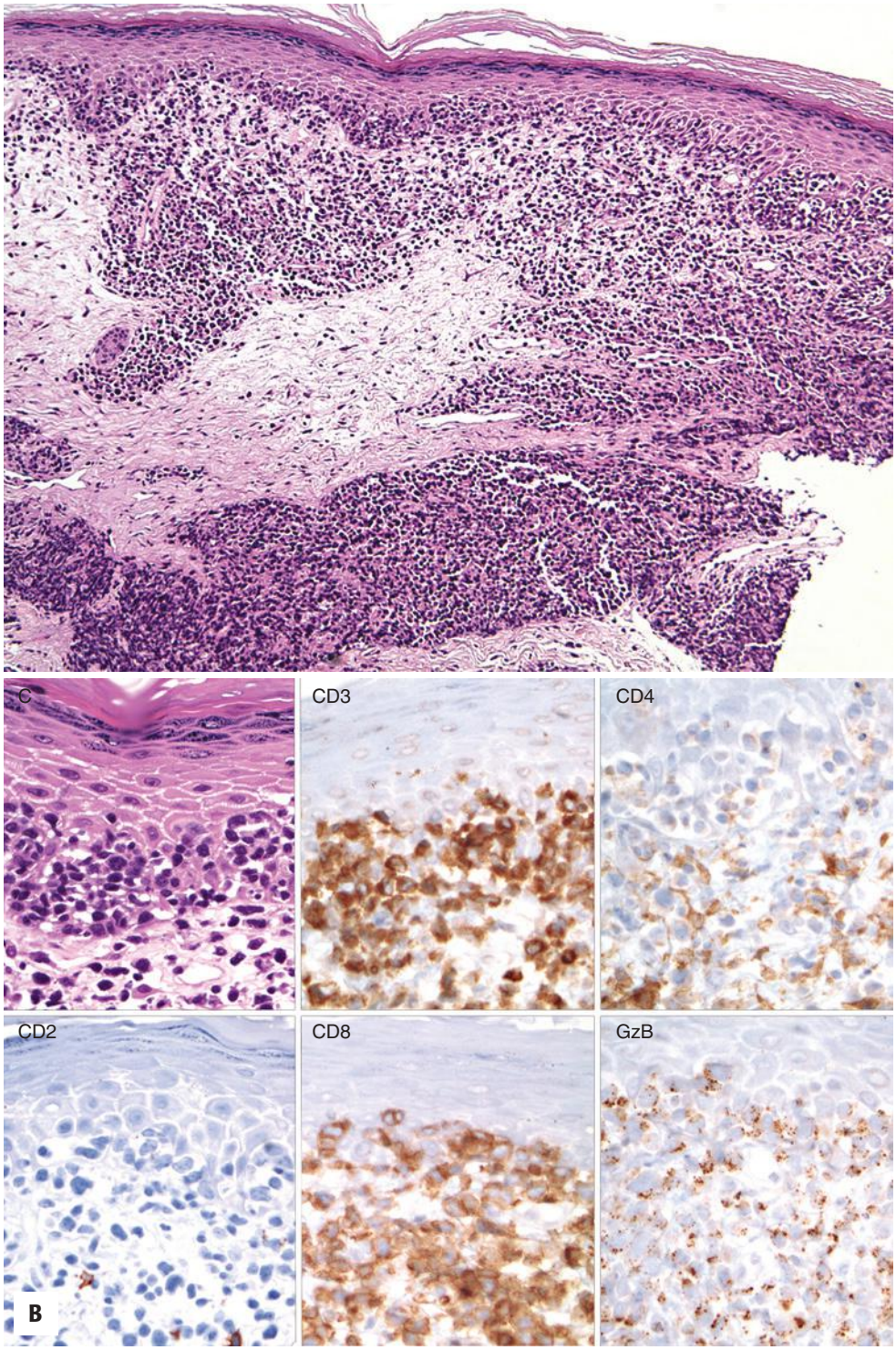


FIG. 9.13

Primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic T-cell lymphoma: microscopic features and immunophenotypic features. A, At low magnification, this skin biopsy shows an epidermotropic and lichenoid lymphoid infiltrate that extends into the deep dermis. B, At high magnification in the *upper left panel,* small to medium-sized tumor cells show striking epidermotropism. The accompanying paraffin immunoperoxidase stains show a CD3$^+$, CD8$^+$, and granzyme B$^+$ phenotype for the neoplastic cells that also lacks CD4 and has aberrant loss of CD2.

small to medium and are pleomorphic. Pleomorphic large cells, if present, comprise less than 30% of the infiltrate. Reactive small lymphocytes and histiocytes may be frequent.

Immunophenotype

The tumor cells of primary cutaneous γδ T-cell lymphoma are typically CD3$^+$, CD4$^-$, CD5$^-$, CD7$^{+/-}$, CD8$^-$, CD56$^+$, TCR-αβ$^-$, and TCR-γδ$^+$. Tumors resembling SPTCL preferentially express the TCRVδ2 epitope, which is the form found in normal cutaneous γδ T cells. All cases have strong expression of cytotoxic granule–associated proteins such as TIA-1 and granzyme B.

The tumor cells of primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic T-cell lymphoma are typically CD3$^+$, CD4$^-$, CD5$^-$, CD7$^{+/-}$, CD8$^+$, CD56$^-$, TCR-αβ$^+$, and TCR-γδ$^-$ (see Fig. 9.13). These cells express cytotoxic granule–associated proteins such as TIA-1 and granzyme B.

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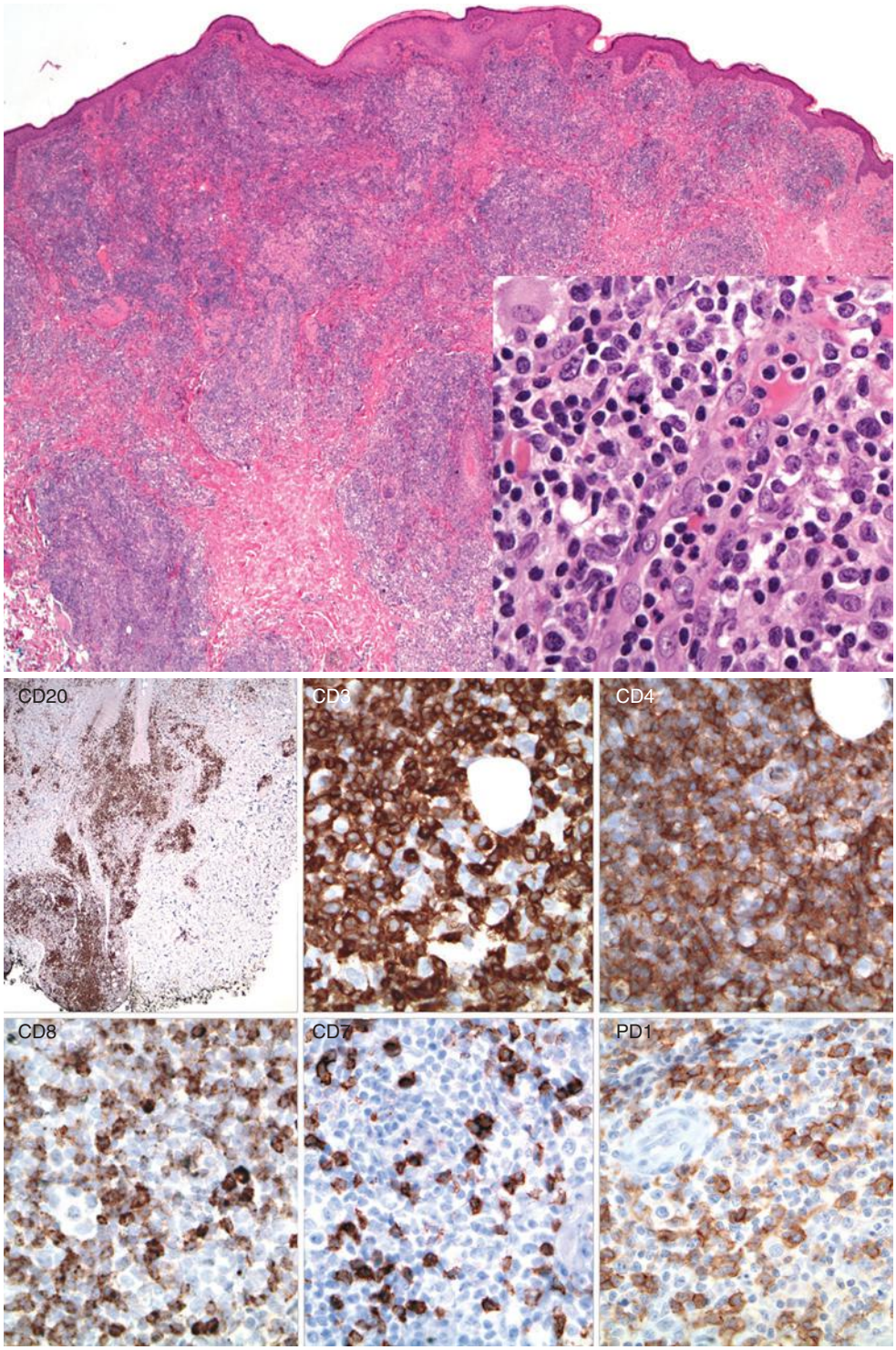


FIG. 9.14

Primary cutaneous CD4⁺ small/medium T-cell lymphoproliferative disorder: microscopic features and immunophenotypic features. At low magnification, this skin excision exhibits a patchy dense dermal lymphoid infiltrate with no significant epidermotropism. High magnification *(inset)* shows mostly pleo morphic small lymphocytes and some medium-sized lymphocytes admixed with histiocytes. At high magnification, the paraffin immunoperoxidase stains show a CD3⁺, CD4⁺, and CD7⁻ phenotype for the tumor cells, some of which coexpress PD1 (CD279). There are intermixed non-neoplastic CD20⁺ B cells and reactive CD7⁺ and CD8⁺ T cells.

The tumor cells of primary cutaneous acral CD8⁺ T-cell lymphoma are typically CD3⁺, CD4⁻, CD5⁺, CD7$^{+/-}$, CD8⁺, TCR-αβ⁺, TCR-γδ⁻, TIA-1⁺, and granzyme B⁻. TIA-1 generally has aGolgi dot-like staining pattern. CD68 may be present in the tumor cells.

The tumor cells of primary cutaneous CD4⁺ small to medium T-cell lymphoproliferative disorder are typically CD3⁺, CD4⁺, CD8⁻, CD30⁻, TCR-αβ⁺, and TCR-γδ⁻ (see Fig. 9.14); they do not express cytotoxic granule–associated proteins. Frequent small B cells are often intermixed. These lymphomas may be related to TFH cells because they have also been shown to express TFH-associated markers such as PD-1, CXCL-13, and BCL6.

Molecular and Cytogenetic Findings

Clonal TCR gene rearrangements are generally found in all four of these rare primary cutaneous PTCL subtypes. If

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clonal TCR-β chain gene rearrangements are detected in primary cutaneous γδ T-cell lymphoma, they are nonproductive. None of these rare subtypes is EBV associated. Specific genetic abnormalities have not been described for any of the four subtypes.

Differential Diagnosis

All four rare subtypes must be distinguished from MF or one of its variants. The presence of typical patches or plaques in the appropriate distribution along with epidermotropism of $CD4^+$ T cells supports a diagnosis of MF. Primary cutaneous γδ T-cell lymphomas with predominant subcutaneous involvement require a distinction from SPTCL. Immunophenotyping demonstrating that the tumor cells are indeed γδ type strongly supports primary cutaneous γδ T-cell lymphoma as opposed to these other rare types and SPTCL. Primary cutaneous $CD4^+$ small to medium T-cell lymphoproliferative disorder can be misidentified as cutaneous lymphoid hyperplasia or as a cutaneous B-cell lymphoma when there are numerous intermixed non-neoplastic B cells. Demonstration of monoclonality and expression of TFH markers on the atypical $CD4^+$ T cells will support the diagnosis. Drug reactions associated with lymphoid hyperplasia, such as anticonvulsants, should also be considered, since these lymphoproliferative lesions can be monoclonal T-cell–dominant reactions. Clinical history is important for arriving at the most appropriate diagnosis.

Primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma should be distinguished from type D LyP. Both may be composed of $CD8^+/CD30^+$ cytotoxic T-cells with epidermotropism but clinical presentation is completely different. The former presents with plaques and nodules with an aggressive and progressive course while LyP shows indolent waxing and waning papules. Pagetoid reticulosis (PR) shows epidermotropic T-cells that may commonly express either CD4 or CD8. However, unlike primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma, PR presents as a localized patch/plaque and cells are limited to the epidermis.

EPSTEIN-BARR VIRUS–POSITIVE T-CELL LYMPHOPROLIFERATIVE DISORDERS OF CHILDHOOD

There are two main EBV-associated T-cell lymphoproliferative disorders that manifest in the pediatric age group and occasionally in young adults: systemic EBV-positive T-cell lymphoma of childhood and chronic active EBV infection (CAEBV). CAEBV has a variety of clinical manifestations, and hydroa vacciniforme–like lymphoproliferative disorder is a localized form. Systemic EBV-positive T-cell lymphoma of childhood and hydroa vacciniforme–like lymphoproliferative disorder have increased frequency in Asians and in indigenous people from Central and South America and Mexico.

Clinical Features

Systemic EBV-positive T-cell lymphoma of childhood occurs shortly after primary acute EBV infection or in the setting of chronic active EBV infection. The clinical course is fulminant with fever, hepatosplenomegaly, cytopenia, rapid progression to multiorgan failure, sepsis, and death within days to weeks. A hemophagocytic syndrome usually develops. Cases of systemic EBV-positive T-cell lymphoma of childhood have been reported previously under such terms as *fulminant EBV-positive T-cell lymphoproliferative disorder of childhood, fulminant hemophagocytic syndrome in children, fatal*

EPSTEIN-BARR VIRUS–POSITIVE T-CELL LYMPHOPROLIFERATIVE DISORDERS OF CHILDHOOD—FACT SHEET

Definition
- Two EBV-associated T-cell lymphoproliferative disorders in the pediatric age group: systemic EBV-positive T-cell lymphoma of childhood and hydroa vacciniforme–like lymphoproliferative disorder

Incidence and Location
- Rare

Morbidity and Mortality
- Systemic EBV-positive T-cell lymphoma of childhood: most cases fulminant with death in days to weeks after onset
- Hydroa vacciniforme–like lymphoproliferative disorder: protracted course often lasting more than a decade but fatal outcome

Race and Age Distribution
- Children and occasionally young adults
- Increased frequency in Asians and in indigenous people from Central and South America and Mexico for both entities

Clinical Features
- Systemic EBV-positive T-cell lymphoma of childhood: occurs shortly after primary acute EBV infection or in the setting of chronic active EBV infection with rapid progression to hemophagocytic syndrome, multiorgan failure, sepsis, and death
- Hydroa vacciniforme–like lymphoproliferative disorder: papulovesicular eruptions on sun-exposed skin that generally ulcerate and scar in patients who exhibit hypersensitivity to sunlight and insect bites, particularly mosquito bites

Prognosis and Therapy
- Systemic EBV-positive T-cell lymphoma of childhood: poor response to any therapy
- Hydroa vacciniforme–like lymphoproliferative disorder: refractory to chemotherapy but cures possible with allogeneic hematopoietic stem-cell transplantation

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EBV-associated hemophagocytic syndrome, and *severe chronic active EBV infection*. It is associated with abnormal EBV serology with low or absent anti-viral-capsid antigen (VCA) immunoglobulin M (IgM) antibodies and high viral loads.

Hydroa vacciniforme–like lymphoproliferative disorder manifests as papulovesicular eruptions on sun-exposed skin that generally ulcerate and scar. Patients exhibit hypersensitivity to sunlight and insect bites, particularly mosquito bites. The clinical course is often protracted for a decade or more before progression to systemic disease, which behaves more aggressively with a fatal outcome. B symptoms accompanied by lymphadenopathy and hepatosplenomegaly occur with systemic involvement.

Pathologic Features

Microscopic Findings

Systemic EBV-positive T-cell lymphoma of childhood preferentially infiltrates the liver and spleen, followed by lymph nodes, bone marrow, skin, and lung. Splenic infiltrates are mainly in the sinuses, and the white pulp is diminished. Hepatic infiltrates are portal and sinusoidal. The tumor cells are often small without significant cytologic atypia, but cases with pleomorphic medium to large cells have been described. Hemophagocytosis is often pronounced.

Hydroa vacciniforme–like lymphoproliferative disorder extends from the epidermis to the subcutis, and there is frequent ulceration. Tumor cells are small to medium without significant cytologic atypia. Angiocentric and angioinvasive foci may be present.

Immunophenotype

Tumor cells of systemic EBV-positive T-cell lymphoma of childhood are typically $CD3^+$, $CD56^+$, and $TIA\text{-}1^+$. The cells are often $CD8^+$ after acute primary EBV infection but $CD4^+$ in the setting of severe chronic active EBV infection. Immunophenotyping may be needed to demonstrate the true extent of infiltrate in cases with minimal cytologic atypia.

Tumor cells of hydroa vacciniforme–like lymphoproliferative disorder have a cytotoxic T-cell phenotype and may express CD56. A few cases have NK-cell derivation.

Molecular and Cytogenetic Findings

Clonal rearrangement of TCR genes can usually be demonstrated in both systemic EBV-positive T-cell lymphoma of childhood and hydroa vacciniforme–like lymphoproliferative disorder. Tumor cells are EBV positive by EBER in situ hybridization, and EBV is monoclonal by terminal repeat analysis.

EPSTEIN-BARR VIRUS–POSITIVE T-CELL LYMPHOPROLIFERATIVE DISORDERS OF CHILDHOOD—PATHOLOGIC FEATURES

Microscopic Findings

- Systemic EBV-positive T-cell lymphoma of childhood: splenic sinus and hepatic portal and sinusoidal infiltrates of usually small tumor cells, often accompanied by striking hemophagocytosis
- Hydroa vacciniforme–like lymphoproliferative disorder: epidermal to subcutaneous infiltrates of small to medium tumor cells, often associated with ulceration

Immunophenotypic Findings

- Systemic EBV-positive T-cell lymphoma of childhood: $CD3^+$, $CD56^+$, and $TIA\text{-}1^+$; $CD8^+$ after acute primary EBV infection but $CD4^+$ in setting of severe chronic active EBV infection/systemic EBV+ T-cell lymphoma of childhood
- Hydroa vacciniforme–like lymphoproliferative disorder: $CD3^+$, $CD8^+$, $CD56^{+/-}$, and $TIA\text{-}1^+$

Molecular and Cytogenetic Findings

- Clonal TCR gene rearrangements for both entities
- EBV-positive tumor cell by EBER in situ hybridization for both
- Clonal EBV by terminal repeat analysis

Differential Diagnosis

- Systemic EBV-positive T-cell lymphoma of childhood: aggressive NK-cell leukemia and HSTCL
- Hydroa vacciniforme–like lymphoproliferative disorder: extranodal NK/T-cell lymphoma, nasal type

Differential Diagnosis

Systemic EBV-positive T-cell lymphoma of childhood has some overlapping features with aggressive NK-cell leukemia; however, the latter tends to occur more frequently in middle-aged adults rather than in the pediatric age group. HSTCL should be included in the initial differential diagnosis but can be excluded by the phenotypic attributes of the tumor cells and their lack of EBV. Hydroa vacciniforme–like lymphoproliferative disorder must be distinguished from cutaneous presentation of extranodal NK/T-cell lymphoma, nasal type. X-linked lymphoproliferative disease caused by *SAP* mutation is characterized by an initial fulminant acute EBV infection with organomegaly and hemophagocytic syndrome. EBV-infected B cells and activated T cells are seen, as opposed to the EBV-infected T/NK cells of these systemic EBV-positive T-cell disorders.


The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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10

Immunodeficiency-Related Lymphoproliferative Disorders

Jonathan W. Said, MD

INTRODUCTION

Lymphoproliferative disorders are innately disorders of the immune system, and the immunodeficiency-related lymphoproliferative disorders are of particular interest because they offer a paradigm for studying the relationships between the immune system and the evolution of neoplastic disease. Although the various entities offer a complicated spectrum of lymphoid proliferations with heterogeneous morphologies, there are similarities across varying immunodeficiency backgrounds. They have a number of features in common, including the tendency to involve extranodal sites and to be infected with the Epstein-Barr virus (EBV).

POSTTRANSPLANTATION LYMPHOPROLIFERATIVE DISORDERS

Posttransplantation lymphoproliferative disorders (PTLDs) occur in patients who have undergone bone marrow or solid organ transplantation and associated immunosuppressive therapy to prevent graft rejection.

Clinical Features

According to the United Network for Organ Sharing (UNOS), the 5-year cumulative incidence of PTLD is 3.26% for children and 0.91% for adults (Organ Procurement and Transplantation Network [OPTN]/UNOS Ad Hoc Disease Transmission Advisory Committee Report to the Board of Directors 2009). PTLD is most common in children younger than 10 years and adults older than 60 years. It is the second most common posttransplant malignancy in children, and the second most common malignancy after skin cancer in adults. The risk of PTLD is greatest in the first year to 18 months but can occur any time after transplant. Treatment decisions are based on the histologic type, grade, stage, and site of tumor; assessment of clinical state, including transplant organ function; and capacity to tolerate therapy.

Patients who are EBV negative before transplantation and then seroconvert are at the greatest risk for PTLD. With early preventive strategies, late PTLD is becoming relatively more common. Greater risk occurs with more complex and multiple organ transplants, which require greater immunosuppression. In bone marrow transplant recipients, PTLD is of donor rather than recipient origin, and there is increased risk with T-cell depletion, severe graft-versus-host disease, anti-CD3 immunotherapy, and degree of HLA antigen mismatch.

Early or nondestructive lesions, such as plasma cell hyperplasia–infectious mononucleosis (IM)-like PTLDs, tend to occur in younger age groups, often children, and involve the head and neck, particularly tonsil and Waldeyer's ring. These lesions usually regress with surgical excision or reduction in immunotherapy. Cases of polymorphic PTLD may regress in response to decreased immunosuppression, but more aggressive lesions will either require chemotherapy (including the use of anti-CD20 rituximab), and/or radiation. In the more advanced forms of PTLD there may be no response or progression despite therapy. Monomorphic PTLDs are often extranodal but may present in bone marrow or lymph nodes. These PTLDs tend to involve older age groups (median age, 56 years) and are often aggressive despite chemotherapy or radiation therapy. T-cell PTLD is uncommon, usually EBV negative, and tends to be aggressive and refractory to therapy.

Pathologic Features

A spectrum of histologic appearances with PTLDs ranges from the early or non-destructive lesions (plasma cell hyperplasia-IM–like), polymorphic PTLD, and monomorphic PTLD, which includes malignant lymphoma and myeloma (Table 10.1).

Early lesions (termed *non-destructive lesions* in WHO 2017) are characterized by preservation of architecture although they form mass lesions. Follicular hyperplasia may

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POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDER—FACT SHEET

Definition

- Heterogeneous group of lymphoid proliferations occurring in the posttransplantation setting, frequently extranodal and usually positive for EBV

Clinical Features

- Early or non-destructive lesions (follicular hyperplasia, plasma cell hyperplasia–infectious mononucleosis [IM]-like PTLDs) usually in younger age group and often involve tonsils or Waldeyer's ring
- More advanced lesions often extranodal, including the transplanted organ

Pathologic Features

- Early lesions (renamed nondestructive lesions in WHO 2017): Follicular hyperplasia EBV+, plasma cell hyperplasia–IM-like PTLD
- Polymorphic PTLD
- Monomorphic PTLD (classified like the corresponding lymphoma)

B-Cell Neoplasms

- Diffuse large B-cell lymphoma
- Burkitt lymphoma
- Myeloma
- Other

T/NK-Cell Neoplasms

- Peripheral T-cell lymphoma, NOS
- Hepatosplenic T-cell lymphoma
- Natural killer (NK) cell leukemia–lymphoma
- Other

Immunohistochemistry

- Early or non-destructive lesions are polyclonal; the remainder are monoclonal for immunoglobulin expression
- EBV is demonstrated by in situ hybridization for EBV-encoded RNA (EBER) or expression of latent membrane protein (LMP-1); EBER is more sensitive and should be used as a screening test

Molecular Genetics

- Plasmacytic hyperplasia is germline for immunoglobulin genes, and EBV polyclonal or oligoclonal
- Both polymorphic and monomorphic PTLDs are monoclonal for immunoglobulin genes and EBV
- Polymorphic PTLD lacks oncogene abnormalities except for BCL6
- Advanced lesions frequently have oncogene or suppressor gene abnormalities

Prognosis and Therapy

- Early and polymorphic lesions may respond to excision or reduction of immunosuppression
- More advanced lesions may progress despite chemotherapy or radiation therapy; treatment with anti-CD20 (rituximab) has been effective in some cases

NOS, Not otherwise specified.

TABLE 10.1

Classification and Key Features of PTLD

Type of PTLD	Histology	Phenotype	Molecular
Early (non-destructive) lesions			
Follicular hyperplasia Plasma cell hyperplasia Infectious Mononucleosis-like	Variable follicular hyperplasia EBV+ Numerous plasma cells Paracortical expansion with numerous immunoblasts	Polyclonal plasma cells T cells Most EBV+	Polyclonal or oligoclonal
Polymorphic			
	Effacement of architecture; mixed population of lymphocytes, plasma cells, immunoblasts	B cells and T lymphocytes admixed with immunoblasts and plasma cells	Clonal Ig gene rearrangements; usually EBV+; plasma cells may show light chain restriction
Monomorphic			
DLBCL Burkitt, myeloma, plasmacytoma, classic Hodgkin lymphoma, T-cell lymphoma	Resembles lymphoma type in nonimmunosuppressed population	Similar to lymphoma subtype; most EBV+; large cells may express EBER and LMP1 T-cell lymphomas usually EBV–	Clonal Ig gene rearrangements; often have other molecular abnormalities (e.g., *MYC, BCL6, NRAS,* p53)

DLBCL, Diffuse large B-cell lymphoma; *EBER*, EBV-encoded RNA*; EBV, Epstein-Bar virus; Ig,* immunoglobulin; *IM,* infectious mononucleosis; *LMP-1*, latent membrane protein *PTLD,* posttransplantation lymphoproliferative disorder.

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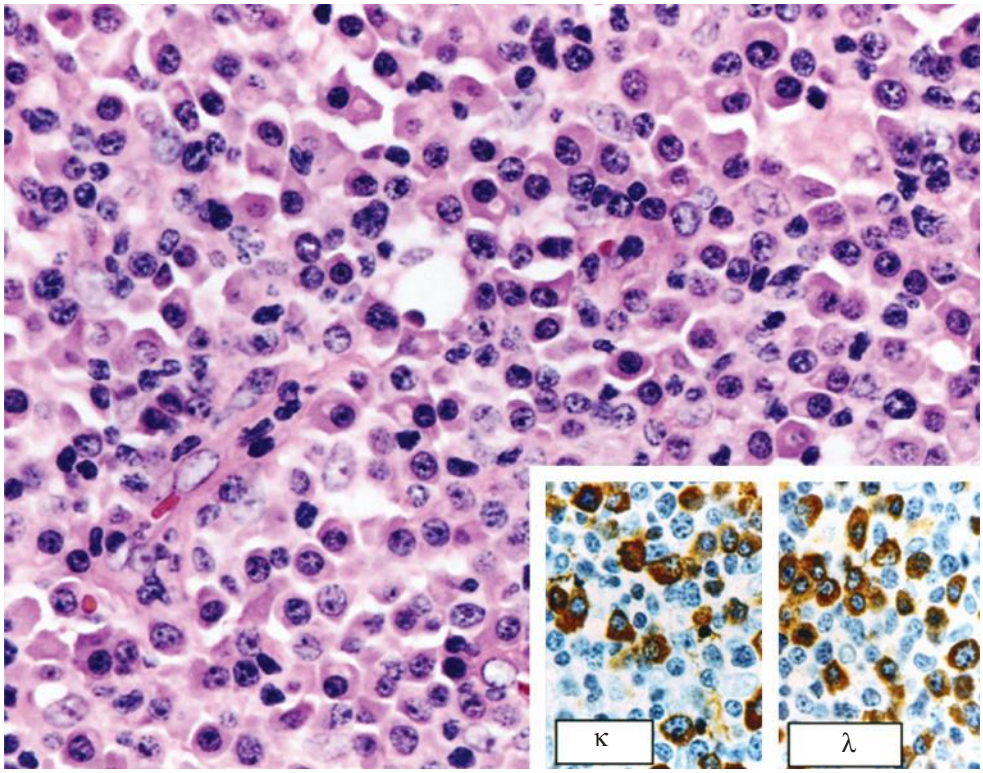


FIG. 10.1

Posttransplantation lymphoproliferative disorder plasma cell hyperplasias. There are sheets of plasmacytic cells with minimal atypical and polyclonal staining for immunoglobulin light chains.

be considered an early lesion if the hyperplastic follicles are positive for EBV. EBER expression may be variable in the follicles and/or interfollicular regions but characteristically involves one or more hyperplastic follicles. Hyperplasias generally involve lymph nodes, tonsils, and adenoids rather than extranodal sites. They tend to regress spontaneously or with excision or reduction of immunosuppression. In plasmacytic hyperplasia-IM–like PTLD, which often involves lymph nodes and tonsils, there is retention of the overall architecture with expansion of interfollicular areas by a proliferation of plasma cells, admixed with small lymphocytes and immunoblasts (Fig. 10.1). The plasma cells are polytypic and do not have clonal gene rearrangements. Large cells or immunoblasts are usually single, and there is minimal cytologic atypia. They form mass lesions without destruction of architecture, and the majority regresses spontaneously with withdrawal of immunosuppressive agents.

In polymorphic PTLD, there is destruction of architecture and a lymphoid infiltrate showing variable plasmacytic differentiation including immunoblasts, which may be found in small clusters but do not fulfill diagnostic criteria for lymphoma. This is the most common form of PTLD in the pediatric age group. They characteristically exhibit a full range of B-cell stages and have clonal immunoglobulin gene rearrangements. Atypical lymphoid cells with irregular nuclear outlines may be present, and there may be foci of necrosis (Fig. 10.2). The number of large cells is variable in polymorphic PTLD and the distinction from monomorphic PTLD is not always clear-cut. Immunoblasts may resemble Hodgkin or Reed-Sternberg cells. In some cases, in which T cells and histiocytes in the background predominate, separation from diffuse large B-cell lymphoma (DLBCL) with a T-cell/histiocyte-rich background may be particularly difficult. Most polymorphic PTLDs are EBV driven, with clonal immunoglobulin gene rearrangements but no other additional genetic abnormalities. They tend to respond to reduction in immunosuppression.

Mucocutaneous ulcer may also occur following transplantation as well as in other immunodeficiency settings. These present as well-circumscribed ulcerated masses in mucocutaneous sites. There is a spectrum of lymphoid cells and immunoblasts that are EBV+ and may resemble Hodgkin cells. The base of the ulcer characteristically consists of inflammatory cells without tissue invasion.

Monomorphic PTLD resembles lymphoma in the immunocompetent population and includes diffuse large B-cell lymphomas, which may have an immunoblastic appearance, Burkitt lymphoma (BL), myeloma/plasmacytoma, classic Hodgkin-type PTLD, and T-cell lymphomas. Indolent small B-cell lymphomas after transplant are generally not considered part of the spectrum of PTLD with the exception of EBV+ marginal zone lymphoma.

Burkitt lymphoma after transplant (Fig. 10.3) often manifests with high-stage disease and bone marrow involvement. It usually occurs late after transplant (average, 4.5 years). Plasmacytoma-like PTLD is relatively rare (Fig. 10.4) and is similar to extramedullary plasmacytoma in other settings, involving extranodal sites such as the gastrointestinal tract or lymph nodes.

Classic Hodgkin-type PTLD is a late-occurring PTLD (mean, 4 years after transplantation, but possibly 7 years or longer). It may be difficult to differentiate these lesions from other forms of PTLD in which there may be Reed-Sternberg

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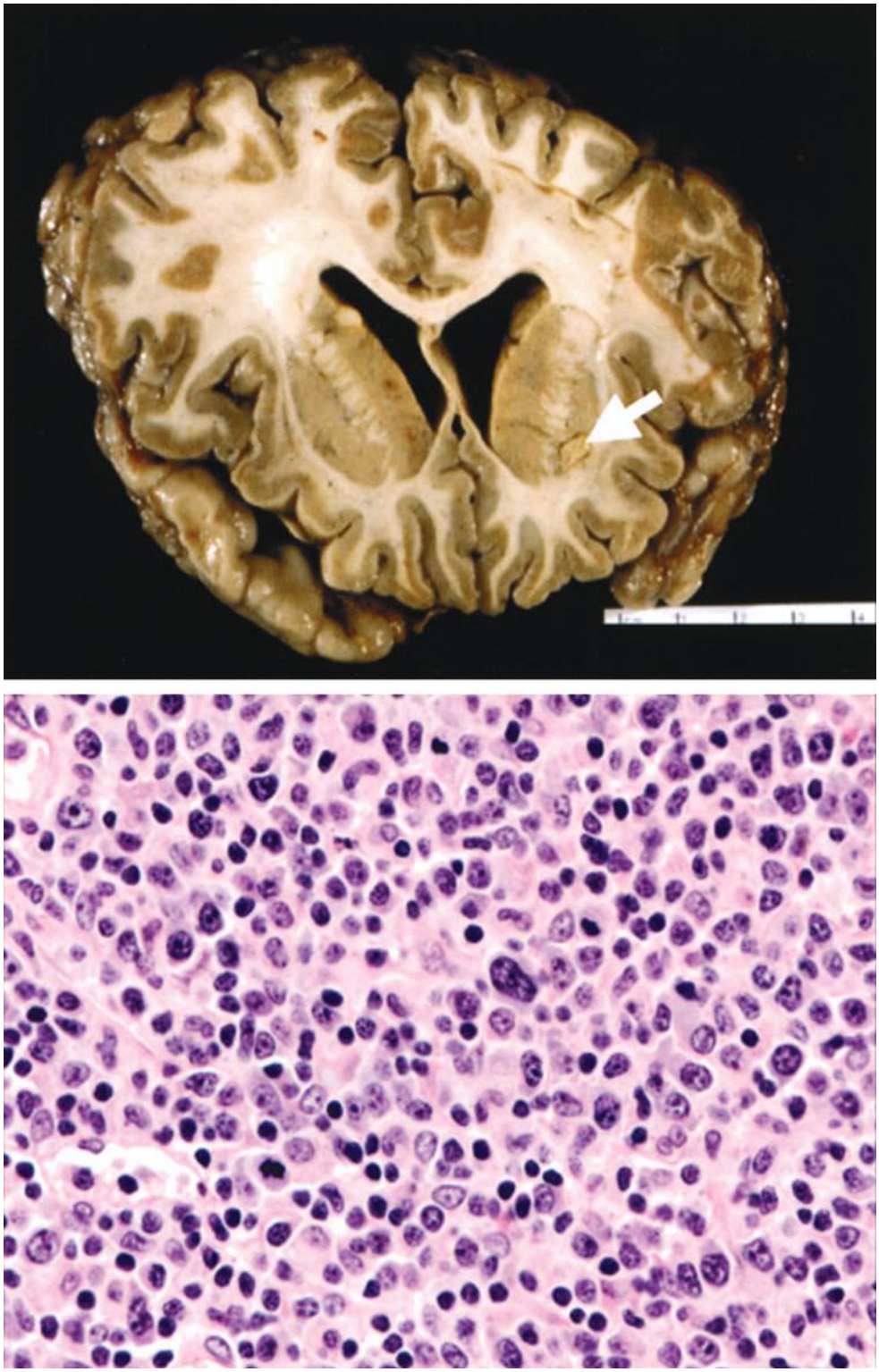


FIG. 10.2

Orthotopic liver transplant recipient treated initially for plasma cell hyperplasia but disease recurred with central nervous system involvement by polymorphic PTLD. A partially necrotic deep-seated lesion is seen in the region of the basal ganglia *(arrow)*. Histologic section reveals a polymorphous proliferation including plasma cells and large immunoblasts.

and Hodgkin-like cells, and the diagnosis of classic Hodgkin lymphoma (CHL) in this setting requires application of stringent diagnostic criteria. In other forms of PTLD resembling HL there may be small and intermediate sized EBV+ cells in addition to the HL cells. Mixed cellularity type is the most common form of HL in this setting, and the Reed-Sternberg cells are EBV+ (Fig. 10.5). Immunostaining may be helpful in the differential. Reed-Sternberg cells should have the classic phenotype CD15+, CD30+, usually negative for Octamer Transcription Factor-2 (OCT-2) or B-cell OCT binding factor-1 (BOB.1).

T-cell PTLDs range in incidence from 4% to 14% of PTLD. They should be classified according to the WHO classification of T-cell lymphomas and include the entire spectrum of

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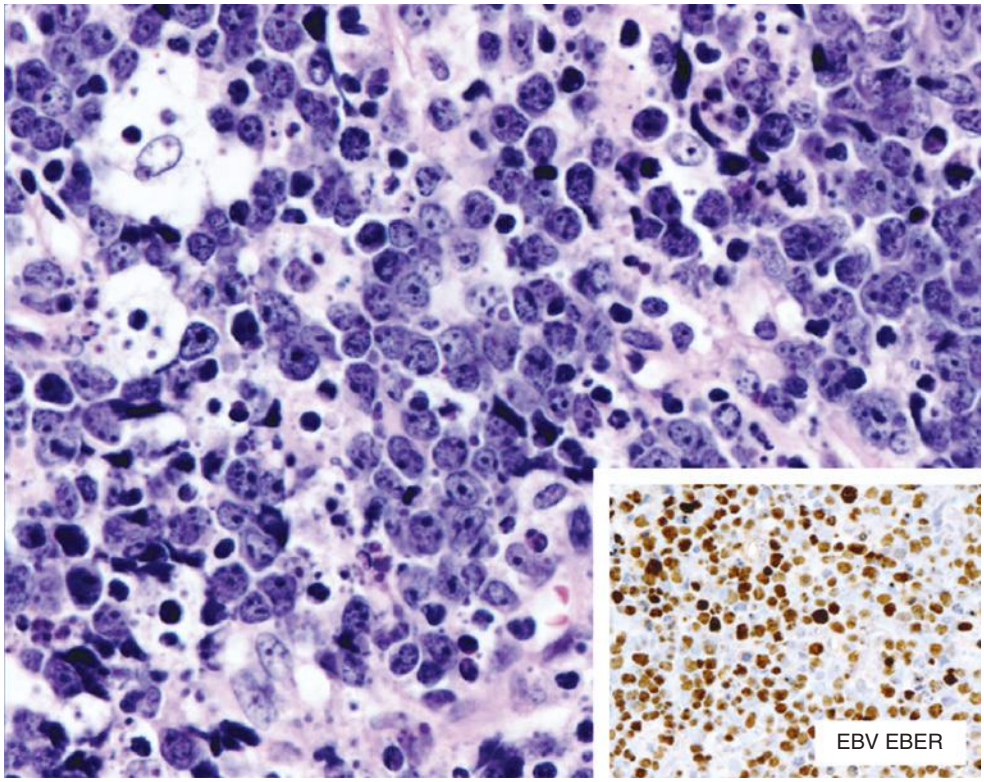


FIG. 10.3

Burkitt lymphoma post transplant with intermediate-sized, noncleaved lymphoma cells and a background that includes numerous phagocytic macrophages. The in situ hybridization for Epstein-Barr virus-encoded RNA (EBER) is strongly positive.

T/NK cell neoplasms. T-cell PTLDs are usually EBV– and occur later than B-cell PTLDs. PTLDs of NK-cell lineage are rare and include T/NK-cell lymphoma nasal type, and large granular lymphocytic leukemia. T-cell PTLDs can occur subsequent to other types of PTLDs.

Prognosis and Therapy

For hyperplasias and polymorphic PTLDs, a graded approach is generally recommended, with observation and reduction in immunosuppression, to single-agent rituximab, and addition of combination chemotherapy in refractory or progressive cases. With early lesions in children, surgical excision may be sufficient, and these cases are only rarely associated with progression to a more aggressive form of PTLD. Monomorphic PTLDs are generally managed as the corresponding lymphoma type, but on occasion have responded to reduction in immune suppression.

Immunophenotype

With the exception of the early plasma cell hyperplasia and IM-like lesions, B-cell PTLD cases often reveal clonally restricted surface or cytoplasmic immunoglobulin light chain expression. Despite their polymorphous histology, most polymorphic PTLDs are clonal in terms of immunoglobulin gene rearrangements and expression of EBV. EBV is usually not a factor in the causation of T-cell PTLD, with the exception of rare cases of extranodal NK/T-cell lymphoma nasal type, chronic active EBV infection, and systemic EBV+ T-cell lymphoma of childhood.

Genetic Features

Posttransplantation lymphoproliferative disorders are monoclonal in regard to immunoglobulin gene rearrangements, with the exception of the early or non-destructive lesions, which are germline. Up to 90% of cases of PTLD are associated with EBV, which can easily be demonstrated with in situ hybridization for EBER. Most cases of PTLD express EBV nuclear antigen-2 (EBNA-2) and LMP1, corresponding to EBV latency patterns II or III.

In plasmacytic hyperplasia–IM-like PTLD, there are no abnormalities in oncogenes or tumor suppressor genes. Polymorphic PTLD usually lacks abnormalities in oncogenes, with the exception of *BCL6*. Monomorphic PTLD usually demonstrates structural abnormalities involving oncogenes and tumor suppressor genes such as *MYC, NRAS,* and *TP53*.

HUMAN IMMUNODEFICIENCY VIRUS–RELATED LYMPHOPROLIFERATIVE DISORDERS

Introduction and Definition

Because the spectrum of lymphoid hyperplasia associated with HIV infection is discussed in Chapter 4, this chapter

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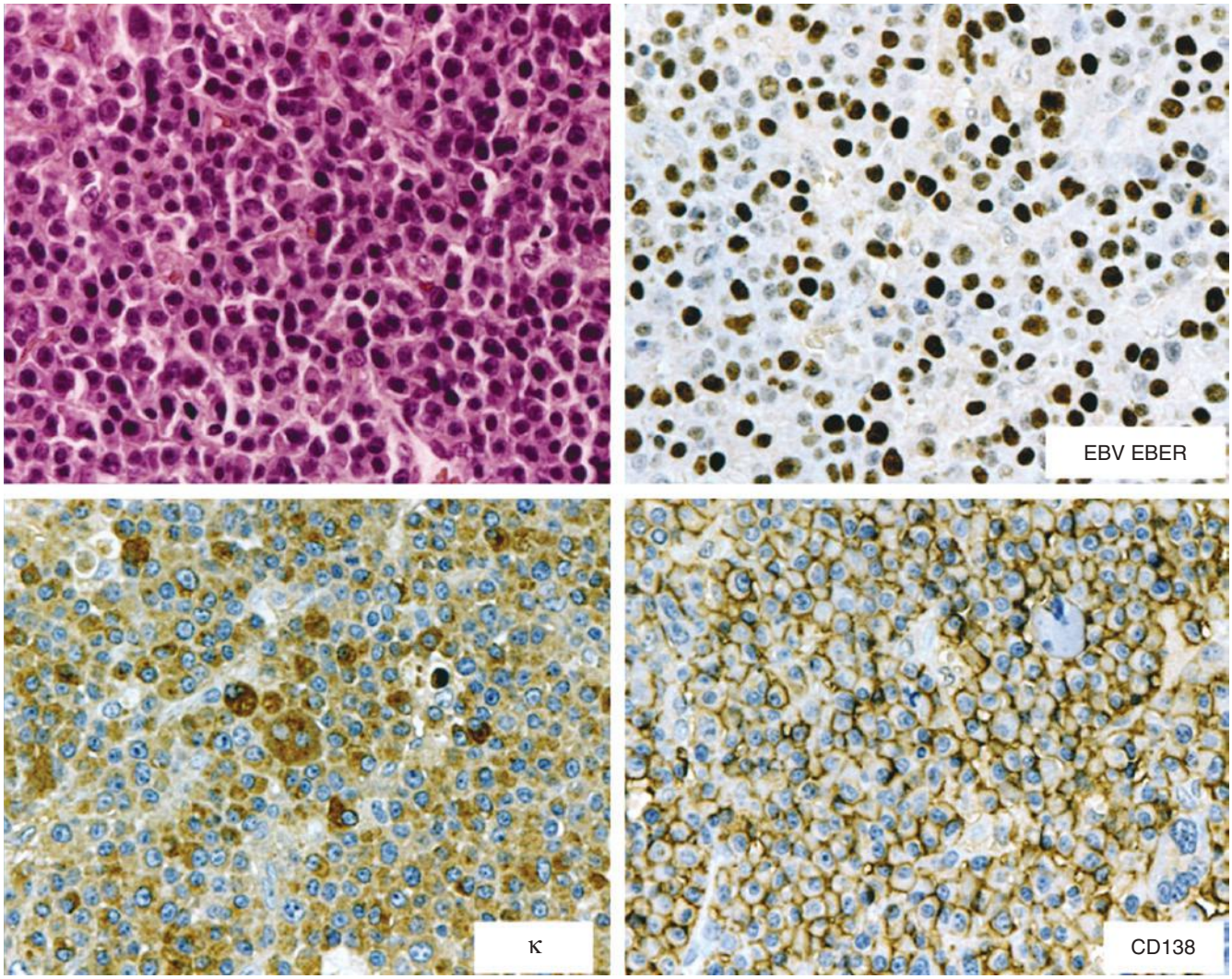


FIG. 10.4

Plasmacytoma-like posttransplantation lymphoproliferative disorder with sheets of uniform plasmacytic cells, positive for CD138, κ light chains, and Epstein-Barr virus. These disorders resemble extramedullary plasmacytomas and are not associated with lytic bone lesions.

restricts discussion to neoplastic lymphoproliferative disorders. Acquired immunodeficiency syndrome (AIDS)-related lymphomas (ARLs) account for approximately 10% of all lymphomas in the United States and Europe. The introduction of highly active antiretroviral multiple-agent therapy (HAART) for HIV has significantly affected the incidence of AIDS lymphoma and has resulted in a 75% decline in mortality. Nevertheless, the incidence of non-Hodgkin lymphoma (NHL) remains higher in HIV+ individuals compared with the general population; paradoxically, the incidence of classic Hodgkin lymphoma appears to have increased with the widespread use of antiretroviral therapy. Before HAART, lymphoma was a late manifestation of HIV infection, occurring in severely immunocompromised individuals with extremely low CD4+ T-cell counts. Tumor pathobiology at the time of clinical presentation is becoming a critical determinant of outcome rather than the immune environment during treatment. The incidence of low-grade lymphomas in HIV-infected patients is also increasing.

The pathogenesis of AIDS lymphomas most likely relates to chronic antigen stimulation and EBV infection resulting in B-cell overproduction and secondary genetic abnormalities in the background of immune deficiency. Polyclonal and oligoclonal B-cell expansion frequently precedes the development of lymphoma. Recent evidence suggests that HIV may directly contribute to lymphomagenesis because HIV p17 protein enhances B-cell clonogenic activity. Unlike lymphomas in the transplant setting, ARLs are associated with both A- and B-type EBV. Viral infection (EBV and human herpesvirus 8 [HHV8]) may be associated with cytokine production including IL6, IL10, and IL13, which may also contribute to lymphoid proliferation and plasmacytoid differentiation of neoplastic cells.

EBV can be demonstrated in approximately 40% of cases of HIV-related lymphoma and is highest in cases with immunoblastic morphology and lymphomas involving the central nervous system (CNS; approaching 100%), compared with BL, where the incidence of infection of tumor cells with EBV resembles sporadic rather than endemic cases (approximately

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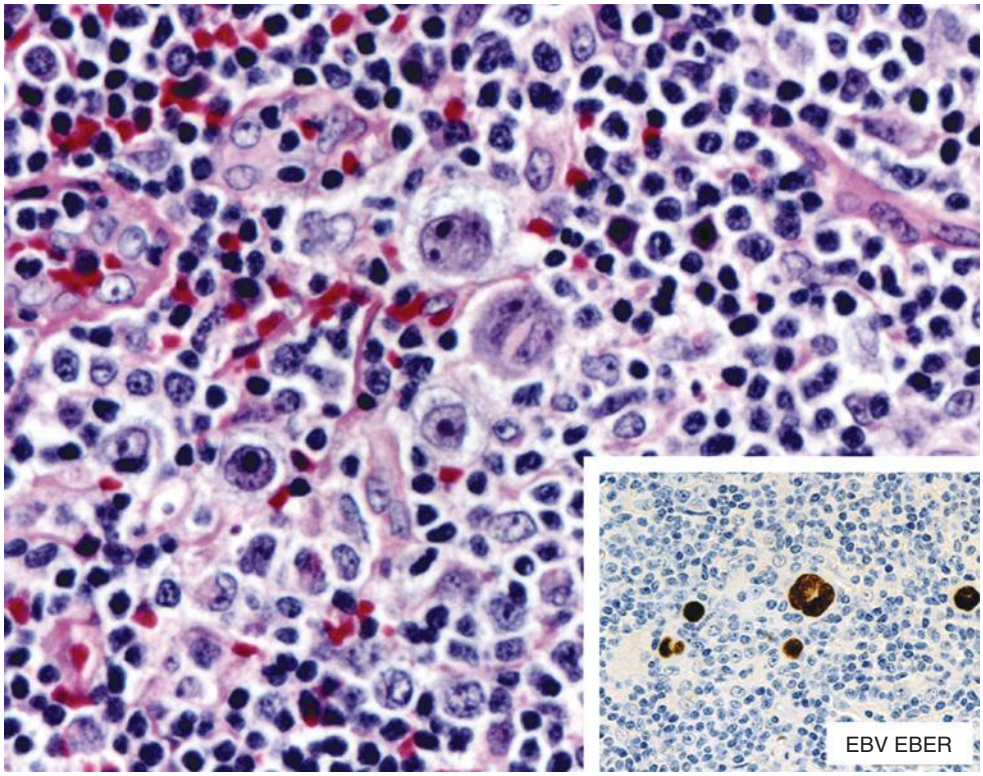


FIG. 10.5

Classic Hodgkin lymphoma, mixed cellularity type, post transplant. Numerous Reed-Sternberg and Hodgkin cells are present, with strong localization of EBERs.

40%, slightly higher in cases with atypical or plasmacytoid morphology). The incidence of EBV infection in AIDS-related centroblastic lymphoma is 20% to 30%. Cases of primary effusion lymphoma in patients with AIDS contain both EBV and HHV8. Plasmablastic lymphomas associated with HIV are usually EBV+, as are many cases of myeloma, including the plasmablastic variant.

Chromosomal translocations can result in the activation of *MYC* and, less commonly, other oncogenes, including *RAS*, *BCL2,* and *TP53*. *MYC* activation and *TP53* inactivation occur more often in cases of BL. *MYC* has also been associated with plasmablastic differentiation in cases of EBV+ plasmablastic lymphoma.

Clinical Features

Systemic B symptoms are usually present in patients with ARLs, and these include unexplained fever, drenching night sweats, and weight loss greater than 10% of normal body weight. Patients frequently have extensive disease at diagnosis, including involvement of the bone marrow, cerebrospinal fluid, and gastrointestinal tract. Any site can be involved by lymphoma; unusual sites include the oral cavity, adrenal glands, heart, kidney, and gallbladder. Multiple tumor masses may be present and can represent synchronous clonal neoplasms. There may be extensive organ infiltration leading to organ failure, particularly in the bone marrow and liver.

The incidence of primary central nervous system (CNS) lymphomas has fallen dramatically since the onset of HAART. These patients usually have one or more space-occupying lesions in the brain that can be deep seated. A computed tomographic scan of the brain may be confused with cerebral toxoplasmosis, although in the latter condition the lesions are usually multiple and smaller. In children with AIDS, primary lymphoma is the most common cause of focal or multifocal brain masses.

Pathologic Features

The World Health Organization (WHO) has classified ARLs into three categories. The first category, lymphomas that also occur in immunocompetent patients, includes BL, diffuse large B-cell lymphoma, mucosa-associated lymphoid tissue (MALT) lymphoma, and classic HL. Cases of peripheral T-cell lymphoma have been reported, as well as rare NK-cell lymphomas.

In the second category of tumors, which occur more specifically with HIV, there is HHV8-related PEL and its extracavitary variant, plasmablastic lymphoma of the oral cavity type, and lymphoma arising in the setting of HHV8-associated multicentric Castleman disease.

The third category, limited to lymphomas also occurring in other immunodeficiency disorders, contains a single-entity, polymorphic B-cell or PTLD-like lymphoma.

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AIDS-RELATED LYMPHOMA CLASSIFICATION—FACT SHEET

Lymphomas Also Occurring in Immunocompetent Patients

- Burkitt lymphoma
- Diffuse large B-cell lymphoma
- Centroblastic
- Immunoblastic (>90% of the cells resemble immunoblasts)
- Plasmablastic
- Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
- T/NK-cell lymphomas, including peripheral T/NK-cell lymphoma NOS, anaplastic large-cell lymphoma, cutaneous T-cell lymphoma, and nasal-type NK/T-cell lymphoma
- Classic HL

Lymphomas Occurring More Specifically in HIV+ Patients

- Primary effusion lymphoma
- Plasmablastic lymphoma of the oral cavity type

Lymphomas Also Occurring in Other Immunodeficiency States

- Polymorphic lymphoma (PTLD-like)

Diffuse Large B-Cell Lymphomas (30% ARL Cases)

- Heterogeneous morphology, most centroblastic or immunoblastic (>90% of the cells resemble immunoblasts in the immunoblastic variant)
- EBV variable, but 100% in the CNS

Burkitt Lymphomas (30% ARL)

- Present at peripheral sites (e.g., the gastrointestinal tract) or with lymphoma-like packing of the marrow
- Subtype with plasmacytoid cytoplasm characteristic of AIDS
- EBV in about 30% to 40% of cases
- Have *MYC* translocations and frequent *TP53* mutations

Primary Effusion Lymphomas

- Present in body cavities without tumor masses
- Have an immunoblastic or anaplastic large cell appearance
- Infected with HHV8 and EBV
- Phenotype negative for CD45 (leukocyte common antigen) and T- and B-cell markers, but express activation antigens and exhibit immunoglobulin gene rearrangements
- Rare solid lymphomas with similar phenotype and appearance to extracavitary primary effusion lymphomas (PEL)

Plasmablastic Lymphomas of the Oral Cavity Type

- First described in the jaw but present at other extranodal sites
- Positive for EBV but negative for HHV8
- May be associated with deregulation of *MYC*
- Phenotype showing strong plasmacytoid differentiation, including expression of CD138

Classic Hodgkin Lymphoma

- More aggressive than classic HL in the general population
- B symptoms, early dissemination, and bone marrow involvement common
- Hodgkin and Reed-Sternberg cells positive for EBV

Immunohistochemical features vary in different forms of ARLs. In general, patients with AIDS-related lymphomas have lower BCL-2 and higher CD10 expression compared with the general population. They also have higher Ki67 scores and express p53 in a greater percentage of cases. Immunoglobulin gene rearrangement studies reveal monoclonal patterns in almost all cases of ARL. Rearrangements of the *BCL6* gene are associated with DLBCL. CNS lymphomas are monoclonal B-cell neoplasms positive for EBV and lack *MYC* rearrangements. Abnormalities in the tumor suppressor gene *TP53* occur in approximately 60% of BL cases and 40% of DLBCL cases. Translocations involving the *MYC* locus also occur in approximately 20% of DLBCL cases.

DIFFUSE LARGE B-CELL LYMPHOMAS

Approximately 30% of cases of ARL are formed by DLBCL, and this subtype is becoming relatively more common since the widespread use of HAART. Morphologically these lymphomas are heterogeneous and include cases with centroblastic morphology (Fig. 10.6), as well as variants with plasmacytoid differentiation (immunoblastic or plasmablastic lymphomas) and anaplastic lymphomas (Fig. 10.7). Most cases of HIV-related DLBCL are of the nongerminal center or activated B-cell type.

The immunoblastic variant of DLBCL (approximately 20% of cases), which is more characteristic of ARLs, is composed almost entirely of immunoblasts. These cells are large with single central macronucleoli and amphophilic or plasmacytic cytoplasm. Multinucleated and Reed-Sternberg–like cells may also be present. Staining of the lymphoma cells for EBV (EBER and LMP-1) can be demonstrated frequently, and, in immunoblastic lymphomas of the brain, the incidence of EBV infection approaches 100%. Characteristically in biopsies from the CNS, the large neoplastic cells are clustered around blood vessels in the brain (Fig. 10.8), and tumor necrosis is frequent.

BURKITT LYMPHOMA

The incidence of BL has decreased relative to large-cell lymphoma since the use of HAART. Patients are usually young in age and are less immunocompromised with peripheral CD4 cell counts exceeding 200/μL. Most cases of BL manifest at extranodal sites or bone marrow. The bone marrow may be heavily infiltrated at presentation, resembling lymphoblastic leukemia, but without spillage into the peripheral blood. The bowel is a common site, and symptoms can include abdominal pain, obstruction, bleeding, or syndromes resembling appendicitis or intussusception.

In BL, there is a diffuse and cohesive proliferation of intermediate-sized, noncleaved cells with basophilic cytoplasm

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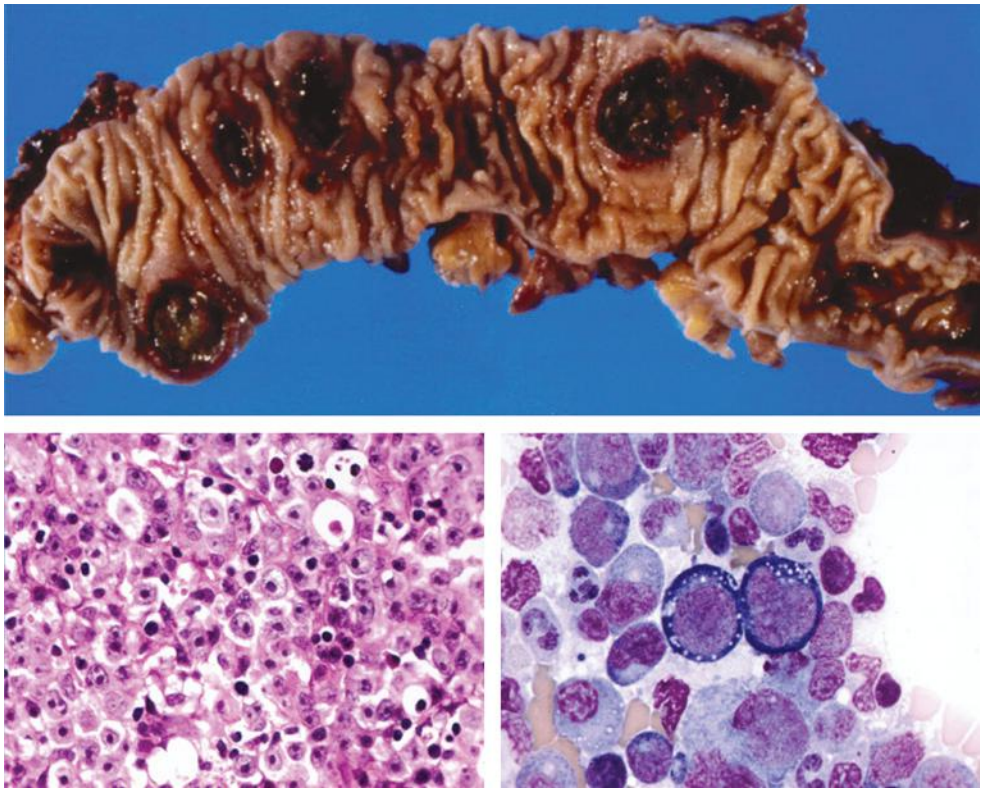


FIG. 10.6

Multifocal diffuse large B-cell lymphoma of the bowel with multiple ulcerated masses. A histologic section reveals diffuse proliferation of large lymphoid cells. Touch imprints reveal large round or oval cells with vacuolated cytoplasm.

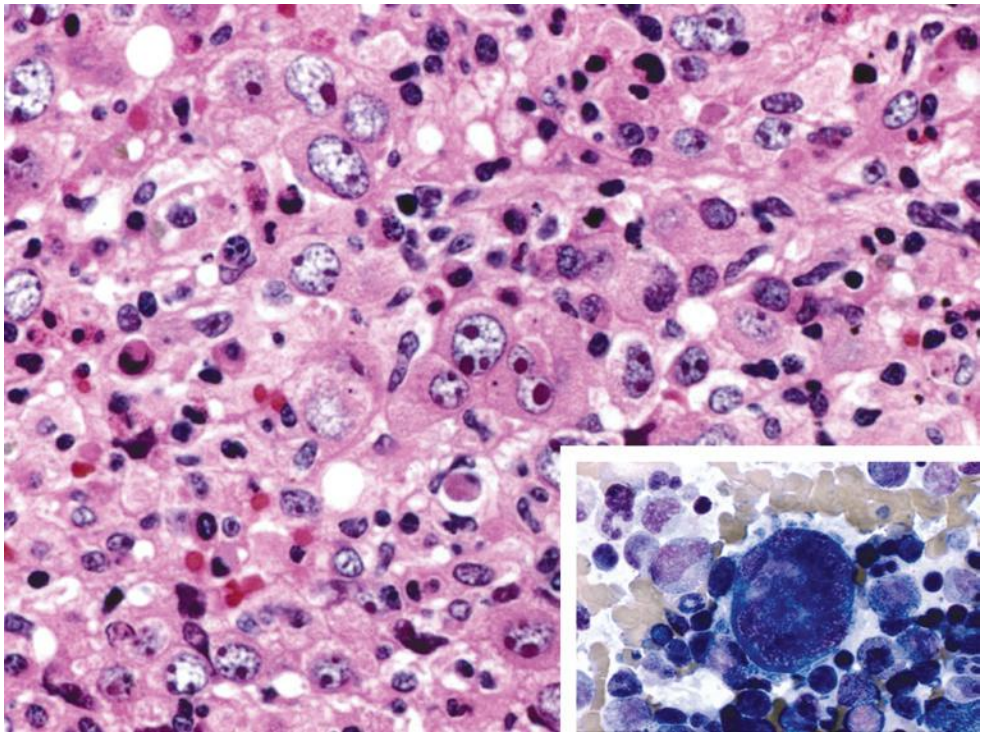


FIG. 10.7

Anaplastic diffuse large B-cell lymphoma with pleomorphic malignant cells with multiple macronucleoli.

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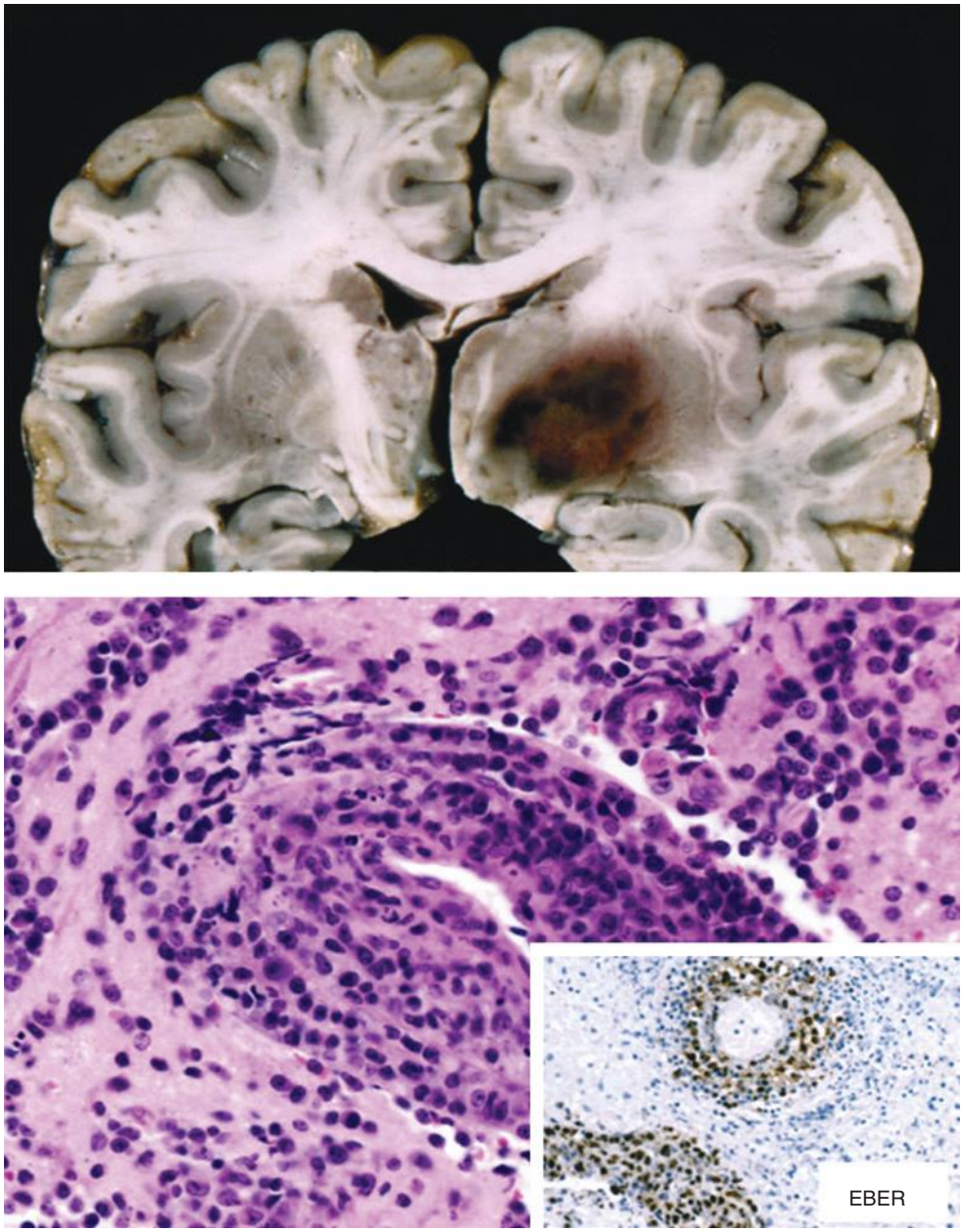


FIG. 10.8
Immunoblastic large-cell lymphoma of the brain with a deep-seated hemorrhagic lesion. Histologic sections reveal perivascular cuffing by immunoblastic cells positive for EBER.

often containing lipid droplets. There is a high proliferation rate, numerous mitoses, and tingible body or starry-sky macrophages. There are usually few admixed reactive T cells. The nuclei are round or oval with multiple nucleoli (Fig. 10.9). Associated with HIV infection, there may be a spectrum of cytologic appearance in BL. The cells may be more pleomorphic in size and shape with fewer nucleoli, and they often have more cytoplasm and a plasmacytoid appearance (Fig. 10.10). This plasmacytoid variant of BL is unique to the setting of HIV. In some cases, features may be intermediate between BL and DLBCL, making pathologic classification difficult. EBV infection is present in 30% to 40% of cases of BL with classic morphology and 40% to 50% in cases of the plasmacytoid variant.

Of BLs, 80% have translocations t(8;14), which involve the juxtaposition of the *MYC* gene on chromosome 8q24 and the heavy chain locus on chromosome 14q32. In the remaining 20%, the translocations with the *MYC* gene involve the kappa or lambda light chain loci on chromosomes 2 and 8, respectively, resulting in t(2;8)(p12;q24) or t(8;22)(q24;q11). Deregulation of *MYC* may also arise from point mutations in the first intron-first exon regulatory regions and amino acid substitution in the second exon. In both HIV-associated and sporadic BL, and unlike endemic cases, the breakpoints occur between exons 1 and 2 of the *MYC* gene and within the Sμ switch region of the *IgH*. *TP53* point mutations are also relatively common in BL (approximately 60% of cases).

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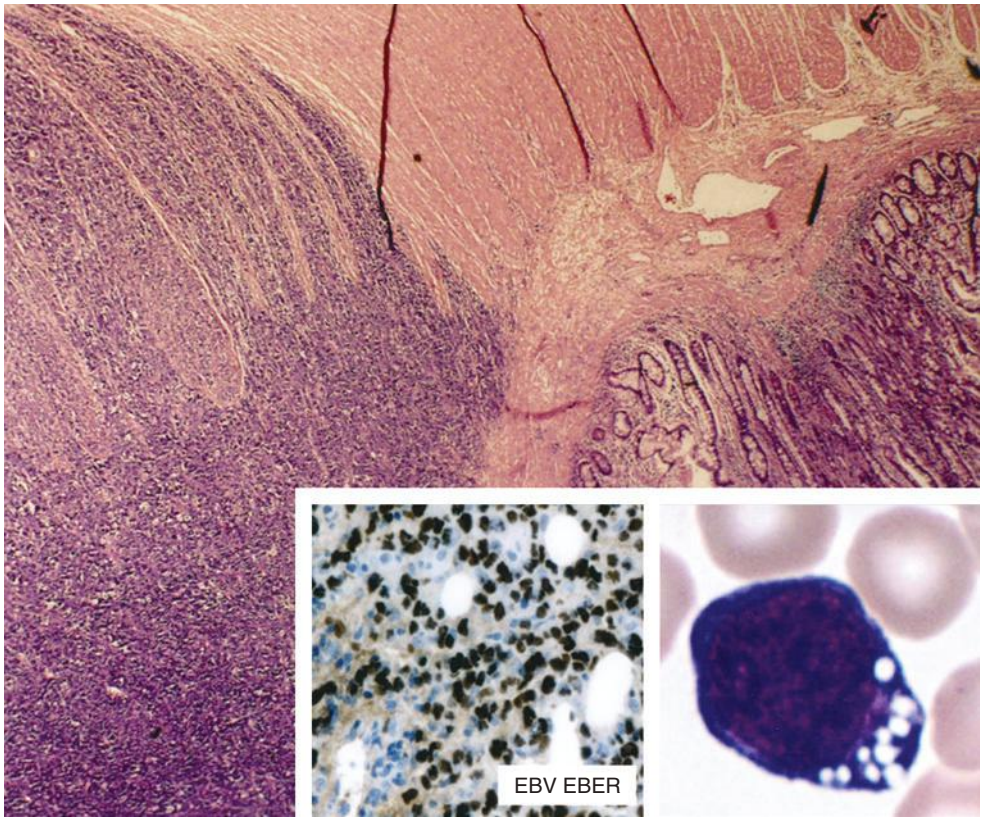


FIG. 10.9

Burkitt lymphoma of the bowel forming a large mass in the bowel wall. Imprints reveal noncleaved cells with lipid vacuoles in the cytoplasm. EBER stain is positive.

EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA OF MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT LYMPHOMA)

There appears to be an increasing incidence of low-grade lymphomas, particularly MALT lymphomas, in patients with AIDS. This incidence may relate to the use of HAART and prolonged survival in patients infected with HIV. MALT lymphomas have been reported in the lung, in which patients have multiple pulmonary nodules consisting of centrocyte-like or monocytoid lymphocytes, and there is infiltration into the bronchiolar epithelium forming characteristic lymphoepithelial lesions (Fig. 10.11). Unlike MALT lymphomas in the immunocompetent population, these may be EBV+. Some of these cases were preceded by lymphocytic interstitial pneumonitis.

PERIPHERAL T/NK-CELL LYMPHOMAS

T–natural killer (T/NK)-cell lymphomas are rarely associated with AIDS, but when they occur they are usually associated with involvement of extranodal sites, particularly the skin. The full spectrum of T/NK-cell lymphomas has been associated with AIDS, including Ki1+ anaplastic large-cell lymphoma (ALCL) and neutrophil-rich ALCL, as well as EBV+ T/NK-cell lymphoma of the nasal type.

Peripheral T-cell lymphomas are usually aggressive neoplasms with pleomorphic large cells (Fig. 10.12). They frequently invade blood vessels and are associated with necrosis. T/NK-cell lymphomas in patients with AIDS are usually positive for CD2, CD3, CD5, CD8, and CD16 and CD56 in the case of the NK-cell phenotype. As with other peripheral T-cell lymphomas, there is frequent loss of T-cell antigens, particularly CD7 and CD5. Most large-cell lymphomas in the skin of patients with HIV express CD30 but are negative for the anaplastic lymphoma kinase (ALK) protein.

MYCOSIS FUNGOIDES

Mycosis fungoides (MF) occurs in the setting of HIV infection and AIDS, and criteria for the diagnosis resemble that in the general population, notably the presence of cerebriform lymphocytes infiltrating the epidermis and the formation of small clusters or Pautrier microabscesses. The cerebriform lymphocytes or Sézary cells are frequently positive for CD8, unlike MF in the general population, which is usually CD4+. The disease may be more aggressive in patients with AIDS and may be associated with involvement of lymph nodes and bone marrow. Difficulties may occur in differentiating MF from numerous dermatologic conditions associated with AIDS.

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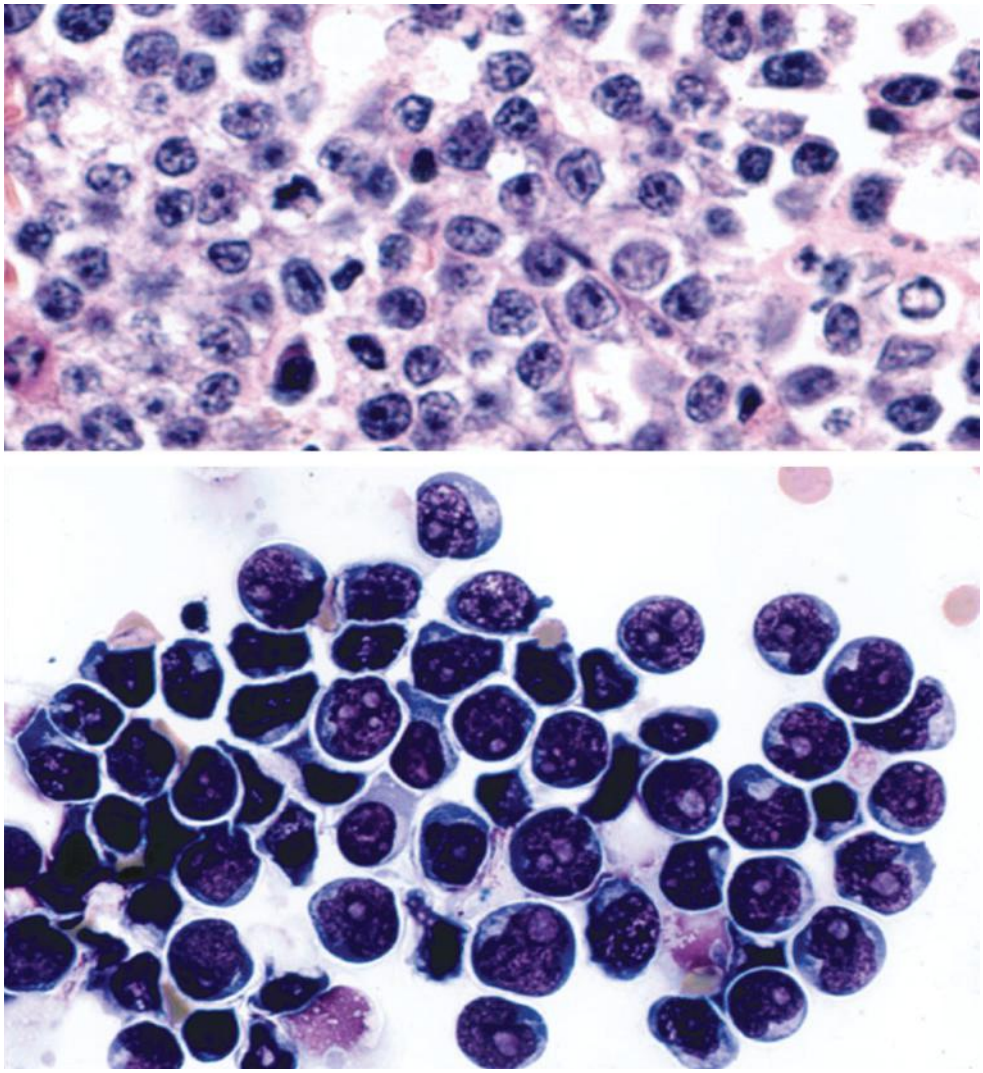


FIG. 10.10
Burkitt-like lymphoma showing cytoplasmic or plasmacytoid Burkitt cells densely packing the bone marrow.

PRIMARY EFFUSION LYMPHOMA AND OTHER KAPOSI SARCOMA–ASSOCIATED, HERPESVIRUS-RELATED NON-HODGKIN LYMPHOMAS

Primary effusion lymphoma, a rare form of lymphoma, occurs usually, but not exclusively, in patients with AIDS and is characterized by presentation in body cavities (e.g., pleural, pericardial, abdominal), usually without an associated solid tumor mass. Malignant cells in PEL are large and pleomorphic, often with plasmacytoid features resembling immunoblasts, or more bizarre and anaplastic in appearance resembling ALCL (Fig. 10.13). Some cells may resemble Hodgkin or Reed-Sternberg cells. Because of the presentation in body cavities, diagnosis is often made in cytologic preparations. With disease progression there may be an associated solid mass, usually involving adjacent soft tissues, and the cells have a morphologic appearance similar to those in the fluids with sheets of large anaplastic or plasmacytoid cells.

Cells from PEL are productively infected with the Kaposi sarcoma–associated herpesvirus, also called HHV8. This virus also causes Kaposi sarcoma (KS), although KS lesions are absent from most patients with PEL. The presence of HHV8 may be readily demonstrated by electron microscopy (Fig. 10.14) but is most commonly demonstrated with immunohistochemistry using antibodies to the HHV8 latency-associated nuclear antigen (LANA). In patients with AIDS, PEL cells are also infected with EBV, as can be demonstrated by EBER in situ hybridization. Most patients with PEL are severely immunocompromised with extremely low CD4 cell counts. The prognosis is poor, with most patients surviving less than 1 year from diagnosis.

In addition to characteristic clinical and cytologic features, cells in PEL also have a distinctive phenotype. Although they are B cells based on molecular studies, they usually are negative for common leukocyte antigen and lack conventional T- and B-cell markers such as CD19, CD20, and surface immunoglobulins. The cells exhibit features of postgerminal center B-cell differentiation, however, and may express activation antigens including HLA-DR, EMA, CD30, CD38, and CD77. Molecular analysis of PEL reveals clonal rearrangements and somatic mutations of the immunoglobulin genes, features of late (postfollicular) B-cell differentiation,

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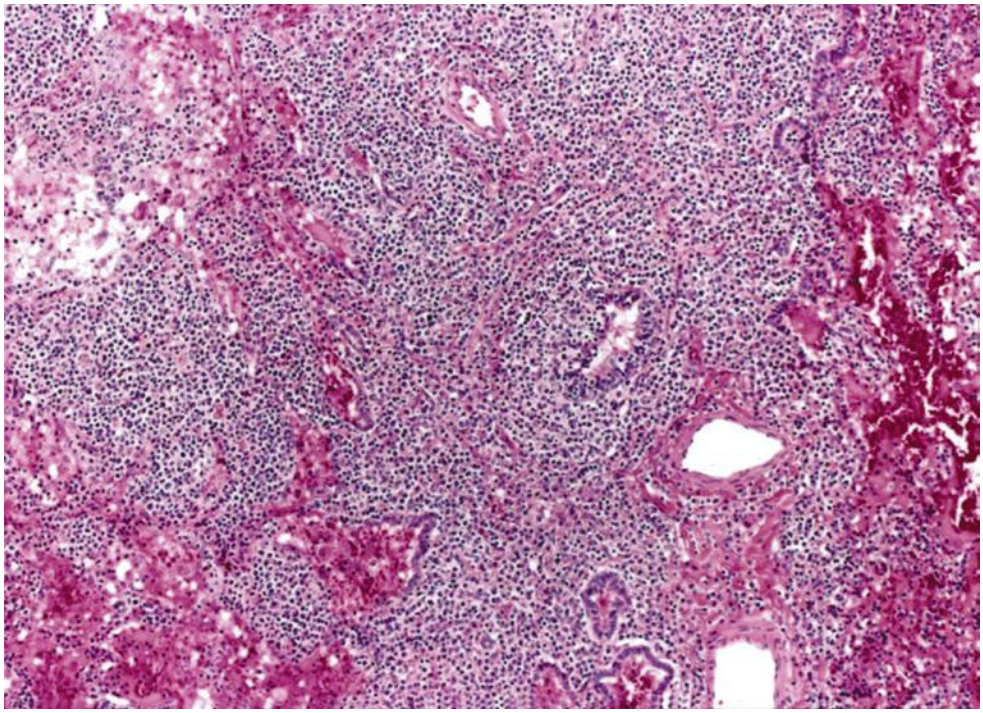


FIG. 10.11

Mucosa-associated lymphoid tissue (MALT) lymphoma of the lung showing small cytoplasmic lymphoid cells surrounding and infiltrating the walls of the bronchioles forming lymphoepithelial lesions.

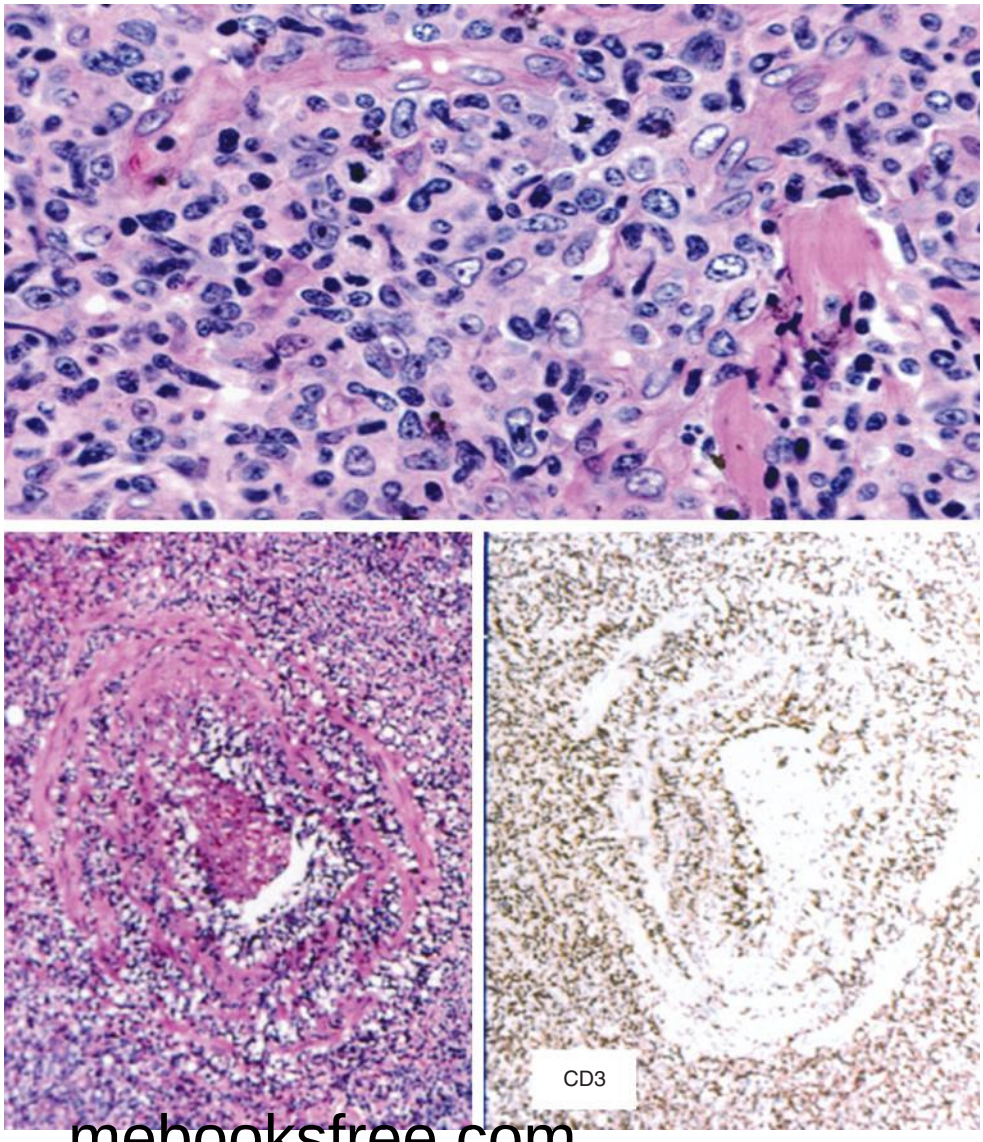


FIG. 10.12

T/natural killer–cell lymphoma with large pleomorphic cells infiltrating the walls of a blood vessel. The cells express pan–T-cell marker CD3.

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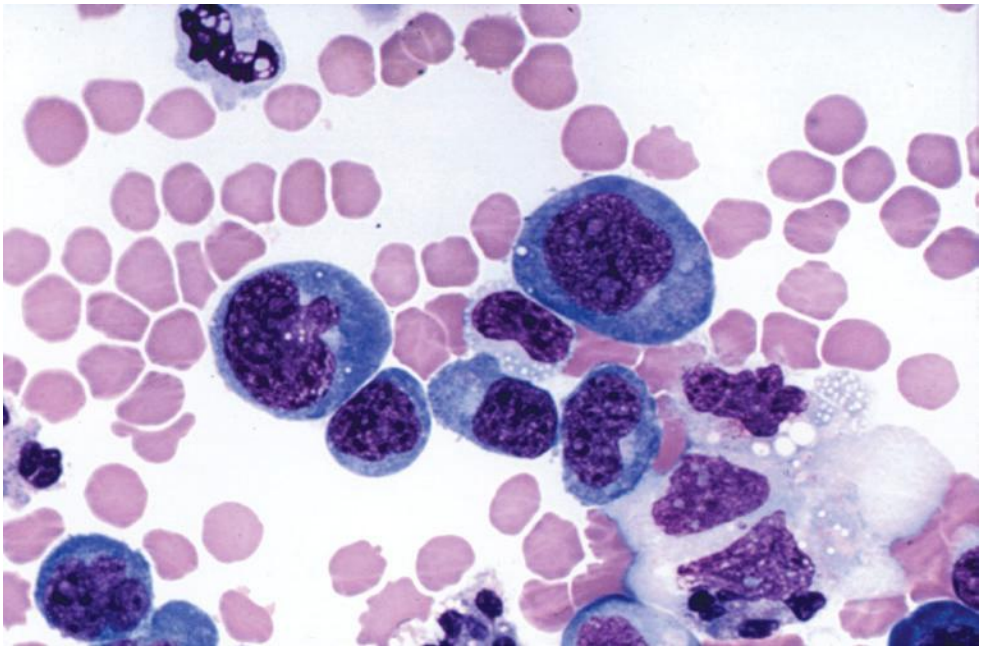


FIG. 10.13
Pericardial fluid cytospin showing large anaplastic and plasmacytoid lymphoid cells characteristic of primary effusion lymphoma (PEL).

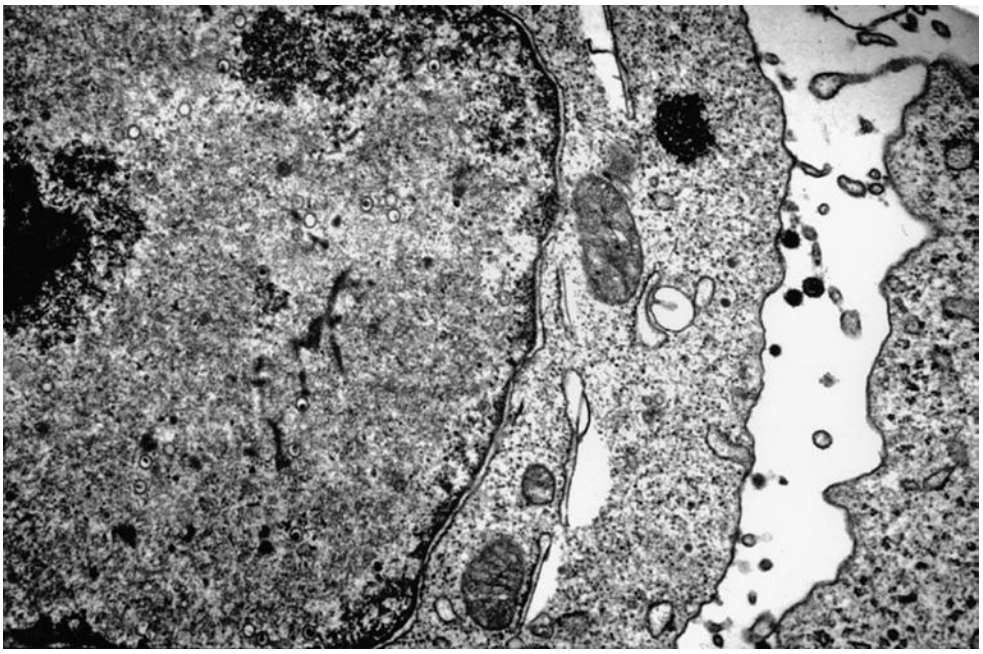


FIG. 10.14
Ultrastructural study showing numerous herpesvirus 8 particles in the nucleus and being shed from the cell surface.

and no involvement of *MYC*. PELs also lack *BCL6* gene rearrangements and *RAS* oncogene or p53 tumor-suppressor gene mutations. Cytogenetic studies reveal multiple chromosomal abnormalities. At least two potential viral oncogenes have been identified, one a cellular type D cyclin similar to the *CCND1* oncogene involved in mantle cell lymphomas, and one homologous to the cellular G protein–coupled receptor family of proteins.

Although HHV8-related lymphomas usually manifest with involvement of body fluids, the virus is rarely associated with solid lymphomas in patients who do not have effusions (called extracavitary PEL). These lymphomas usually occur at extranodal sites, including the gastrointestinal tract. In other respects, they are similar to PEL, with similar morphologic features (Fig. 10.15). The phenotype is also similar, although the solid lymphomas more often express B-cell–associated markers such as CD20 and CD79a.

HHV8 is also associated with multicentric Castleman disease (MCD) in patients with HIV infection and AIDS (discussed in Chapter 4). In this setting, monotypic plasmablasts expressing immunoglobulin M λ can develop and are associated with B-cell follicles. They may form lymphoma-like clusters often originating in the mantle zone regions (previously called microlymphomas) or develop into HHV8+ DLBCL with effacement of architecture (Fig. 10.16). It is thought that activation of the interleukin-6 signaling pathway

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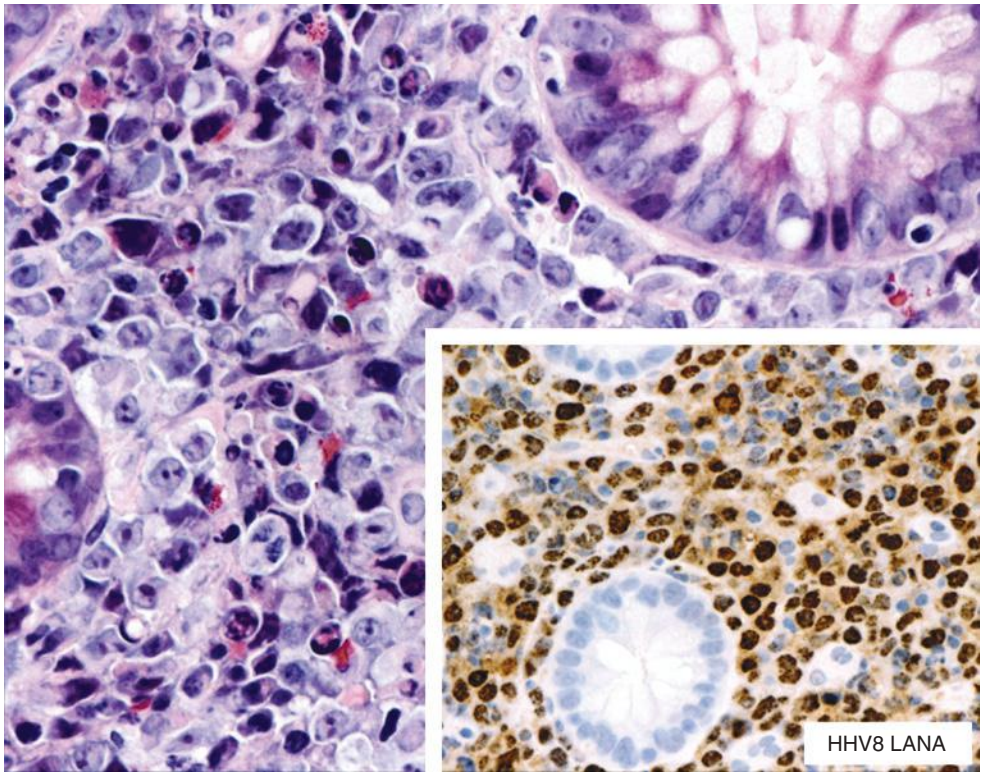


FIG. 10.15

Extracavitary primary effusion lymphoma (PEL) with human herpesvirus 8 (HHV8)-positive solid lymphoma forming a mass in the bowel wall. The large, pleomorphic cells are shown infiltrating the colon epithelium.

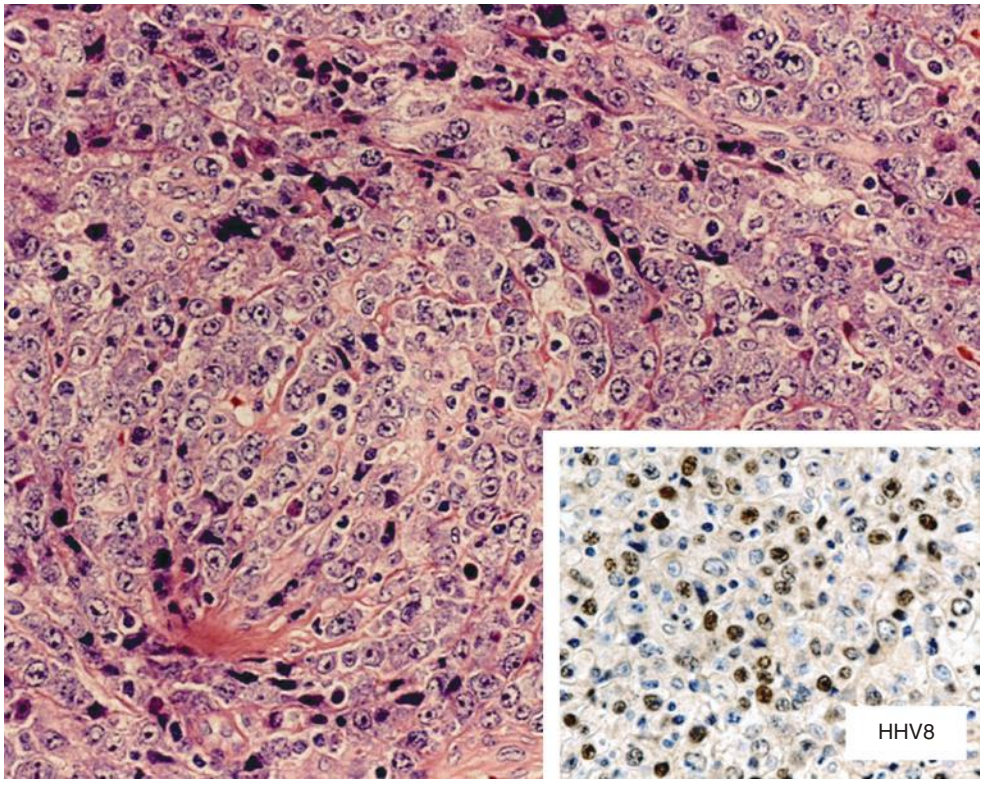


FIG. 10.16

Diffuse large B-cell lymphoma human herpesvirus 8 (HHV8)-positive lymphoma arising in the spleen in a patient with multicentric Castleman disease. The malignant cells have a plasmablastic appearance with prominent central nucleoli.

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may be important in differentiation of HHV8-infected naive B cells into plasmablasts and the development of lymphomas. HHV8+ DLBCL associated with MCD occur in both HIV+ and HIV– patients and, unlike the plasmablastic lymphomas of the oral cavity type, are negative for EBV.

PLASMABLASTIC LYMPHOMA

Plasmablastic lymphoma was first described in the jaw and oral cavity of patients infected with HIV but subsequently has been described at other predominantly extranodal sites (Table 10.2). Histologically, plasmablastic lymphomas are large cell lymphomas with plasmablastic differentiation evidenced by large blastic nuclei and prominent, often central nucleoli, as well as amphophilic or plasmacytoid cytoplasm (Fig. 10.17). The phenotype of plasmablastic lymphoma is also distinctive, with plasmacytoid differentiation evidenced by weak or absent expression of leukocyte common antigen CD45 and B-cell antigen CD20. Some cases are positive for pan B-cell marker CD79a. There may be cytoplasmic immunoglobulin, and gene rearrangement studies reveal a clonal B-cell population. The cells are positive for plasma cell antigens such as VS38c or CD138. There is no association with HHV8, but the neoplastic cells are positive for EBV EBER and variably positive for EBV latent membrane protein (LMP1). It has been suggested that dysregulation of *MYC* may be responsible for the plasmablastic morphology in these cases.

POLYMORPHIC B-CELL LYMPHOMA (PTLD-LIKE)

Patients with AIDS can develop polymorphic B-cell lymphomas that resemble the lymphoid proliferations seen in the posttransplantation setting. The median age at presentation is 35 years. As with cases of PTLD, these lymphomas are associated with EBV infection and often occur at extranodal sites including the lung, salivary glands, and skin, although they may involve lymph nodes. Morphologically, these lymphomas resemble polymorphic PTLDs, with a heterogeneous population of lymphocytes, plasma cells, and immunoblasts

TABLE 10.2
Differential Diagnosis of PEL and Plasmablastic Proliferations in HIV

Type	Plasmablastic Lymphoma of the Oral Cavity Type	Plasmablastic Myeloma	MCD-Associated Plasmablastic Proliferations	PEL
Clinical features	Extranodal mass often but not always in the oral cavity	Features of myeloma including lytic bone lesions, paraproteinemia, plasma cells in the marrow and peripheral blood	Clinical features of MCD including malaise and adenopathy	Malignant effusions; may be an associated mass lesion
Histology	Sheets of cells with large blastic nuclei, prominent central nucleoli, and plasmacytoid cytoplasm; some cases may have a more centroblastic appearance	Sheets of plasmablasts admixed with plasma cells	Clusters or sheets of plasmablasts in association with features of MCD. Plasmablastic aggregates previously termed microlymphomas usually start in follicular mantles	Large cells with anaplastic and plasmablastic features
Phenotype	Weak expression of CD45 and CD20; may express CD79a and cytoplasmic immunoglobulin CD138+	Absent CD45 and CD20, cytoplasmic immunoglobulin, CD138+	Plasmablasts express IgM λ and are EBV–	Absent CD45 and CD20; activation antigens including CD30, CD38, HLA-DR
EBV	EBV-encoded RNA (EBER)+ EBV LMP-1 variable	EBV+ or –	Negative	EBER+, EBV LMP–
Molecular	MYC deregulation common	May have myeloma-associated abnormalities	Polyclonal, oligoclonal, or clonal with progression to HHV8+ DLBCL	Clonal Ig gene rearrangements, complex cytogenetics
HHV8	Negative	Negative	Positive	Positive

EBV, Epstein-Barr virus; *HHV8,* human herpesvirus 8; *HIV,* human immunodeficiency virus; *Ig,* immunoglobulin; *MCD,* multicentric Castleman disease; *PEL,* primary effusion lymphoma.

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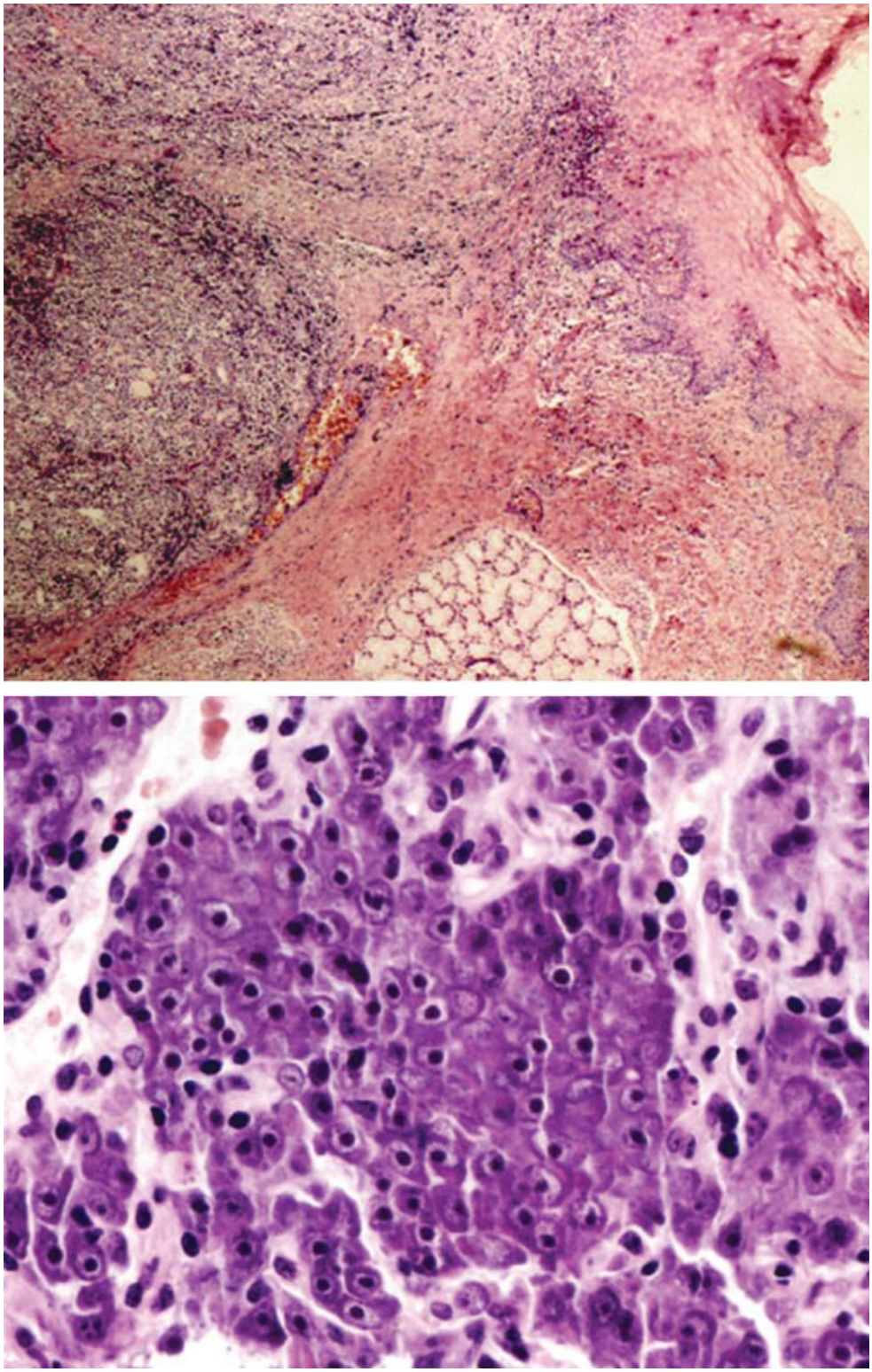


FIG. 10.17

Plasmablastic lymphoma of the oral cavity type showing a submucosal infiltrate of large plasmablastic cells.

(Fig. 10.18). Cytologic atypia and necrosis are variable. The proliferations are composed mainly of CD20+ B cells admixed with lesser populations of T cells, and, in most cases, there is monotypic expression of immunoglobulin light chains detectable with immunohistochemistry or a monoclonal immunoglobulin gene rearrangement by standard molecular techniques. The rearranged bands in Southern blots may be faint and superimposed on a polyclonal background. EBV sequences in the cases studied have been of type A, which is the characteristic pattern in PTLDs. There may be improvement or resolution with antiretroviral therapy, but some require chemotherapy.

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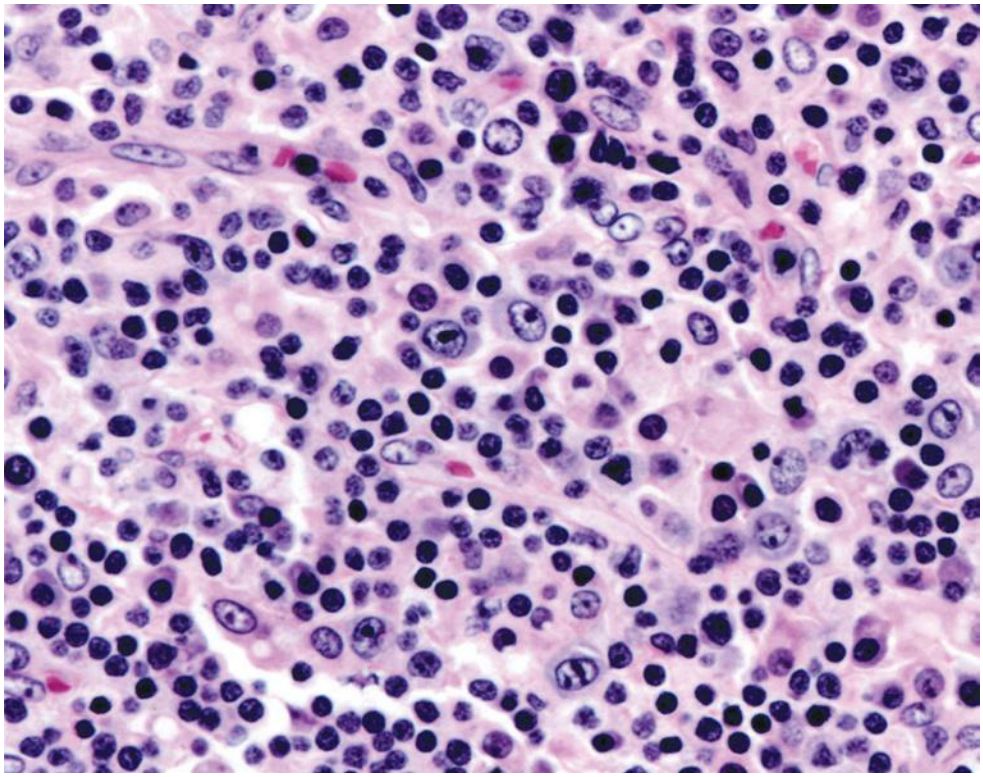


FIG. 10.18

Polymorphous infiltrate resembling polymorphic posttransplantation lymphoproliferative disorder (PTLD) in a patient with acquired immunodeficiency syndrome (AIDS). There is a spectrum of lymphoid cells including frequent plasma cells and immunoblasts. Despite the polymorphous appearance, this lymphoma revealed monoclonal rearrangement of the immunoglobulin genes.

MULTIPLE MYELOMA

Patients with AIDS have an increased incidence of myeloma, which resembles myeloma in the general population but may be more aggressive, with blastic or anaplastic appearance and involvement of extraskeletal sites. In patients with HIV the myeloma cells may be positive for EBV and, in cases with plasmablastic morphology, it may be difficult to differentiate these cases from plasmablastic lymphomas. Clinical features indicating myeloma include paraproteinemia, lytic bone lesions, and neoplastic plasma cells in the marrow (generally 30% or more), peripheral blood, or extramedullary sites such as the liver or spleen.

CLASSIC HODGKIN LYMPHOMA

Although classic HL is not an AIDS-defining neoplasm, there is an increased risk for HL in patients with HIV, and the incidence of HL in HIV+ individuals appears to be increasing since the widespread use of antiretroviral therapy. HIV-associated HL and NHL occur in patients with similar age, gender, HIV risk factors, degree of immunodeficiency, and incidence of previous AIDS. Patients with HIV tend to have more aggressive HL with early dissemination, often stage four at presentation (Fig. 10.19). B symptoms are usually present, and bone marrow involvement is seen at presentation in approximately half of the patients. The clinical presentation of HIV-associated HL is often atypical, and the disease is more aggressive than in patients who are HIV−. The most common subtype of HL in patients with HIV is mixed cellularity, although the full spectrum may be seen, including nodular sclerosis.

The phenotype of HL in patients with AIDS is somewhat different in that the background lymphoid proliferation tends to be CD4 depleted, and the Hodgkin and Reed-Sternberg cells are invariably positive for EBV EBER or EBV-latent membrane proteins, or both. Patients may respond to conventional HL chemotherapy but are at risk for opportunistic infections and other complications of AIDS. The prognosis is substantially better in the post-HAART era.

BONE MARROW INVOLVEMENT IN AIDS-RELATED LYMPHOMA

Bone marrow involvement is common in all forms of ARL. Hemoglobin levels are similar in patients with ARL, with and without bone marrow involvement, but thrombocytopenia of less than 100,000/μL is more common in patients with lymphoma in the marrow. Patients with marrow involvement have a higher incidence of lymphomatous meningitis and positive cerebrospinal fluid.

In DLBCL, there may be tumor-like aggregates in the marrow or single-cell dispersion among the other hematopoietic elements. Extensive marrow infiltration is associated with impaired survival. BLs can appear with packing of the marrow resembling lymphoblastic leukemia (see Fig. 10.10). Bone marrow involvement by HL is common at presentation,

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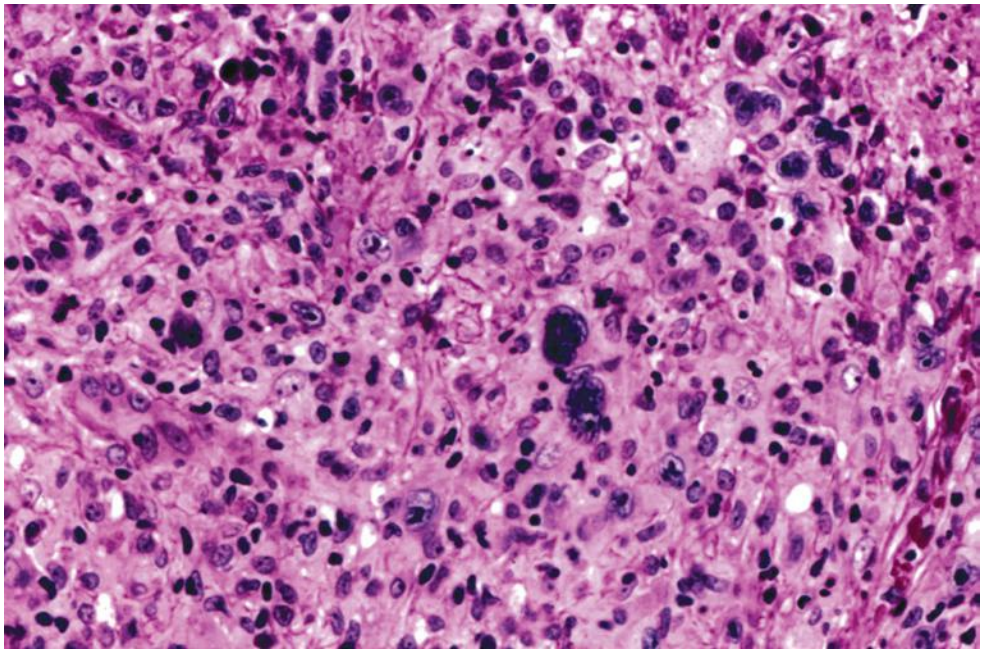


FIG. 10.19

Hodgkin lymphoma presenting as stage IV disease with bone marrow and liver infiltrates. Section from the liver biopsy reveals pleomorphic Hodgkin cells in a mixed background including numerous histiocytes.

and criteria for diagnosis are the same as in the general population.

Prognosis and Therapy

The patient's immune status at the time of diagnosis is an important factor in determining the outcome of ARL. Adverse prognostic factors include CD4 cell counts less than 100/mm^3, age greater than 35 years, stage III or IV disease, history of injection drug use, and high International Prognostic Index score. Therapy may involve chemotherapy and radiation, as well as antiretroviral therapy and hematopoietic growth factor support. Autologous hematopoietic cell transplantation can be performed safely and effectively in patients with HIV-associated lymphomas, with success rates comparable to those in the HIV− population.

LYMPHOPROLIFERATIVE DISEASES ASSOCIATED WITH PRIMARY IMMUNE DISORDERS

Primary immune disorders may be due to immune dysregulation, DNA repair defects, low immunoglobulins, and combined immunodeficiencies (Table 10.3). They form a broad group of heterogeneous lymphoproliferative disorders that includes lymphoproliferative disease in association with ataxia telangiectasia, Wiskott-Aldrich syndrome, common variable immunodeficiency (CVID), severe combined immunodeficiency, X-linked lymphoproliferative disorder, and autoimmune lymphoproliferative syndrome (ALPS), among others. The majority of B-cell proliferations in patients with primary immune disorders are associated with EBV infection in the setting of an underlying primary immune defect. A full spectrum of B-cell proliferations may occur, including hyperplasias, mucocutaneous ulcer, polymorphic B-cell lymphoproliferative disorders, and Hodgkin and non-Hodgkin lymphomas.

ATAXIA TELANGIECTASIA

Ataxia telangiectasia is a rare autosomal-recessive disorder caused by inheriting biallelic mutations of the ataxia telangiectasia mutated (*ATM*) gene. A strong association with lymphoid malignancies suggests that this gene may be involved in lymphomagenesis. Lymphoid neoplasms in patients with ataxia telangiectasia are of both B- and T-cell origin and include HL, non-Hodgkin lymphoma, and several forms of leukemia. *ATM* normally functions as a tumor suppressor, and it is activated primarily in response to double strand breaks. *ATM* alterations, including loss of heterozygosity at 11q22-23 (location of the *ATM*), also occur in diverse sporadic lymphoproliferative disorders. These disorders include patients with T-cell prolymphocytic leukemia, B-cell chronic lymphocytic leukemia, and mantle cell lymphoma.

AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME (ALPS)

Autoimmune lymphoproliferative syndrome is an immune dysregulation immunodeficiency disorder with defective T-cell signaling, defective apoptosis, expansion of T-cell subsets, and predisposition to hemophagocytic lymphohistiocytosis.

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TABLE 10.3
Types of Primary Immunodeficiencies

Type	Abnormality	Examples	Clinical Effect
Immune dysregulation	Defects in T and/or NK signaling or apoptosis	Autoimmune lymphoproliferative syndrome (ALPS)	Predisposition to B-cell lymphoproliferative disorders and autoimmunity
Immune dysregulation	Defects in SH2D1A (Src homology-2 domain protein 1A)	X-linked lymphoproliferative disorder (XLP)	Fulminant infectious mononucleosis B-cell lymphoproliferative disorders
Low immunoglobulins	In most cases underlying molecular abnormality is unclear	Common variable immunodeficiency (CVID)	Infectious and autoimmune diseases Lymphadenopathy Splenomegaly Granulomatous disease Lymphoma
Combined T- and B-cell immunodeficiencies	X-linked deficiency of WASp protein involved in cytoskeletal stabilization of lipid rafts	Wiskott-Aldrich syndrome	Autoimmune disease Infections
Combined T- and B-cell immunodeficiencies	Recombination-activating gene (RAG 1/2) mutations	Severe combined immunodeficiency (SCID) Omenn syndrome	Immunodeficiency Granulomatous disease Autoimmunity

LYMPHOPROLIFERATIVE DISEASES ASSOCIATED WITH PRIMARY IMMUNE DISORDERS—FACT SHEET

Ataxia Telangiectasia

- Both T- and B-cell lymphomas and leukemias are associated with *ATM* mutations in patients with ataxia telangiectasia
- *ATM* gene alterations occur in sporadic lymphoproliferative disorders

Combined Variable Immunodeficiency

- There is a spectrum of lymphoid proliferations, including atypical proliferation, which can be confused with lymphoma
- Granulomatous proliferation can be confused with sarcoid
- Increased circulating CD8+ T cells should not be confused with large granular lymphocytic leukemia
- Lymphoma should be confirmed by immunohistochemistry and molecular studies
- MALT lymphomas occur in the lung and at other sites

Autoimmune Lymphoproliferative Syndrome (ALPS)

- Associated mostly with mutations in the *FAS* gene or its ligand
- Paracortical expansion of CD4–, CD8– T cells may cause partial architectural effacement
- Lymphoid proliferations possibly confused with lymphoma, including HL
- Clinicopathologic communication including suspicion of an underlying immunodeficiency disorder may be crucial

Immunomodulator Agent-Related Lymphoproliferative Disorders

- Occur in patients with severe autoimmune disease, most often rheumatoid arthritis treated with immunosuppressive drugs such as methotrexate and tumor necrosis factor (TNF)-α antagonists
- Usually extranodal disease
- Spectrum of polymorphous lymphoplasmacytic infiltrates, which may resemble diffuse large B-cell lymphoma and classic HL
- Usually regresses or resolves after withdrawal of methotrexate therapy
- May be associated with EBV

ALPS is associated with germline mutations in the *FAS* gene (type 1a) or its ligand (type 1b). Both *Fas* (CD95) and its ligand play a pivotal role in apoptosis, and defective lymphocyte apoptosis caused by *FAS* gene mutations can result in a severe ALPS, manifested by moderate to massive splenomegaly and lymphadenopathy. Clinically there is hypergammaglobulinemia, autoimmunity, B-cell lymphocytosis, and failure of lymphocyte apoptosis that leads to expansion of $CD4^-/CD8^-$ T cells that express the α/β T-cell receptor (TCR) (Fig. 10.20). A related condition in patients with similar clinical symptoms due to a functional *FAS* deficiency without *FAS* gene mutations has been termed *ALPS type 2* (ALPS 2). A third type of autoimmune lymphoproliferative syndrome (i.e., ALPS 3) has no known gene mutations.

Lymph node biopsies in patients with ALPS reveal marked paracortical hyperplasia, with expansion of interfollicular areas by $CD3+/CD4^-/CD8^-$ (double negative) T cells, many of which stain for cytotoxic granules. The paracortical proliferation may be extensive enough to cause partial architectural effacement, which in association with high proliferative index and unusual immunophenotype may suggest a diagnosis of

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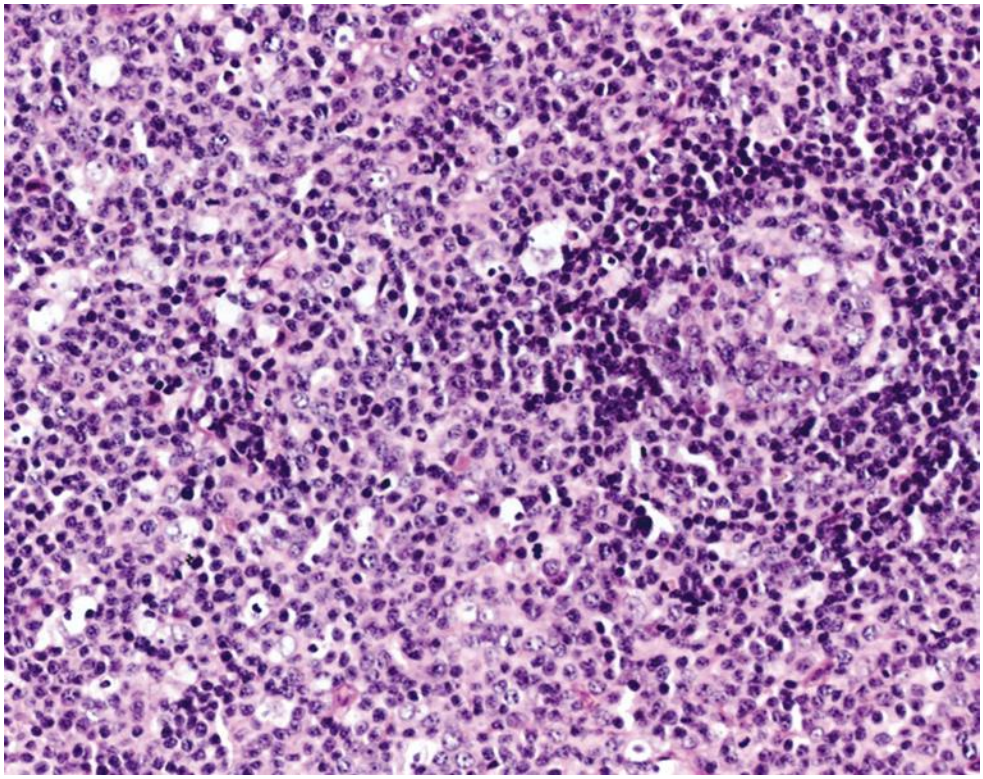


FIG. 10.20

Lymph node from a patient with autoimmune lymphoproliferative syndrome type I reveals lymphoma-like paracortical expansion by T cells with CD4–CD8– phenotype.

lymphoma (see Fig. 10.20). Clinical features, such as young age and suspicion of an immune disorder, as well as absence of a clonal gene rearrangement, may be helpful in making the correct interpretation. In addition, there may be a polyclonal plasmacytosis and florid follicular hyperplasia, follicular involution, and progressive transformation of germinal centers. Similar changes may be present in the spleen. Although some cases may resemble HL or EBV-related lymphoproliferative disease with increased large cells, caution should be used in the diagnosis because there may be spontaneous regression, even in the presence of a clonal lymphoid population. Lymphomas occurring in association with ALPS include HL, non-Hodgkin lymphomas including Burkitt and DLBCL, as well as peripheral T-cell lymphomas. Splenic sequestration can lead to multilineage cytopenias, but splenectomy may lead to life-threatening sepsis and should be avoided. ALPS may also be associated with Rosai-Dorfman disease (sinus histiocytosis with massive lymphadenopathy).

LYMPHOPROLIFERATIVE DISORDERS IN PATIENTS TREATED WITH IMMUNOSUPPRESSIVE DRUGS FOR AUTOIMMUNE DISEASES OR RELATED CONDITIONS (IMMUNOMODULATOR AGENT–RELATED LYMPHOPROLIFERATIVE DISORDERS)

In autoimmune diseases such as rheumatoid disease, the disease itself may be linked to the development of EBV-associated lymphoproliferative disorders. In addition, the use of immunomodulator agents including TNF and integrin antagonists in the treatment of these conditions has been associated with their pathogenesis. Lymphoproliferative disorders usually occur in patients with prolonged or severe autoimmune disease (most often rheumatoid disease) who are being treated with immunosuppressive drugs such as methotrexate. The condition can also recur with re-introduction of methotrexate after a period of withdrawal. Cases are also described in association with newer immunomodulator agents such as TNF-α antagonists. Lymphoid proliferations are usually extranodal and involve the gastrointestinal tract, skin, lung, and soft tissues, among other sites. There is a broad spectrum of histologic appearances ranging from mucocutaneous ulcer, polymorphous lymphoplasmacytic infiltrates (Fig. 10.21), diffuse large B-cell lymphoma (most common), classic HL, Hodgkin-like lymphoid proliferations, BL, and peripheral T-cell lymphoma, NOS. Follicular lymphomas are unusual.

Most of the lymphoid lesions have a morphology and phenotype that can be classified similar to conventional lymphomas in the general population. The Hodgkin-like lesion contains EBV+ large cells resembling Hodgkin and Reed-Sternberg cells in a polymorphous background, which includes lymphocytes, plasma cells, and histiocytes. Immunohistochemical analysis may be helpful because the large cells express CD20 and CD30 but are negative for CD15. The Hodgkin-like cells exhibit a complete range of B-cell markers including BOB.1 and OCT2. This polymorphic, plasmacytic appearance can be a clue that one might be dealing with an immune suppression–related lymphoproliferative process and, in the appropriate clinical context, should prompt EBV studies.

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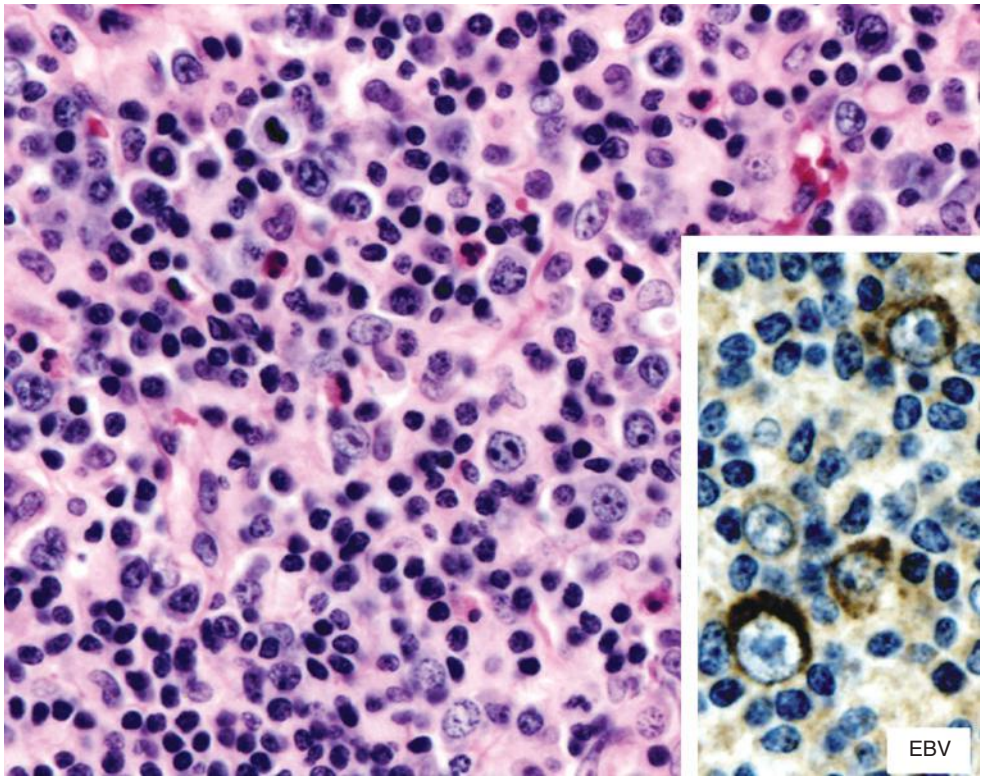


FIG. 10.21

Polymorphic B-lymphoproliferative disorder in a patient with severe rheumatoid disease treated with methotrexate. There is a mixed cellular infiltrate, including scattered immunoblasts that are positive for Epstein-Barr virus EBV-encoded RNA (EBV EBER) and EBV latent membrane protein (LMP) (inset).

The importance of recognizing lymphoproliferative disorders in the setting of methotrexate therapy is that most cases show at least partial regression on withdrawal of methotrexate, particularly in the lymphoplasmacytic infiltrates, the Hodgkin-like lesions, and those that are positive for EBV.

EBV+ mucocutaneous ulcer may be seen usually in the setting of rheumatoid arthritis treated with methotrexate and should not be confused with more aggressive forms of the disease. Clinically there are ulcerated lesions most commonly involving but not restricted to the oropharyngeal mucosa without formation of a mass lesion. Histologically there are circumscribed shallow ulcers with a polymorphous population of lymphoid and plasma cells with variable numbers of EBV+ and CD30+ immunoblasts that frequently resemble Hodgkin or Reed-Sternberg cells (Fig. 10.22). There may be pseudoepitheliomatous hyperplasia, but the base is delineated by a sharply defined zone of small lymphocytes, which are CD3+ T cells. There may be clonal immunoglobulin gene rearrangements, but the lesions generally behave in an indolent fashion with spontaneous regression and do not involve lymph nodes.

COMMON VARIABLE IMMUNODEFICIENCY

Primary immunodeficiencies dominated by low immunoglobulins are associated with infectious manifestations and a predisposition to B-lymphoid hyperplasias and lymphomas often due to an inability to suppress EBV-transformed clonal B-cell populations.

CVID, which is also known as late-onset hypogammaglobulinemia, is recognized clinically by variably reduced levels of serum immunoglobulin, loss of antibody production, and increased incidence of infections. Most patients are adults aged 20 to 40 years.

Localized or systemic granulomatous disease occurs in up to 20% of patients with CVID and should not be confused with sarcoidosis. A spectrum of lymphoproliferative disorders is encountered, with the majority of cases showing reactive or atypical lymphoid hyperplasia. Recognition of atypical lymphoid proliferations is particularly important, because these patients may have extensive or disseminated disease, which may be misinterpreted clinically and on pathologic examination as lymphoma. Lymphoid infiltrates in the lung can lead to interstitial pneumonia or follicular bronchitis or bronchiolitis.

Biopsy specimens of lymph nodes usually show lymphoid hyperplasia, which may be atypical or granulomatous inflammation. In cases of atypical lymphoid hyperplasia, the nodal architecture may appear partially effaced in routine histologic sections, but compartmentalization into B- and T-cell zones and the presence of residual germinal centers can be identified with immunohistochemical studies. Some cases reveal chronic granulomatous inflammation, which may be necrotizing.

The incidence of malignancy is increased in CVID (up to 15% of subjects), and there is a 30-fold increase in non-Hodgkin lymphoma. Lymphomas are more common in older patients (range 13 to 88 years) and is more common in females. Lymphoma can arise in a background of lymphoid hyperplasia.

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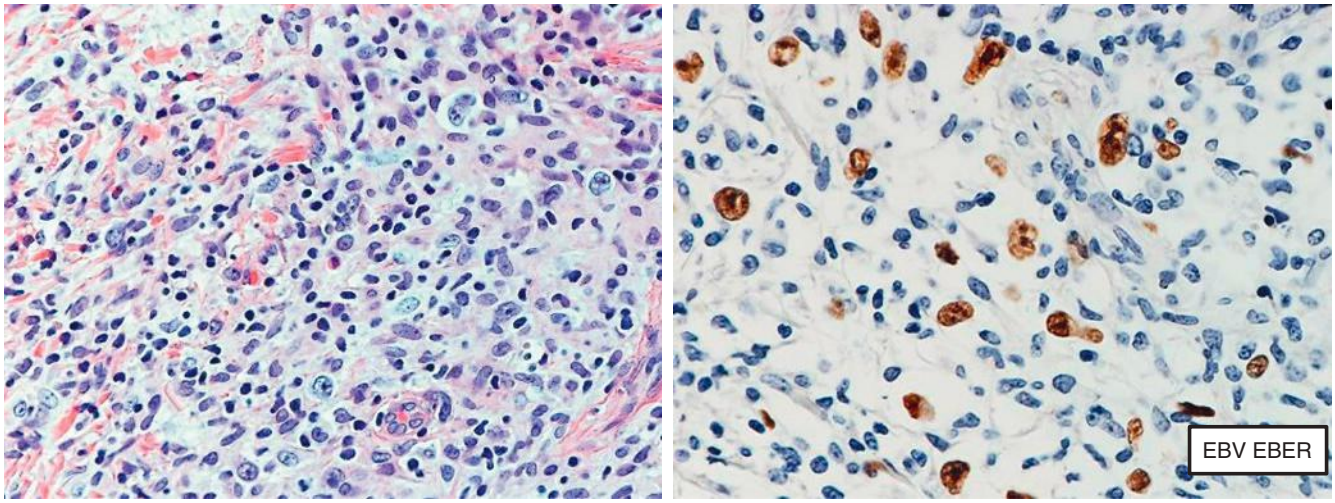


FIG. 10.22

Mucocutaneous ulcer with a mixed lymphohistiocytic proliferation in which there are scattered large pleomorphic cells, some of which resemble Hodgkin cells. These cells are positive for Epstein-Barr virus EBV-encoded RNA (EBV EBER).

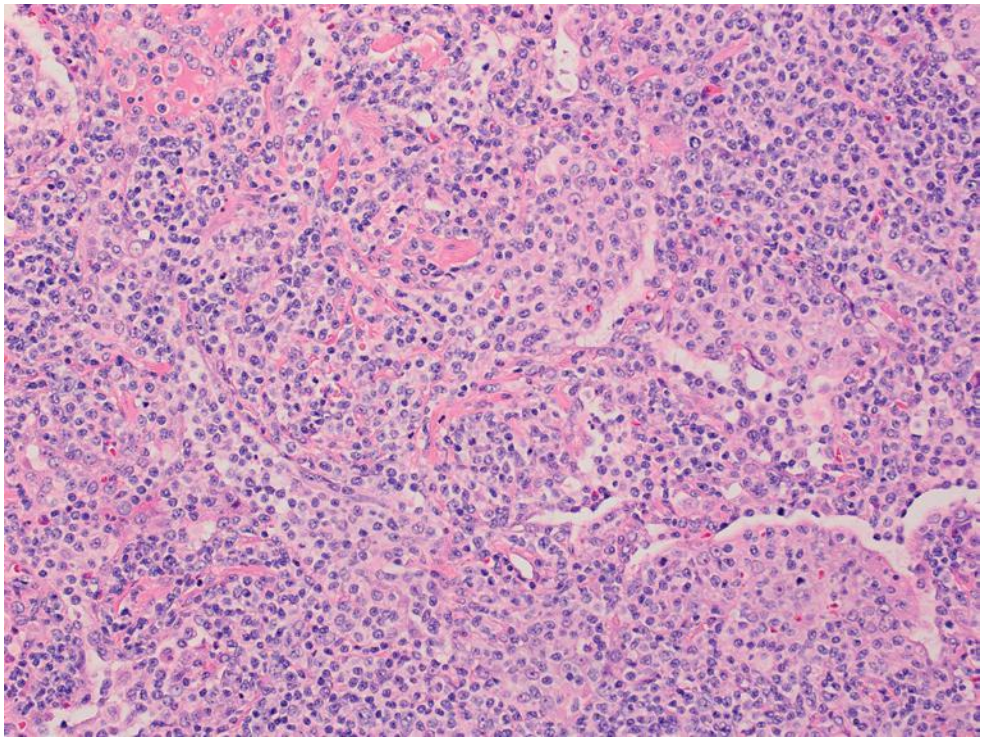


FIG. 10.23

Pulmonary mucosa-associated lymphoid tissue (MALT) lymphoma in a patient with common variable immunodeficiency (CVID). There is a dense infiltrate of lymphoid cells, including nests of centrocyte-like cells with clear cytoplasm.

Lymphomas of extranodal MALT type can occur in the lung (Fig. 10.23) and at other sites. Lymphomas in CVID are usually not associated with infection by EBV. Diagnoses of lymphoma in this setting are based on conventional criteria. A subset of CVID patients has increased circulating CD8+ large, granular lymphocytes that are reactive and should not be confused with T-cell large, granular lymphocytic leukemia. Sinusoidal cytotoxic T-cell infiltrates may also occur in the liver.

The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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11

Hodgkin Lymphoma*

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■ INTRODUCTION

In this chapter we review the definition and current classification of Hodgkin lymphoma (HL), describe the histologic and immunohistochemical features, and discuss the differential diagnosis of this intriguing disease. HL has some unusual clinical, morphologic, and biological features that set it apart from most other malignant neoplasms. Histologically it is characterized by a small minority of neoplastic cells, termed *Hodgkin and Reed-Sternberg* (H/RS) *cells*—and lymphocyte-predominant (LP) cells in nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL)—in an exuberant inflammatory background. Clinically, this is frequently mirrored by symptoms more suggestive of an inflammatory or infectious disorder, including fever and night sweats, as result of the production of inflammatory cytokines. Although the first autopsy cases later attributed to this disease were already reported in 1832 by Thomas Hodgkin and Carl Sternberg in 1898 and Dorothy Reed in 1902 described the microscopic findings including the cytologic characteristics of the giant cells bearing their name, it was until the second half of the 20th century that cytogenetic studies confirmed the neoplastic nature of the disease, and elegant molecular studies using single microdissected H/RS cells confirmed their derivation from clonal B cells. However, because of the clinical and pathologic peculiarities of this disease as well as for historical reasons, HL remains separated from the non-Hodgkin lymphomas in today's World Health Organization (WHO) classification.

■ DEFINITION

Hodgkin lymphoma is a clonal, malignant lymphoproliferation originating from germinal-center B cells. The malignant cells usually represent only a small minority (0.1% to 2%) of the total cellular population of involved tissues. The WHO classification discerns two major groups of HL: classical HL (CHL) and NLPHL, which differ both clinically and pathologically. A histopathologic diagnosis of CHL is based on the identification of diagnostic H/RS cells in an appropriate inflammatory background, and the composition of the background dictates the subclassification. Although CHL usually shows a very typical morphology, current diagnostic criteria include the characteristic immunophenotype of the neoplastic population, with positivity for the CD30 and CD15 antigens, lack of CD45, and an inconsistent and heterogeneous expression of lineage-specific lymphoid markers. In contrast, NLPHL is characterized by the presence of rare neoplastic cells expressing a broad range of B-cell markers including CD20, termed *LP cells* in a background usually dominated by B cells arranged in follicular fashion.

■ ETIOLOGY AND POSTULATED CELL OF ORIGIN

The cause of CHL is still unresolved, but owing to the unique epidemiologic and clinical features of the disease, an infectious agent has long been suspected. Epstein-Barr virus (EBV), an oncogenic gamma herpesvirus, is present in clonal episomal (nonintegrated) form in the neoplastic cells of a significant proportion of CHL, but as evidenced by EBV^- cases, EBV is not mandatory for the development of the disease and likely represents only a cofactor in positive cases. EBV^+ cases consistently express latent membrane protein 1 (LMP1), the only viral latent protein with proven oncogenic properties. The search for other candidate viruses has failed to provide evidence for an infectious agent in EBV^- cases.

Due to their unique morphology and phenotype, the origin and lineage of H/RS cells have been controversial and the subject of intense investigations for decades. At different times, the H/RS cell has been postulated to derive from a lymphocyte, interdigitating dendritic cell, histiocyte, or granulocyte. The controversy was finally settled by molecular analyses performed on isolated single H/RS cells, which confirmed that these cells are derived from germinal-center B cells incapable of immunoglobulin transcription. Their mutational profile, complemented by LMP1 expression in

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EBV$^+$ cases, results in the constitutive activation of multiple prosurvival and anti-apoptotic pathways, which enables them to survive and grow in the absence of B-cell–receptor signaling. Whether true bona fide cases of T-cell–derived HL exist is still controversial.

The LP cell of NLPHL also is considered to originate from a germinal-center B cell. In contrast to CHL, however, LP cells retain many B-cell markers, show immunoglobulin transcription on the messenger RNA (mRNA), but usually not on the protein level, and reside in or around atypical germinal centers.

Clinical Features

Classical Hodgkin Lymphoma

CHL accounts for approximately 0.5% to 1% of all cancer cases and 15% to 25% of all lymphomas in the United States, with an age-adjusted annual incidence rate of 2 to 4 per 100,000 population. About 8500 new cases of CHL are diagnosed annually in the United States, with a male-to-female ratio of 1.3 to 1.5 : 1. CHL has a bimodal age distribution in industrialized countries, with the first peak occurring between ages 15 and 35 years and the second peak beyond 55 years. The nodular sclerosis (NS) subtype of CHL dominates in the first peak, whereas the mixed cellularity (MC) type occurs more often in the older age group. In developing countries, is less common and shows a different age distribution, with a first incidence peak in childhood, lack of the young adulthood peak, and a more frequent association with EBV.

Clinical Presentation

CHL is a node-based disease in 90% of patients. Patients frequently present with localized disease limited to one or two lymph nodes, most often in the cervical region (75% of cases). Mediastinal (thymic) involvement may be the presenting sign, with or without peripheral (particularly supraclavicular) lymphadenopathy, and is most commonly the NS subtype. Some patients are asymptomatic, and a mass is detected on routine chest radiograph, whereas others present with symptoms related to the mediastinal tumor, such as superior vena cava syndrome. Below the diaphragm, retroperitoneal and inguinal lymph node involvement is common, whereas extra-axial lymph nodes are rarely involved. The incidence of splenic involvement is approximately 10% across all subtypes but reaches 30% for the MC subtype. Bone marrow infiltration is rare in CHL, occurring in less than 5% of nonimmunosuppressed patients. Primary extranodal classical CHL is extremely rare, and a diagnosis of extranodal classical CHL requires extensive immunohistochemical studies to rule out morphologic mimics.

Approximately 30% to 40% of patients present with B symptoms, which, although more prevalent in advanced stages, may also occur in early-stage disease. Other symptoms include pruritus, rarely the cyclic Pel-Ebstein type of fever, or pain in involved lymph nodes after consumption of alcohol. Laboratory findings are mostly nonspecific and may include leukocytosis, increased lactate dehydrogenase (LDH) and eosinophilia. In addition, many patients with CHL show evidence of reduced T-cell–mediated immunity.

CHL in Immunosuppressed Patients

The risk for developing CHL is increased in patients with some types of immunodeficiencies, including HIV infection/AIDS and in the posttransplantation setting. Virtually all cases in immunocompromised patients are EBV$^+$. The relative risk of CHL among HIV-positive individuals ranges from 7.6 to 8.9 as compared to the non–HIV-infected population. These patients have an increased incidence of unfavorable histologic subtypes (MC and LD), whereas the NS type is uncommon. The incidence of NS in HIV-positive patients decreases with decreasing CD4 counts. Paradoxically, the number of cases of CHL increased since the introduction of highly active anti-retroviral therapy (HAART). It appears that improved immunity related to increased CD4 counts in patients treated with HAART results in increased numbers of cases of CHL, especially the NS type.

Most HIV-positive patients with HL exhibit B symptoms and frequently have widespread disease (stage III/IV, Ann Arbor stage III/IV). Extranodal involvement is much more common than in HIV-negative patients. Extranodal sites include the bone marrow, which may be the site of primary diagnosis, as well as liver and spleen. In contrast to CHL in immunocompetent individuals, the spread of CHL is often not contiguous. For example, liver without splenic involvement or lung without mediastinal involvement may be present in HIV-positive patients with CHL. As noted previously, there is a strong association with EBV (80% to 100%). Prognosis in patients with high-stage HIV is unfavorable, although it has improved in patients who receive combined antineoplastic therapy and HAART. In posttransplantation patients, CHL needs to be discriminated from polymorphic EBV$^+$ posttransplantation lymphoproliferative disease with Hodgkin-like features (see below).

Nodular Lymphocyte-Predominant Hodgkin Lymphoma

The clinical features of nodular lymphocyte-predominant HL (NLPHL) are distinct from those of CHL and are described in detail below.

Staging of Hodgkin Lymphoma

Staging of patients with HL is important in determining the extent of the disease, which, in turn, dictates the therapeutic regimen that the patient will receive. HL disseminates in a highly predictable manner through lymphatic channels involving contiguous lymph node stations and other organs in a stepwise fashion. With the advent of advanced diagnostic imaging techniques, nowadays including positron emission tomography/computed tomography (PET/CT), pathologic staging including splenectomy and lymphangiography has

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TABLE 11.1

Cotswolds Revision of the Ann Arbor Staging Classification

Stage	Definition
I	Involvement of a single lymph node region of lymphoid structure (e.g., spleen thymus, Waldeyer's ring)
II	Involvement of two or more lymph node regions on the same side of the diaphragm (the mediastinum is a single site; hilar lymph nodes are lateralized); the number of anatomic sites should be indicated by suffix (e.g., II_3)
III	Involvement of lymph node regions or structures on both sides of the diaphragm
III_1	With or without splenic, hilar, celiac, or portal nodes
III_2	With paraaortic, iliac, or mesenteric nodes
IV	Involvement of extranodal site(s) beyond those designated E

A, No symptoms; *B,* fever, drenching sweats, or weight loss; *X,* bulky disease: > one third widening of the mediastinum at T5-6, or maximum of nodal mass >10 cm; *E,* involvement of a single extranodal site, or contiguous or proximal to known nodal site of disease.

become obsolete. Staging of HL is performed according to the Ann Arbor staging classification, as modified in the Cotswolds meeting (Table 11.1). Trephine bone marrow biopsy is not indicated in early-stage patients, who have an extremely low rate of marrow involvement.

Prognosis and Therapy

Modern therapeutic regimens have transformed HL into a curable disease in 80% to 90% of cases and represent one of the first success stories of medical oncology. The natural history of the disease is characterized by slow but relentless tumor progression with extensive organ involvement, which frequently leads to death due to infectious complications. Prior to the availability of adequate staging procedures and before modern therapies were established for treating HL, prognosis was related to the histologic subtype. NLPHL was associated with the best prognosis, whereas LD CHL with the worst, although one can assume that many of the cases classified as LD CHL would be classified as aggressive NHL or so-called gray zone lymphomas nowadays. NS subtype was generally associated with a better outlook than the MC subtype (because of lower stage disease). With proper staging and modern therapies, the prognostic relevance of subtyping CHL has all but disappeared, including the separation of NS CHL into grade 1 and 2 as determined by the number and cytologic features of H/RS cells, which was advocated by the British National Lymphoma Investigation (BNLI) group. However, NLPHL remains distinct both in terms of clinical course and therapy and shows the best prognosis. Of note, NLPHL shows relapses, frequently multiple, in approximately 20% of patients and is more akin to a low-grade B-cell NHL in its clinical course.

Stage remains the most important single prognostic parameter in HL. Ann Arbor stages I/II are designated as early-stage and stages III/IV as advanced-stage disease in all types of HLs and determine therapy. Nevertheless, more than 50% of stage IV patients can achieve complete response and

HODGKIN LYMPHOMA—FACT SHEET

Definition
- A monoclonal B-cell lymphoma composed of a minority (<1% to 2%) of malignant cells called Reed-Sternberg (RS) and Hodgkin (H) cells (or LP cells in NLPHL) accompanied by an inflammatory cell infiltrate
- HL encompasses two distinct clinical and biological entities
  - CHL
  - Nodular lymphocyte-predominant HL (NLPHL)
- CHL consists of 4 histologic subtypes based on characteristics of the background inflammatory infiltrate and cytology of the H/RS cells
  - nodular sclerosis (NS)
  - mixed cellularity (MC)
  - lymphocyte rich (LR)
  - lymphocyte depleted (LD)

Incidence and Location
- HL accounts for approximately 0.5% to 1% of all malignancies and 15% to 25% of all lymphomas
- NLPHL accounts for approximately 3% to 5% of all cases of HL, the remainder being CHL
- Approximately 8500 new HL cases are diagnosed annually in the United States
- Usually arises in lymph nodes, most often cervical nodes (75% of cases)
- Mediastinal involvement common in CHL, NS subtype

Gender, Race, and Age Distribution
- Male predominance (except for NS CHL)
- Higher incidence in Caucasians
- Most cases occur in young adults but are seen in all age groups

Clinical Features
- CHL has a bimodal age distribution in industrial countries: first peak between ages 15 and 35 years, with a second peak in the older age group
- Patients may be asymptomatic except for lymph node enlargement or may present with B symptoms
- More than 60% of patients with CHL have localized disease (Ann Arbor stages I and II)
- NLPHL is an indolent disease with male predominance (male-to-female ratio is 2.4 : 1) of usually localized stage (80%) and infrequent B symptoms (10%)

Prognosis and Therapy
- Prognosis is related to disease stage, which also determines the appropriate therapy
- Eighty percent to 90% of patients are cured with current therapy protocols, and newer therapeutic developments aim at reducing long-term side effects, especially second cancers
- Prognosis for NLPHL is excellent, but recurrences, often multiple and over a prolonged period, occur in 20% of patients

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frequently durable remissions. Other parameters for adverse prognosis include age, male sex, bulky disease, liver involvement, and some laboratory features such as anemia, leukocytosis, lymphopenia, and elevated LDH. Bone marrow involvement does not confer a worse prognosis compared with other advanced disease stage patients. In addition to the histologic subtypes mentioned, some pathologic features have been analyzed for prognostic relevance in CHL, including the composition of the background infiltrate, EBV status, immunophenotype of the tumor cells such as expression of CD20 or BCL2, and gene expression profile, which demonstrated a worse prognosis for patients with a signature dominated by macrophage genes. However, for many of these markers, repeat studies led to conflicting results, preventing them from being considered in clinical decision making. An important new prognostic feature is a good response to chemotherapy as assessed by functional imaging modalities (e.g., fluorodeoxyglucose [^{18}F] positron emission tomography [FDG-PET]) to guide risk-adapted therapy. Prognostic factors for NLPHL are detailed below.

Treatment

CHL is very sensitive to radiation, and moderate doses can induce complete remission in most limited stage patients. However, to reduce long-term complications of therapy, recent years have seen a shift toward abbreviated, less toxic chemotherapy regimens with or without limited radiotherapy. In patients with advanced disease, multiagent chemotherapy is the mainstay of treatment, whereas autologous stem-cell transplantation and novel treatments such as the anti-CD30-antibody conjugate brentuximab vedotin (consisting of an anti-CD30 antibody with monomethyl auristatin E, an anti-microtubule agent), or blockade of the Programmed death 1/programmed death-ligand 1 (PD1/PD-L1) axis with specific antibodies are reserved for relapsed or refractory disease.

Patients with localized NLPHL and favorable prognostic features are usually treated with involved field radiotherapy without chemotherapy. It is not entirely clear whether treatment following excisional biopsy of stage I NLPHL is required. In patients with advanced disease, multiagent chemotherapy similar to that for CHL is generally recommended, frequently with addition of rituximab based on the expression of CD20, although an internationally accepted standard is still lacking (also see below).

Pathologic Features

Histopathologic Classification of Hodgkin Lymphoma

In contrast to most NHLs and other tumors, the enlargement of lymph nodes in HL is caused by the overwhelming number of benign inflammatory cells, whereas the malignant cell level is often less than 1% and thus may be difficult to identify. Despite the recognition of the B-cell nature of HL, the classification of the disease relies primarily on the morphologic and in part immunophenotypic features of the involved tissues, and genetic analysis does not play a role in its diagnosis. In contrast to NHL, the classification of HL therefore has remained remarkably stable for almost 40 years, and most cases are classified much as they were 30 years ago, after the development of the Rye classification.

This first universally accepted classification scheme, adopted in 1965, described four subgroups. The four histologic types in the Rye classification included the following: (1) lymphocyte predominant (or LP), in which there is a predominance of small lymphocytes, and few of the neoplastic RS cells; (2) nodular sclerosis (or NS), a disorder with frequent mediastinal disease and the only subtype found in almost equal distribution in young women and men; (3) mixed cellularity (or MC), which as the term implies, is characterized by a mixed inflammatory cell infiltrate including small lymphocytes, eosinophils, plasma cells, histiocytes, and neutrophils; and (4) lymphocyte depletion (or LD), characterized by few lymphocytes and many RS cells. The prognostic significance of these histologic types has become less relevant with the advent of modern therapeutic regimens, and survival is now known to be associated with the stage of disease rather than with the histologic subtype of disease.

The Lukes and Butler/Rye classification remained unchanged until it was supplanted in 1994 by the Revised European American Lymphoma (REAL) classification and its updated version, the World Health Organization (WHO) scheme in 2001 (Table 11.2). Although the REAL and WHO schemes were founded on the Lukes and Butler/Rye classifications, several significant changes were made, including the following: (1) a name change from Hodgkin disease (HD) to Hodgkin lymphoma (HL), based on our better understanding of the nature and histogenesis of this lymphoproliferation; and (2) HL was divided into two distinct clinical and biological entities: NLPHL and CHL. These two types of HLs differ from each other in their natural history, epidemiology, clinical features (Table 11.3), immunophenotypes, genetic features, and association with EBV. Most important, NLPHL shows a preserved B-cell program with expression of a broad range of B-cell markers, whereas CHL lacks most B-cell markers and immunoglobulin transcription despite the presence of

TABLE 11.2

Classification of Hodgkin Lymphoma[a]

- Nodular lymphocyte-predominant Hodgkin lymphoma
- Classical Hodgkin lymphoma
  - Nodular sclerosis classical Hodgkin lymphoma
  - Lymphocyte-rich classical Hodgkin lymphoma
  - Mixed cellularity classical Hodgkin lymphoma
  - Lymphocyte-depleted classical Hodgkin lymphoma

[a]According to the fourth edition of the WHO classification, update 2016.

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TABLE 11.3
Clinical Features of NLPHL and CHL

	CHL	NLPHL
Age distribution	Bimodal	Unimodal
Percentage male sex	NS, 50%; MC, 70%	70%
Sites involved	Peripheral lymph nodes, mediastinum, abdomen, spleen	Peripheral lymph nodes
Stage at diagnosis	Often II or III	Usually I
B symptoms	40%	Rare
Association with EBV	25% to 70%	None
Course	Aggressive, curable	Indolent, late relapses
Risk of large B-cell lymphoma	1%	2% to 5%

CHL, Classical Hodgkin lymphoma; *EBV*, Epstein-Barr virus; *MC*, mixed cellularity; *NLP*, nodular lymphocyte-predominant; *NS*, nodular sclerosis.

CLASSICAL HODGKIN LYMPHOMA—PATHOLOGIC FEATURES

Microscopic Findings
- Hodgkin/Reed-Sternberg (H/RS) cells or variants must be present to make a diagnosis of HL
- H/RS cells and variant RS cells are the malignant cells and constitute the minority (usually <1%) of the nodal infiltrate
- Tissue architecture, inflammatory cells, and types of H/RS cells determine the histologic subtype of HL
- Classical H/RS cells are most abundant in MC CHL
- Lacunar cell variants of H/RS cells are characteristic of NS CHL

Immunohistochemical Findings
- Classical H/RS cells express CD30, CD15, MUM1, fascin, and weakly PAX-5, but usually lack expression of other B-cell antigens and CD45
- Depending on subtype and epidemiologic features, 20% to 100% of CHL is positive for EBV, demonstrable by staining for LMP1 or Epstein Barr virus–encoded RNA (EBER) in situ hybridization

Genetics
- H/RS and RS variant cells are B cells of germinal-center derivation and carry clonally rearranged immunoglobulin (IG) genes
- The variable region of the IG heavy chain genes carries a high load of somatic mutations without signs of ongoing mutations
- Especially EBV^+ cases carry so-called crippling mutations of the IG genes leading to a premature stop codon or promoter region alterations
- H/RS cells have no detectable IG mRNA or protein due to nonproductive rearrangements or defects in the immunoglobulin transcription machinery

clonally rearranged immunoglobulin genes. In the revised fourth edition of the WHO classification from 2008 (see Table 11.2), LP (lymphocyte-predominant) cells replaced the term *lymphocytic and/or histiocytic* (L & H) *Reed-Sternberg cell variants* in NLPHL, and cases that could not be classified into one of the subgroups were placed into the MC category. No changes were introduced in the 2016 update of the WHO classification, but the presence of different growth patterns in NLPHL, including cases with diffuse areas and predominance of T cells, was noted, since variant patterns are associated with higher stage and more frequent relapses. In addition, the term *T-cell/histiocyte-rich large B-cell lymphoma* (THRLBCL)*-like transformation* was introduced for cases that progress to a completely diffuse pattern without follicular dendritic cells and lack of small B cells and that clinically run a more aggressive course. In addition, morphologic as well as clinical similarities between lymphocyte-rich CHL and NLPHL are acknowledged.

Furthermore, research of the past 2 decades has clarified the separation of diffuse large B-cell lymphoma (DLBCL) variants from CHL and led to the recognition of true biologic gray zone cases between the two entities, which were included in the fourth edition of the WHO classification in 2008 as B-cell lymphoma unclassifiable, with features intermediate between DLBCL and CHL.

CLASSICAL HODGKIN LYMPHOMA

REED-STERNBERG CELLS AND VARIANTS

The presence of Reed-Sternberg (RS) cells or variants (lacunar cells) is required for a diagnosis of CHL. Not only must RS cells be found, but they must also be present in a cellular environment of one of the four subtypes of CHL. The background milieu is of utmost importance not only in establishing a diagnosis but also in subclassifying CHL. Of note, RS cell impostors may be found not only in some non-Hodgkin lymphomas but also in some benign lymphoid proliferations. However, with a few exceptions such as peripheral T-cell lymphomas and THRLBCLs, the accompanying inflammatory cells characteristic of CHL are not found.

Two types of RS cells are recognized in CHL: (1) classical and (2) lacunar cells (Fig. 11.1). The classical binucleate or owl eye cell is most abundant in MC CHL, although it is also found in LR CHL and occasionally in the NS and LD types. Lacunar cells predominate in NS CHL (see Fig. 11.1) but may also be seen in the LR classical type and in small numbers in the MC type.

Classical RS cells are large (up to 100 μm) binucleated, multinucleated, or lobulated giant cells with prominent, viral inclusion-like eosinophilic nucleoli surrounded by a halo in each nucleus. The nucleoli are seen in at least two nuclei in multinucleated cells or in each of two lobes in lobulated RS cells (see Fig. 11.1). These cells have abundant eosinophilic or amphophilic cytoplasm. Mononuclear variants are called Hodgkin cells. Apoptotic H/RS cells—referred to as mummified cells and characterized by pyknotic chromatin, barely

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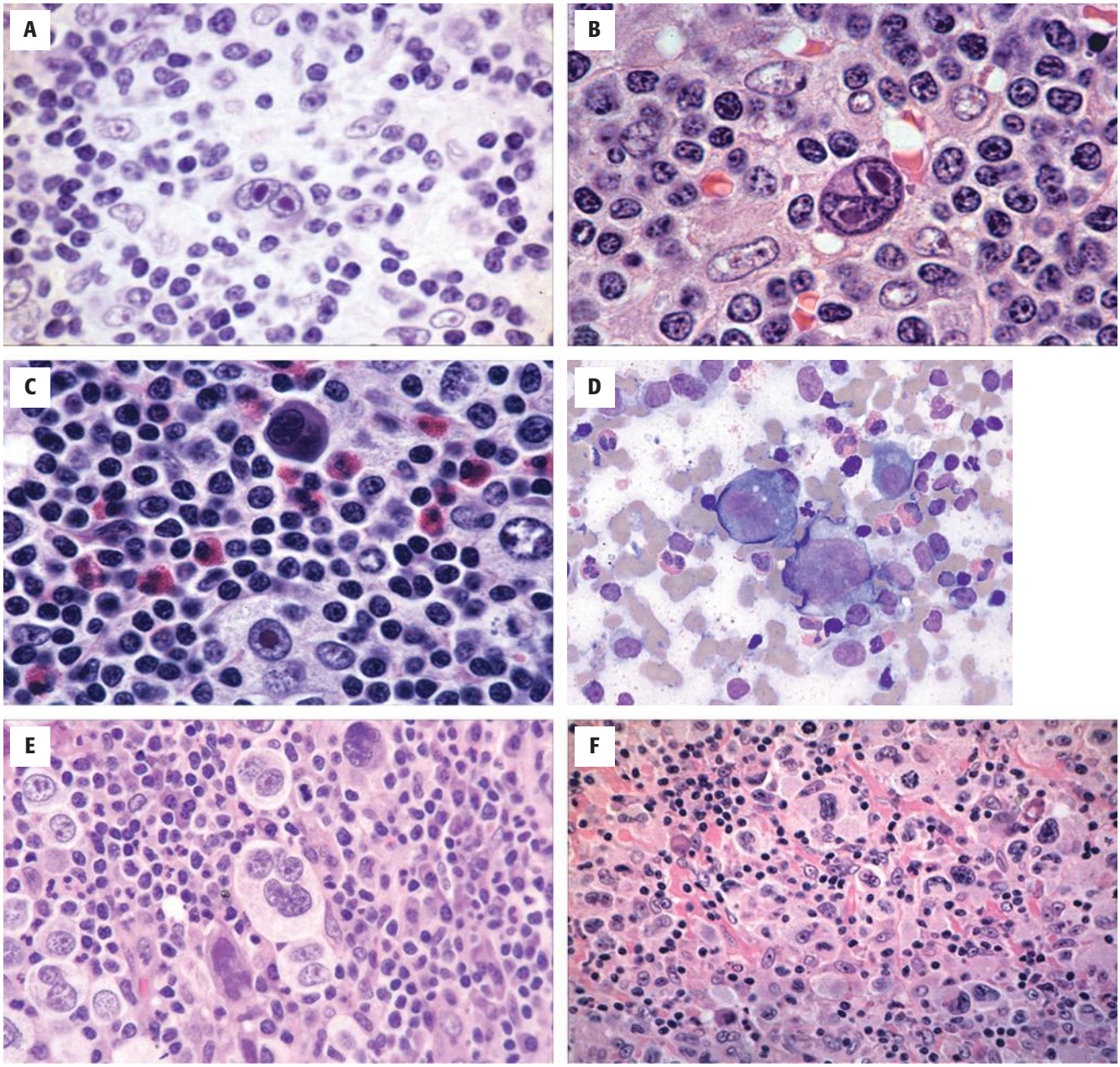


FIG. 11.1

Classical Hodgkin lymphoma, Reed-Sternberg cells and variants: microscopic features and immunophenotype. A, Classical owl-eye cell. The cell is large and binucleate, with each nucleus containing a large inclusion-like nucleolus. B, Bilobed RS cell. C, Hodgkin cell, which is a uninucleated cell with cytologic features of an RS cell, and a mummified cell characterized by purple-staining cytoplasm and a pyknotic nucleus. D, Appearance of RS cells in fine needle aspirate. Note the inflammatory background with many eosinophils. E, Lacunar cell variants of RS cell with characteristic multilobate nuclei containing small nucleoli. F, Tissue fixed in B5 showing lacunar cells with ample cytoplasm.

recognizable nucleoli, and eosinophilic and occasionally retracted cytoplasm—are present in all types of CHLs (see Fig. 11.1). Lacunar cells derive their name from the clear spaces or lacunae in which the nuclei reside. This phenomenon is caused by the retraction of cytoplasm around the nucleus, especially in formalin-fixed tissue, although some spider web–like cytoplasmic strands may still be attached to the cell membrane, thus leaving a space around the cells that is a useful artifact in establishing a diagnosis. In B5-fixed tissue, lacunar cells are not surrounded by empty spaces, but the cells have ample pale-staining cytoplasm (see Fig. 11.1F). Lacunar cells also can be recognized cytologically due to their hyperlobated, smaller nuclei and their nucleoli, which are smaller than those seen in classical RS cells. Lacunar cells can occasionally be more pleomorphic, with prominent nucleoli. They may appear bizarre, resembling cells of anaplastic large-cell lymphoma, or can be mononuclear and thus indistinguishable from cells of large-cell lymphoma (Fig. 11.2). Pleomorphic lacunar cells are especially prominent in cases of the syncytial variant of NS HL.

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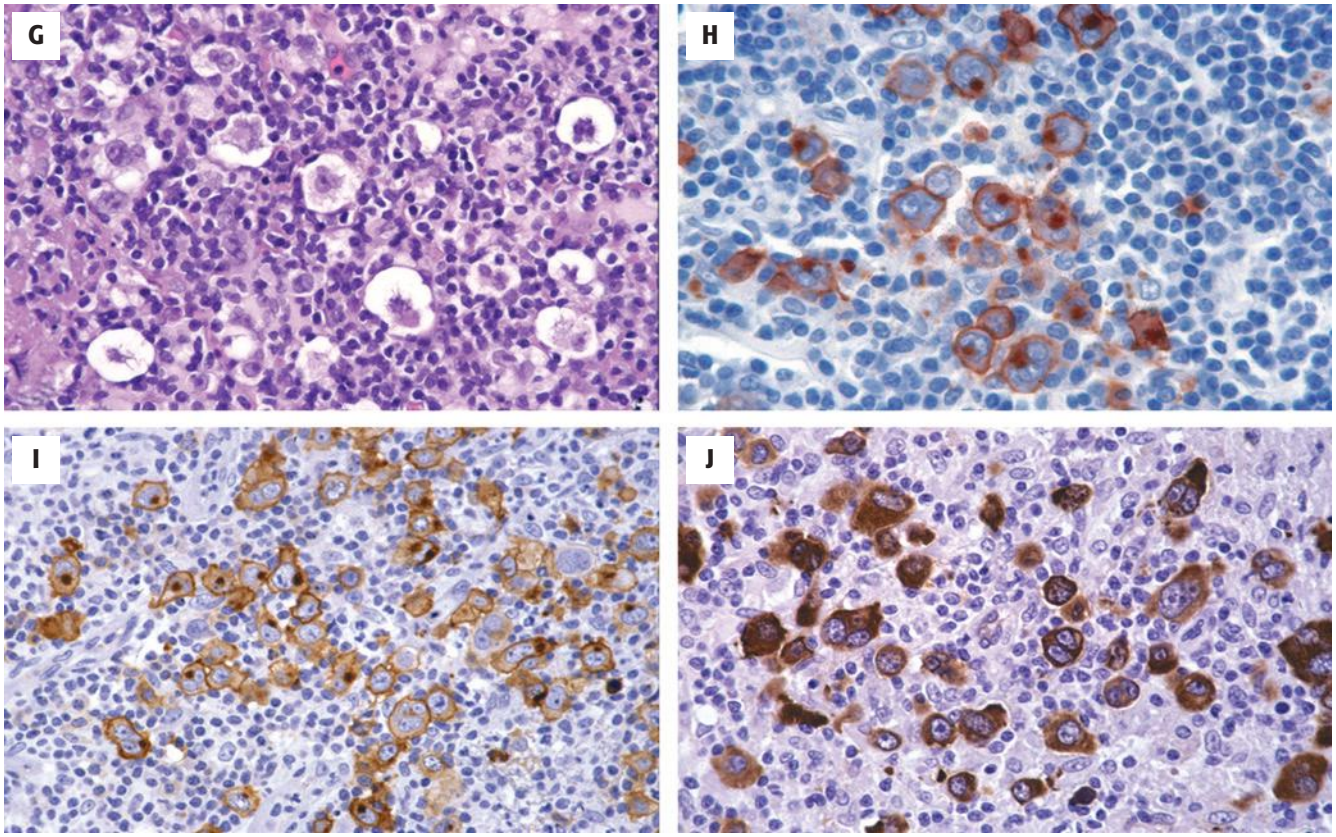


FIG. 11.1, cont'd

G, Formalin-fixed tissue shows retraction of cytoplasm away from cell membrane of lacunar cells. H, Classical RS cells stained with CD15. I, CD30. J, Fascin. CD15 and CD30 stain the cell membrane or Golgi region, or both, whereas fascin diffusely stains the cytoplasm.

NODULAR SCLEROSIS CLASSICAL HODGKIN LYMPHOMA

Nodular sclerosis classical Hodgkin lymphoma (or NS CHL) is a subtype of CHL characterized by collagen bands that subdivide the lymph node into cellular nodules of inflammatory cells admixed with lacunar cell variants of H/RS cells.

Clinical Features

NS CHL is the most common subtype of CHL in developed countries, accounting for approximately 60% to 80% of CHL cases. It is particularly common in individuals of high socioeconomic status and is often found in adolescents and young adults. The median age at diagnosis is 28 years. NS CHL is the only subtype of CHL in which there is no male predominance, with the male-to-female ratio being about equal. Presentation with a mediastinal mass is common (approximately 80% of patients), and more than 50% of patients with a mediastinal mass have bulky disease (an adverse prognostic factor). Most patients have stage II disease, and B symptoms are present is approximately 40% of cases. Lung involvement is found in approximately 10% of patients, usually by contiguous spread from mediastinal disease, or, less often, there may be miliary lung involvement secondary to vascular spread. The spleen is involved in approximately 10% of cases, bone marrow disease is found in 3% of cases, and the liver is affected in 2%.

Microscopic Features

The characteristic histologic features of NS CHL include sclerosis, nodules, and lacunar cell variants of H/RS cells (Fig. 11.3). The presence of fibrosis is a defining feature, but the amount can be extremely variable. The lymph node may show focal, partial, or total involvement. Sclerosis consists of sparsely cellular fibrocollagenous bands emanating from a thickened fibrous capsule, dividing the lymph node into cellular nodules that are often visible at gross examination of the biopsy. The fibrous bands show green birefringence in polarized light, but polarization is not essential for diagnosis. The diagnostic classical RS cells may be present but are rare. To make a diagnosis of NS CHL, at least one fibrocollagenous band forming a partial nodule must be seen. Fibrosis can range from thin and delicate lacing to thick fibrous bands,

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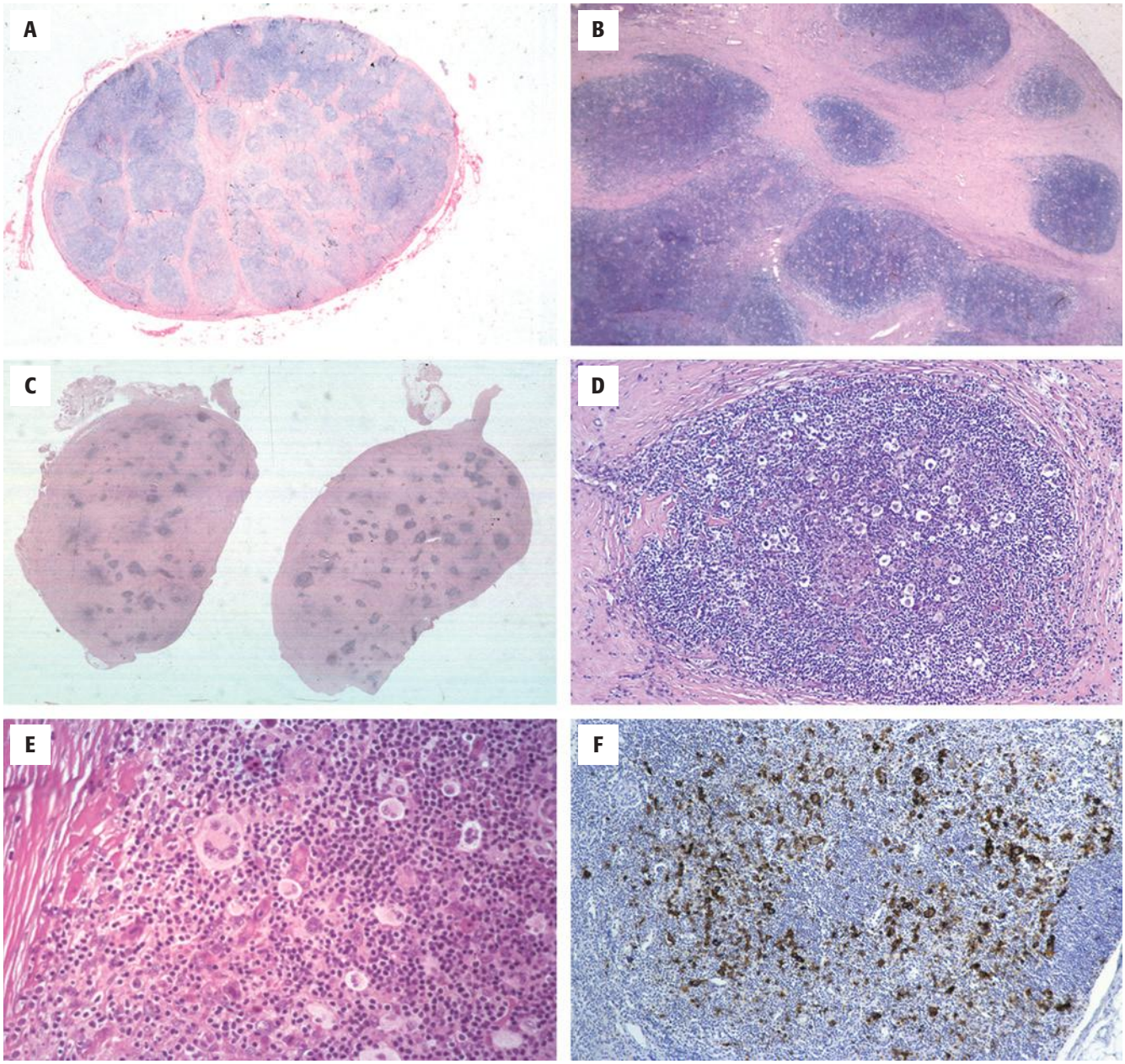


FIG. 11.2

Nodular sclerosis classical Hodgkin lymphoma. Varying degrees of sclerosis. A, The capsule is slightly thickened, and the node is divided into cellular nodules by thin fibrotic bands (low magnification). B, Fibrotic bands are thicker. C, Obliterative sclerosis with little cellular tissue containing lacunar and inflammatory cells. D, A nodule formed by a fibrous band contains lacunar cells interspersed among inflammatory cells. E, High magnification showing edge of sclerotic band and scattered lacunar cells characteristic of grade 1 nodular sclerosis. F, Cellular phase of nodular sclerosis classical Hodgkin lymphoma. A vague nodular pattern containing $CD30^+$ lacunar cells but no sclerotic bands. Images in A, B, C, D, and F are low magnification.

and in some cases, there is almost complete obliterative sclerosis with only a small amount of cellular tissue remaining (Fig. 11.3). These cases can pose significant diagnostic difficulties, especially if only limited amounts of material are available, such as cutting needle biopsies from the mediastinum. A variable inflammatory component is present within the nodules, which contain variable numbers of lacunar cells (Fig. 11.3). Lacunar cells may occur singly, in small aggregates, or in sheets. As stated earlier, the appearance of lacunar cells depends on the type of fixative used (prominent lacunae appearing with formalin fixation and absent or less obvious lacunae with B5-fixed tissue). The syncytial variant of NS causes the greatest problem in differential diagnosis (Figs. 11.2 and 11.4). This variant is characterized by cohesive sheets or aggregates of lacunar cells that may be monomorphic, giving the appearance of large-cell lymphoma, or they may be pleomorphic, mimicking cases of anaplastic large-cell lymphoma, or even resemble metastatic neoplasms. The aggregates of lacunar cells often have admixed histiocytes and may contain areas of necrosis, which may be coagulative

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NODULAR SCLEROSIS CLASSICAL HODGKIN LYMPHOMA—FACT SHEET

Definition
- A subtype of CHL characterized by collagen bands that subdivide the lymph node into nodules of inflammatory cells admixed with lacunar cell variants of RS cells

Incidence and Location
- Approximately 60% to 80% of CHL in the United States and Western Europe
- Mediastinal mass in approximately 80% of patients
- Supraclavicular or cervical lymph node involvement

Gender and Age Distribution
- Equal male-to-female ratio (only type of HL without male predominance)
- Median age is 28 years; may be seen in children and older adults

Clinical Features
- Majority of patients with stage II disease
- Approximately 40% of patients with B symptoms
- Bulky disease in approximately 50% of patients with mediastinal involvement, associated with prognosis
- Lung involvement in 10% of patients
- Spread to spleen in 10% of cases
- Bone marrow involvement in 3% of patients

NODULAR SCLEROSIS CLASSICAL HODGKIN LYMPHOMA—PATHOLOGIC FEATURES

Gross Findings
- Lymph node sometimes showing varying numbers of nodules surrounded by sclerotic bands
- Thickened fibrous capsule

Microscopic Findings
- Lymph node may show focal, partial, or total involvement
- Nodules are composed of an inflammatory cell infiltrate with scattered lacunar cell variants of H/RS cells (grade 1) in most cases (75% to 85%)
- Collagen bands (sclerosis) surround nodules
- Syncytial variant of NS CHL falls into grade 2 category and is characterized by cohesive sheets or aggregates of lacunar cells in background of inflammatory cell infiltrate
- Grading is not required for routine clinical purposes

Immunohistochemical Features
- H/RS cells have an immunophenotype identical to that in other types of CHL
- EBV-encoded LMP1 or EBER is present in H/RS cells in 10% to 25% of cases
- Background lymphocytes are T cells, but residual germinal centers may be present

Pathologic Differential Diagnosis of Syncytial Variant of Nodular Sclerosis
- Anaplastic large-cell lymphoma
- Primary mediastinal large B-cell lymphoma and mediastinal gray zone lymphoma
- Mediastinal seminoma
- Metastatic melanoma or carcinoma
- Necrotizing granulomas (especially cat-scratch disease)

or suppurative, sometimes giving a granulomatous appearance (see Fig. 11.4). Such necrotic lesions are occasionally misinterpreted as infectious granulomas. A two-tiered grading scheme for NS CHL according to the proportion of H/RS cells or the characteristics of the background infiltrate has been proposed by the British National Lymphoma Investigation (BNLI), although at present, this scheme is not strictly required for routine clinical purposes. Most cases (75% to 85%) are grade 1, and the remaining cases are classified as grade 2. In grade 1 cases, 75% or more of the nodules contain scattered lacunar cells in a background of a predominance of lymphocytes, or else the nodules contain a mixture of inflammatory cells (lymphocytes, eosinophils, histiocytes, plasma cells, and neutrophils) in varying proportions. Cases are classified as grade 2 if more than 25% of the nodules show pleomorphic or reticular lymphocyte depletion; more than 80% of the nodules show the fibrohistiocytic variant of lymphocyte depletion; or more than 25% of the nodules show numerous bizarre, anaplastic-appearing RS cells without lymphocyte depletion. Many of these cases were previously classified as lymphocyte-depleted (or LD) CHL. Grade 2 NS appears to be similar to the syncytial variant of NS and to cases in which there are sheets of sometimes bizarre and anaplastic lacunar cells and few lymphocytes present, and which may be confused with nonlymphoid tumors or anaplastic large-cell lymphoma. Although it was initially believed that grade 2 NS was associated with a worse prognosis than grade 1, this finding has not been substantiated in more recent studies based on improved therapies.

Occasional cases have characteristic lacunar cells, sometimes arranged in a vague nodular pattern but with no fibrous bands. Such cases have been referred to as the *cellular phase of NS CHL* (see Fig. 11.3). Rare cases of NS CHL involve only interfollicular areas of lymph nodes with prominent follicular hyperplasia that can mask the focal involvement by CHL. Such cases usually cannot be further subclassified and are called *interfollicular CHL*. In subsequent biopsy specimens, such cases are found to be either NS or MC CHL.

MIXED CELLULARITY CLASSICAL HODGKIN LYMPHOMA

MC CHL is a subtype of CHL with scattered classical H/RS cells in a diffuse or rarely vaguely nodular growth pattern of a mixed inflammatory cell background without nodular fibrosis. Cases that do not fit into the other subtypes are placed into this category.

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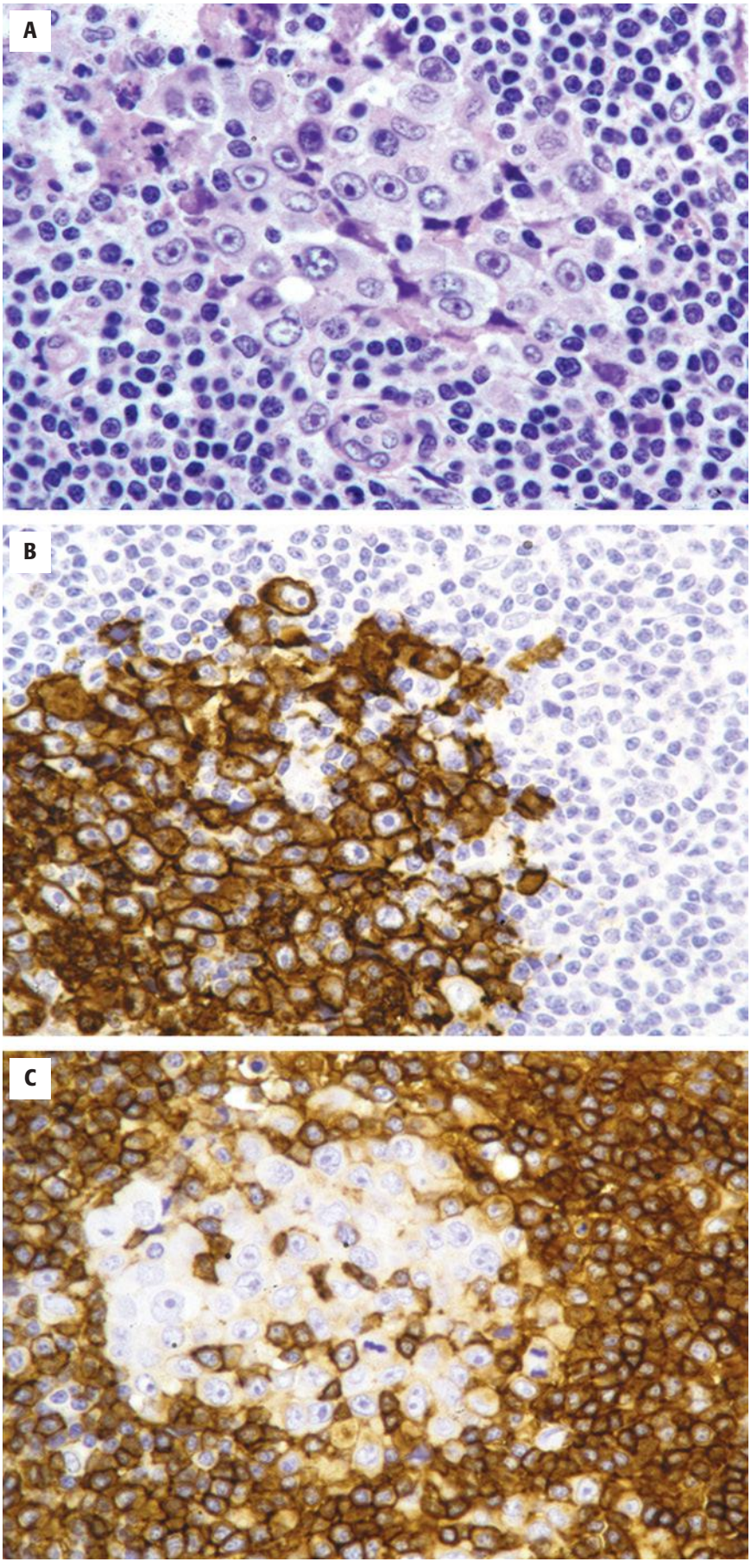


FIG. 11.3

Syncytial variant of nodular sclerosis classical Hodgkin lymphoma (grade 2). A, Mediastinal biopsy specimen showing a cluster of large cells (lacunar cells). B, The cluster of cells shows intense membrane staining with CD15. C, The lacunar cells are negative with CD45, whereas the small background lymphocytes are $CD45^{+}$. All images are high magnification.

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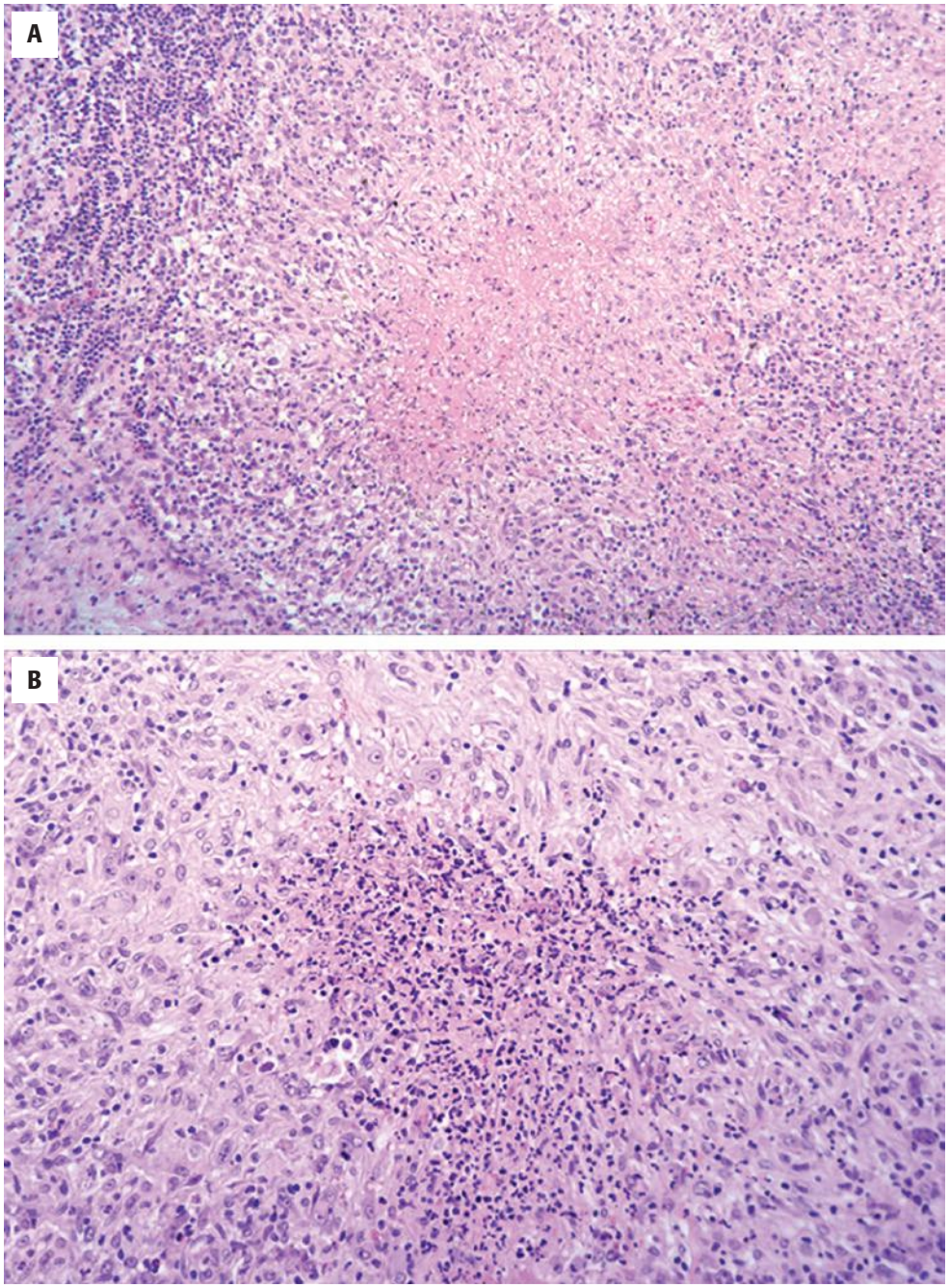


FIG. 11.4

Syncytial variant of nodular sclerosis classical Hodgkin lymphoma with necrosis. A, Coagulative necrosis surrounded by lacunar cells resembling a granuloma (low magnification). B, Suppurative necrosis surrounded predominantly by epithelioid histiocytes resembling cat-scratch disease. Rare lacunar cells with prominent nucleoli are present (low magnification). The latter cells were $CD15^+$ and $CD30^+$ (not shown). Cells with prominent nucleoli are not found in cat-scratch disease.

Clinical Features

Approximately 15% to 25% of all cases of CHL are of the MC type. There is a male predominance (approximately 70%) with a median age at presentation of 38 years. It is uncommon in young adults but is seen more frequently in children and after 50 years of age. A bimodal age distribution is not present. Both MC and LD subtypes are found more often in nonindustrialized nations and are also the more frequent in HIV-positive patients. Presentation with high-stage disease (stage III/IV) and B symptoms is common. Unlike NS CHL, MC rarely involves the mediastinum. The spleen is involved in approximately 30% of cases, the bone marrow in 10%, and the liver in 3%. In HIV-positive patients, the bone marrow is positive in 15% to 30% of patients at the time of diagnosis. The prognosis of MC CHL is similar to that of other subtypes of CHL of similar stage.

Microscopic Features

As the name mixed cellularity implies, there is a mixed inflammatory cell infiltrate composed of lymphocytes, eosinophils, granulocytes, plasma cells, epithelioid histiocytes, and fibroblasts in varying proportions admixed with classical H/RS cells, which can be found readily (Fig. 11.5). Although

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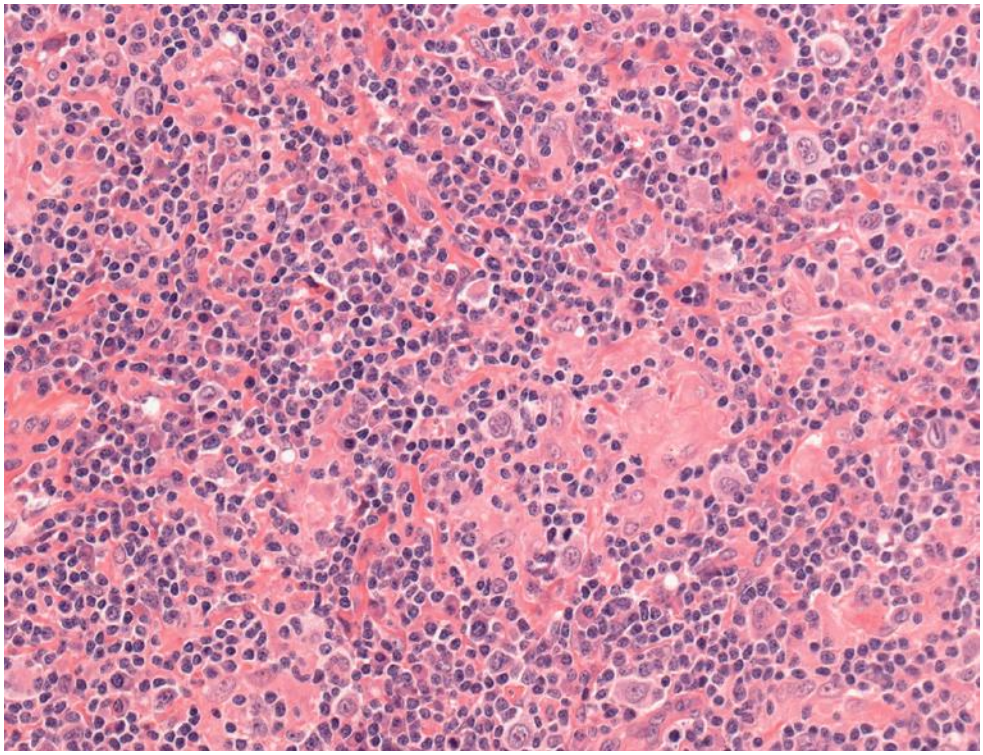


FIG. 11.5

Mixed-cellularity classical Hodgkin lymphoma. At low magnification, classical RS and Hodgkin cells are evident within a mixed inflammatory infiltrate including lymphocytes, histiocytes, eosinophils, and plasma cells.

MIXED CELLULARITY CLASSICAL HODGKIN LYMPHOMA—FACT SHEET

Definition

- A subtype of CHL with scattered H/RS cells in a diffuse or rarely vaguely nodular growth pattern of a mixed inflammatory cell background without nodular fibrosis
- Includes cases that do not fit into other subtypes

Incidence and Location

- Approximately 15% to 25% of all cases of CHL
- More often found in nonindustrialized nations
- Bimodal age distribution absent
- Peripheral lymph nodes involved
- Mediastinal involvement uncommon

Gender and Age Distribution

- Male predominance (approximately 70%)
- Median age at presentation, 38 years; uncommon in young adults
- Most common subtype in HIV infection

Clinical Features

- Presentation often with high-stage disease (stage III/IV)
- B symptoms common
- Splenic involvement and disease below the diaphragm more common than in NS
- Prognosis similar to that of other types of CHLs and stage dependent

MIXED CELLULARITY CLASSICAL HODGKIN LYMPHOMA—PATHOLOGIC FEATURES

Microscopic Findings

- Lymph node architecture effaced
- Mixed inflammatory cell infiltrate consisting of varying proportions of lymphocytes, eosinophils, plasma cells, epithelioid histiocytes, neutrophils, and fibroblasts
- Classical RS and Hodgkin cells readily found
- Absence of band-forming sclerosis
- May focally involve interfollicular areas of lymph nodes with follicular hyperplasia (interfollicular CHL)

Immunohistochemical Features

- H/RS cells have immunophenotype identical to that in other types of classical HLs
- Background lymphocytes are predominantly T cells
- EBV-encoded LMP1 or EBERs are detected in H/RS cells more often (75%) than other types of CHLs

Differential Diagnosis

- Peripheral T-cell lymphoma
- T-cell/histiocyte-rich large B-cell lymphoma
- Infectious mononucleosis
- Hypersensitivity reactions (diphenylhydantoin)

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lacunar cells may be observed occasionally, the presence of clusters of lacunar cells should prompt a differential diagnosis of cellular phase of NS. In some cases, there are many epithelioid histiocytes that form small granuloma-like clusters and may even obliterate the whole node, leading to a differential diagnosis of NHL rich in histiocytes or even inflammatory conditions or storage disorders. Band-forming sclerosis and a thickened capsule characteristic of NS CHL are absent, although interstitial fibrosis may be identified by reticulin stains. Similarly, necrosis is not a feature of this subtype. The lymph node architecture is usually completely effaced, but both MC and NS CHL may involve interfollicular areas of lymph nodes showing prominent follicular hyperplasia that can mask the CHL. Such cases are called interfollicular CHL. Focal collections of histiocytes may be a clue to the presence of interfollicular CHL, and a search for classical H/RS cells should be undertaken. Immunostaining must be performed in such cases to differentiate binucleate immunoblasts, which may be seen in viral infections such as infectious mononucleosis that may resemble classical RS cells.

LYMPHOCYTE-RICH CLASSICAL HODGKIN LYMPHOMA—FACT SHEET

Definition
- A subtype of CHL in which there is a predominance of small lymphocytes admixed with scattered H/RS cells
- The growth pattern either nodular or rarely diffuse
- Eosinophils and neutrophils rare or absent

Incidence and Location
- Approximately 5% of all cases of CHL
- Peripheral, most often cervical lymph nodes involved

Gender and Age Distribution
- Median age at diagnosis is greater than for other types of CHLs and similar to that for NLPHL
- Male predominance (approximately 70%)

Clinical Features
- Features similar to those of NLPHL except that multiple relapses less common
- Peripheral lymphadenopathy, most often cervical
- Mediastinal disease uncommon (15%)
- Stage I/II disease at presentation
- B symptoms usually absent

LYMPHOCYTE-RICH CLASSICAL HODGKIN LYMPHOMA

LR CHL is a subtype of CHL in which there is a predominance of small lymphocytes. It typically has a nodular or, less commonly, a diffuse cellular background. It is also characterized by the absence of eosinophils and neutrophils.

Clinical Features

LR CHL shares many of the clinical aspects of NLPHL, which are distinct from those of other forms of CHLs. This subtype was defined as its own subtype of CHL and separated from NLPHL in the studies from the European Task Force on Lymphoma, which performed immunophenotyping on a large series of cases initially diagnosed as NLPHL. Approximately 5% of all cases of CHL are of LR classical type, but the number may increase with an improved recognition of this subtype. The median age of patients at diagnosis is similar to that of NLPHL but significantly higher than that of other types of CHLs, and there is a male predominance (approximately 70%). Disease-free survival and overall survival are similar for both LR CHL and NLPHL, in addition to being similar to survival of other types of CHLs with limited stage disease. As in patients with NLPHL, patients with LR CHL usually lack B symptoms and bulky disease, have mediastinal involvement in only approximately 15% of cases, and have low-stage disease (usually stage I/II). In contrast to NLPHL, however, LR CHL is less often associated with multiple relapses (27% vs. 5%, respectively).

Microscopic Features

As the term *LR CHL* implies, the histologic features are characterized by a vast predominance of small lymphocytes, which may be arranged rarely in a diffuse, or much more frequently a nodular, growth pattern, along with a paucity of other inflammatory cells. Necrosis and fibrosis are absent. The nodular type of LR CHL was first described in 1992 and called follicular HL; subsequently, the term *follicular* was changed to *nodular* in the REAL and WHO schemes. The nodules, which are expanded mantle zones of B-cell follicles, have irregular borders and contain a predominance of small lymphocytes, thereby resembling nodules in NLPHL (Fig. 11.6). Some nodules contain eccentrically placed, regressed germinal centers; less often they contain normal-sized or hyperplastic germinal centers (Fig. 11.6). In contrast, germinal centers are infrequently found within nodules of NLPHL, and the RS cells in nodular LR CHL are mostly of the classical type. Some lacunar cell variants may be seen, and occasionally cells with morphologic features of LP cells are present. Most of the RS cells are found within the mantle zone, some at the junction of mantle and internodular areas, whereas a few RS cells may be present in the internodular areas that are composed predominantly of residual paracortical T cells. Rarely, eosinophils and neutrophils may be present in the internodular areas.

LYMPHOCYTE-DEPLETED CLASSICAL HODGKIN LYMPHOMA

LD CHL is a rare diffuse type of CHL depleted of small benign lymphocytes with numerous or, less often, few H/RS cells.

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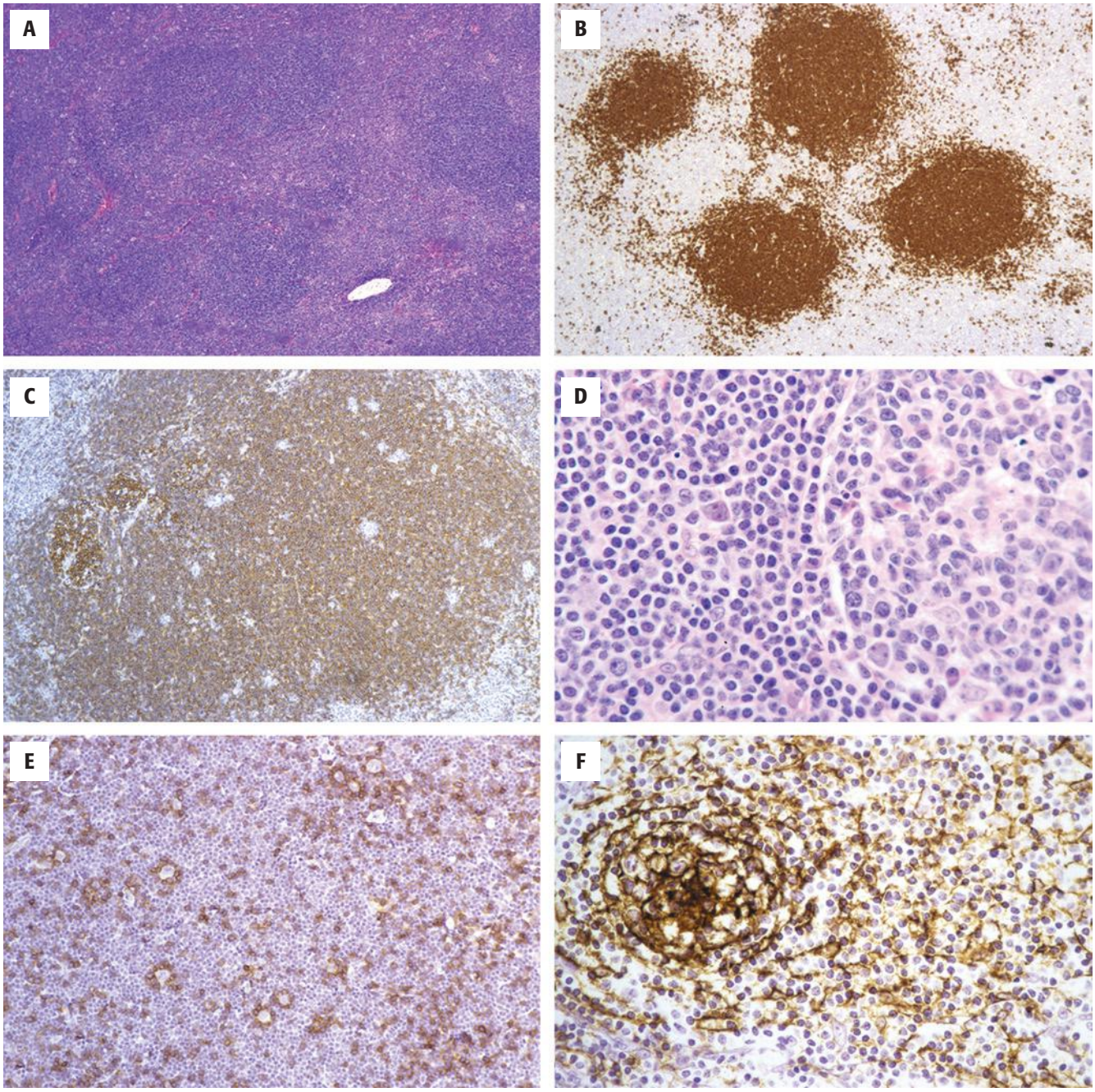


FIG. 11.6

Nodular lymphocyte-rich classical Hodgkin lymphoma. A, At low magnification, multiple vague nodules are evident. B, At low magnification, the CD79a-stained sections show nodules of expanded mantle cell lymphocytes. The nodules have irregular borders. C, CD79a-stained section with eccentrically placed germinal center with surrounding mantle zone (low magnification). D, Germinal center with adjacent classical RS cells within the mantle zone (high magnification). E, $CD3^+$ rosettes encircle classical Reed-Sternberg cells (low magnification). F, CD21 stain shows a concentric dense follicular dendritic meshwork within a residual germinal center extending into surrounding mantle zone in a more loosely arranged fashion (low magnification).

Clinical Features

LD CHL is rarely diagnosed in industrialized nations, accounting for less than 1% of patients in recent series. It is more common in HIV-positive patients and reported more often in developing countries. The majority of LD CHL cases were diagnosed before immunophenotyping became available, and most now would be classified as examples of NHL, the syncytial variant of NS CHL, or occasionally sarcomas, carcinomas, or melanomas. Because of the paucity of cases of true LD CHL, reliable clinical data are sparse. Most of the patients have B symptoms with abdominal involvement and advanced-stage disease (splenic, liver, and bone marrow involvement), and some studies have reported a male predominance and older age of the patients at diagnosis.

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LYMPHOCYTE-RICH CLASSICAL HODGKIN LYMPHOMA—PATHOLOGIC FEATURES

Microscopic Findings

- Lymph node architecture effaced
- Nodular (most cases) or diffuse growth pattern
- Vast predominance of small lymphocytes
- Paucity of other inflammatory cells
- Absence of necrosis and fibrosis
- Nodular growth pattern
  - Composed of small mantle cell lymphocytes
  - Nodules may contain small, residual germinal centers
  - Nodules contain scattered classical RS or lacunar cells or occasionally cells morphologically indistinguishable from LP cells
  - Nodules have irregular borders
- Diffuse growth pattern
  - Composed of small T lymphocytes with or without histiocytes
  - Scattered classical RS cells

Immunohistochemical Features

- Nodular growth pattern
  - Nodules composed of B-mantle cells ($CD20^+$, $CD79a^+$, IgD^+, IgM^+)
  - T-cell rosettes encircle H/RS cells
  - $CD57^+$ cells in germinal center and mantle zone, rarely encircling H/RS cells
  - RS cells with immunophenotype identical to that in other types of classical HLs
  - $CD21^+$ follicular dendritic meshwork in germinal center extending into mantle zone
- Diffuse growth pattern (rare)
  - Background lymphocytes are T cells
  - RS cells have immunophenotype identical to that in other types of CHLs
  - Absence of $CD57^+$ cells and no $CD21^+$ follicular dendritic meshwork

Differential Diagnosis

- Nodular LR CHL
  - Nodular LPHL
  - Indolent B-NHL with nodular growth pattern

Diffuse LR CHL

- TC/HRLBCL

LYMPHOCYTE-DEPLETED CLASSICAL HODGKIN LYMPHOMA—FACT SHEET

Definition

- A diffuse type of CHL depleted of small benign lymphocytes with numerous or occasionally few H/RS cells

Incidence and Location

- Least common type of CHL in industrialized countries (<1% of cases in Western countries)
- More often seen in HIV-positive individuals

Clinical Features

- Reliable clinical data are not available because cases previously diagnosed as LD CHL now mostly classified as non-Hodgkin lymphomas or syncytial variant of NS CHL
- Older patients (except for HIV-positive individuals), male predominance
- Advanced stage (III/IV)
- Frequent B symptoms
- Disease below the diaphragm common
- Spleen, liver, and bone marrow involvement common

LYMPHOCYTE-DEPLETED CLASSICAL HODGKIN LYMPHOMA—PATHOLOGIC FEATURES

Microscopic Findings

- Lymph node architecture effaced
- Depletion of small, benign lymphocytes
- Hypocellular or hypercellular with many bizarre, pleomorphic RS cells
- Disorderly fibrosis
- Background of fibrillary matrix or amorphous pink-staining material
- Necrosis common

Immunohistochemical Features

- RS cells have immunophenotype identical to that of other types of CHLs
- RS cells in most HIV-associated cases are EBV+

Differential Diagnosis

- Anaplastic large-cell lymphoma
- Aggressive large B- or T-cell lymphoma
- Syncytial variant (grade 2) of nodular sclerosis HL
- Soft-tissue sarcomas

Histologic Features

As the name implies, there is a depletion of small lymphocytes, and the two histologic types of LD CHL described in the original Lukes and Butler classification are the reticular and the diffuse fibrosis types (Fig. 11.7). These two histologic subtypes are still recognized in cases currently diagnosed as LD CHL. The more common reticular type is hypercellular and consists predominantly of an abundance of pleomorphic large cells, some resembling or indistinguishable from classical H/RS cells and admixed histiocytes (Fig. 11.7B). Some cases with pleomorphic H/RS cells have a sarcomatous appearance. Few lymphocytes, eosinophils, histiocytes, and plasma cells are present. Necrotic areas are common, and disorderly fibrosis and a fibrillary matrix may be present as well. The other, less common, diffuse fibrosis subtype is hypocellular and consists of disorderly fibrosis (but lacking a nodular pattern) with amorphous pink-staining eosinophilic material containing few cellular elements in the background (Fig. 11.7A). There may be many fibroblasts or reticulin fibrosis and few inflammatory cells. Classical H/RS cells are rare, and necrosis is often present. Coarse fibrous bands are absent. A diagnosis of LD CHL requires immunohistochemical confirmation of the CHL phenotype.

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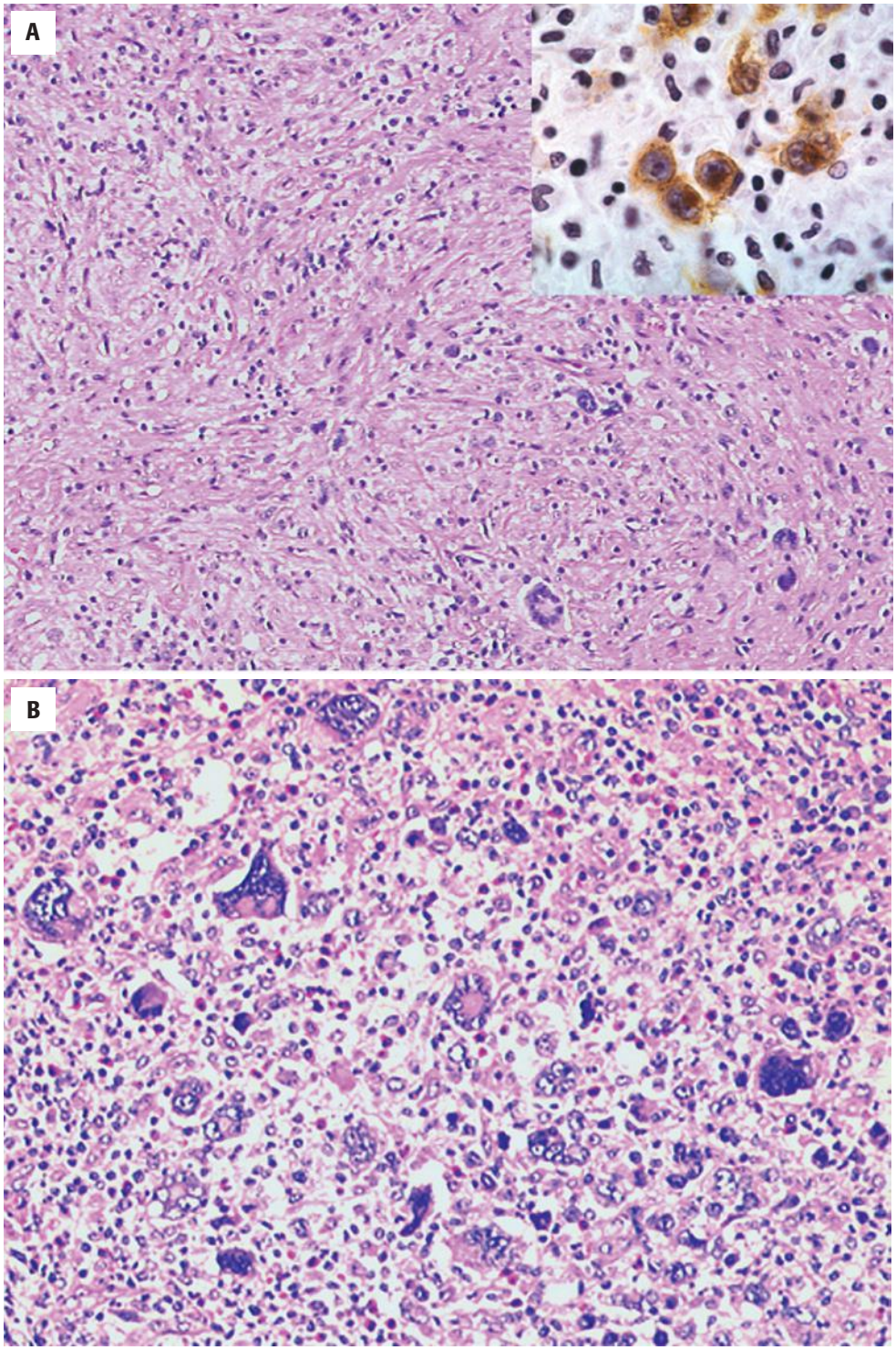


FIG. 11.7

Lymphocyte-depleted classical Hodgkin lymphoma. A, Diffuse fibrosis subtype. There is a paucity of lymphocytes and other inflammatory cells among fibroblasts and amorphous pink-staining material containing large pleomorphic Reed-Sternberg cells. Insert, the large cells are $CD15^{+}$. B, Reticular variant showing numerous bizarre, anaplastic Reed-Sternberg cells.

Diagnosis of Relapsed CHL and Diagnosis at Extranodal Sites

Relapsed CHL usually retains the initial histologic subtype but may show morphologic progression with an increase in the number and pleomorphism of tumor cells. However, the assignment of subtype should be based on the initial biopsies before therapy. Nevertheless, a diagnosis of relapsed CHL should always be confirmed immunohistochemically to rule out secondary neoplasms, especially with changed morphology. Persistent mass lesions after therapy are sometimes biopsied and may show only hyalinized fibrous tissue. However, a diagnosis of residual CHL requires the presence of H/RS cells, and areas of hypocellular sclerosis should not be regarded as evidence of residual disease.

As mentioned earlier, CHL is a nodal disease in most instances. The criteria for a diagnosis of CHL at extranodal sites depend in part on whether a diagnosis of CHL has been established at another site. In liver and bone marrow staging

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biopsies, the presence of atypical mononuclear cells of appropriate immunophenotype is sufficient for a diagnosis of CHL involvement. In the bone marrow, areas of fibrosis should be regarded as suspicious for involvement and examined by further step sectioning and immunostaining.

In contrast to patients with an established diagnosis of CHL, a primary diagnosis of CHL at an extranodal site should only be established with great caution. Stage IE is extremely rare in CHL, and many cases from older series probably represented extranodal NHL or EBV-driven lymphoproliferations. A commonly involved extranodal site sometimes representing diagnostic difficulties is the thymus. Thymic manifestations of NS CHL may present as a large mediastinal mass with formation of cysts interpreted as thymoma in imaging. Because thymic CHL can be accompanied by prominent reactive epithelial proliferations, a misdiagnosis of thymoma or multilocular inflammatory thymic cyst must be avoided by appropriate immunostaining.

Ancillary Studies

Immunophenotype

Immunophenotyping in paraffin sections is exceedingly useful in both the diagnosis and subclassification of CHL. Due to the unique features of CHL, both the immunophenotype of the H/RS cells as well as the reactive background cells are of importance. Despite the significant morphologic variability, the immunophenotype of the neoplastic cells is constant among the four subtypes of CHL. Nevertheless, a panel of antibodies is necessary to characterize H/RS cells, because no single marker is sufficiently sensitive or specific. The H/RS cell phenotype is unique in comparison with other lymphoid cells. In addition to a variety of activation-associated antigens such as CD30, CD25, and HLA-DR, antigens normally found on other cell types such as granulocytes (CD15) or follicular dendritic cells (fascin) are expressed, whereas many B-lineage–specific antigens are lacking or only inconsistently found. Antibodies routinely used when a diagnosis of CHL is suspected should at least include CD15, CD30, CD3, and CD20. Additional B- and T-cell antibodies such as CD79a, PAX-5, MUM-1/IRF-4 and CD4, epithelial membrane antigen (EMA), BCL6, fascin, EBV-latent membrane protein 1 (LMP1), ALK, and in situ hybridization for EBER may be added depending on the differential diagnosis. Other markers that are not routinely used but are occasionally helpful include B-cell transcription factor OCT-2 with its cofactor BOB.1, PU.1, and J-chain (Table 11.4).

Immunophenotype of the Reed-Sternberg Cell in Classical Hodgkin Lymphoma

In most cases, classical RS cells express CD15, CD30, and fascin, and they are CD45 (common leukocyte antigen) negative (see Table 11.4). CD45 may be difficult to interpret due to the surrounding reactive lymphoid cells and therefore is less useful in diagnostic practice. H/RS cells are strongly positive for the postgerminal center marker MUM-1 and weakly express PAX-5. These two nuclear markers are also helpful to highlight the cytologic features of the neoplastic cells. The immunophenotype of classical H/RS cells may differ slightly from case to case, and it is important to remember that not all neoplastic cells in each case will be positive for a specific marker.

CD30 is positive in almost 100% of cases, whereas CD15 is found in 75% to 85% of cases of CHL. CD30 is usually strongly expressed by virtually all neoplastic cells, but CD15 may be more restricted and heterogeneous in intensity (see Fig. 11.1). Both markers typically show distinct membranous and Golgi-area staining. Not surprisingly, considering the B-cell derivation of CHL, some cases of CHL may express pan–B-cell markers, most often CD20. CD20 positivity has been reported in 5% up to 80% of cases, most probably reflecting differences in fixation, antigen retrieval, and interpretation, with most studies being in the 20% to 40% range. However, it is important to note that CD20 expression usually is weak and heterogeneous and restricted to a subpopulation of neoplastic cells. If strong, homogeneous expression of CD20 or other B-cell markers such as CD79a is detected, a diagnosis of large B-cell lymphoma or a so-called gray zone lymphoma should be considered (see below). J-chain is always absent, whereas other B-cell transcription factors such as Oct-2, BOB.1, and PU.1 are usually lacking or only partially expressed, as a reflection of the downregulation of the B-cell program. In addition, H/RS cells in CHL are negative for BCL6 in most cases. Expression of T-cell markers such as CD3 has been described in a minority of cases, although interpretation of staining is difficult due to surrounding T cells. Cytotoxic granule-associated proteins such as TIA-1, granzyme B, or perforin are usually weakly expressed by a minority of cases of CHL. EMA and ALK protein are consistently absent from the neoplastic cells.

CD30, although the most reliable marker, is not specific for H/RS cells because it is expressed by all cases of anaplastic large-cell lymphomas (ALCLs), activated B and T cells, some plasma cells, and embryonal carcinoma. CD15 is also expressed by granulocytes and may be difficult to interpret in granulocyte-rich cases. Fascin, in addition to staining RS cells in almost all cases of CHL, is an excellent marker for various types of dendritic cells (interdigitating and follicular) and endothelial cells (see Fig. 11.1). It may also stain cells in some cases of ALCLs and a variable number of large B- and T-cell lymphomas. The staining with fascin in all these cells is diffuse and cytoplasmic.

The frequency of EBV in H/RS cells in CHL varies according to the histologic subtype. It is most often (approximately 75%) associated with the MC type and least often (10% to 40%) with NS CHL. Most cases of LD type, which are seen in HIV-positive individuals, are EBV^+. EBV infection in CHL is consistently of latency type 2. Therefore, the presence of EBV can be assessed by immunostaining for LMP1, which, if present, decorates most or all H/RS cells.

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TABLE 11.4
Differential Diagnosis of Hodgkin Lymphoma: Immunophenotypes of Malignant Cells

	CHL	NLPHL	TC/HRLBCL	DLBCL	ALCL[a]
CD45	–	+	+/–	+/–	+/–
CD15	+/–	–	–	–	–
CD30	+	–	–/+	–/+[b]	+
Fascin	+	–	–	–/+	+/–
CD20	–/+	+	+	+	–
CD79a	–/+	+	+	+	–
PAX-5	+[d]	+	+	+	–[f]
OCT-2	– (or weakly +)	+	+	+	–
MUM-1/IRF-4	+	+/–	–/+	–/+	+
BOB.1	– (or weakly +)	+	+	+	–
PU.1	–	+/–	–	+	–
CD3, CD7, CD8	–	–	–	–	–/+
CD2, CD4, CD5	–	–	–	–	+/–
CD43	–	–	–	–	+/–
EMA	–	+	–/+	–	+/–
ALK	–	–	–	–	+ (60%–80%)
BCL-6	–[c]	+	+/–	+/–	–
Clusterin	–	–	–	–	+
EBV(LMP1, EBER)	+/–	–	–	–	–
TIA-1, granzyme B, perforin	– (rare cases weakly +)	–	–	–	+
CD21 (FDC meshwork)	–	+[e]	–	–	–

ALCL, Anaplastic large-cell lymphoma; *CHL,* classical Hodgkin lymphoma; *DLBCL,* diffuse large B-cell lymphoma; *NLPHL,* nodular lymphocyte-predominant HL; *TC/HRLBCL,* T-cell/histiocyte–rich large B-cell lymphoma.
[a]ALK$^+$ ALCL and ALK$^-$ ALCL show phenotypic differences.
[b]Especially in primary mediastinal large-cell lymphoma, DLBCL with anaplastic features, and some cells of conventional DLBCL.
[c]Rarely positive.
[d]Rare cases negative.
[e]Absent in diffuse LPHL.
[f]Rare cases positive.

Alternatively, in situ hybridization for EBER can be employed, which strongly stains the nuclei of all H/RS cells in EBV$^+$ cases but may also detect non-neoplastic small bystander cells. The presence of rare small lymphocytes positive for EBER in a case with negative tumor cells should not be taken as an indication for EBV positivity. The determination of latency type can be helpful for the differential diagnosis of CHL from EBV-driven Hodgkin-like lymphoproliferations in immunosuppressed individuals, with the latter frequently showing latency type 3 with positivity for Epstein Barr virus nuclear antigen 2 (EBNA-2), as well as variable expression of B-cell markers.

Immunophenotype of Background Lymphocytes

The reactive background lymphocytes of CHL, with the exception of the nodular LR CHL, are predominantly T cells (Table 11.5). The majority expresses CD4 and shows signs of activation, but their phenotype is more consistent with T-helper 2 (T_H2) cells and regulatory T cells, indicating an

TABLE 11.5
Immunophenotype of Small Lymphocytes in CHL, NLPHL, and Nodular LR CHL

	CHL (Except NLR CHL)	NLPHL	NLR CHL
B cells	–/+	+	+
T cells	+	Ring LP cells	Ring RS cells
PD-1+ cells	–	Ring LP cells	Mostly in germinal center, rarely ring RS cells
CD57+ cells	–	Random distribution, often ring RS cells	Mostly in germinal center, rarely ring RS cells

CHL, Classical Hodgkin lymphoma; *HL,* Hodgkin lymphoma; *NLPHL,* nodular lymphocyte-predominant HL; *NLR,* nodular lymphocyte-rich.

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inadequate response to the tumor. $CD8^+$ cytotoxic T cells are infrequent in CHL, except in HIV-positive patients. In contrast to the other subtypes of CHL and similar to NLPHL, the nodular pattern of LR CHL is characterized by a predominance of B cells. In both NLPHL and nodular LR CHL, the nodules of small lymphocytes are polyclonal B cells of mantle-cell origin expressing CD20, CD79a, IgM, and IgD, whereas the background small lymphocytes in all other types of CHLs are T cells (see Table 11.5). Another similarity between NLPHL and nodular LR CHL is the rosetting or ringing of T cells around H/RS and LP cells, respectively, which can be clearly seen in the B-cell–rich background in both subtypes and which is evident on low magnification (see Figs. 11.6 and 11.8). However, programmed death (PD)-1 (CD279) and CD57-positive follicular T-helper cells form rings around LP cells in NLPHL. This is not a feature of LR CHL. Rosetting of T cells around H/RS cells is also present in the other types of CHLs but is not easily detectable because of all the background T cells. Due to the presence of altered B-cell follicles in both disorders, proliferation of follicular dendritic cells (FDCs) can be detected with stains for CD21 or other FDC markers. In nodular LR CHL, CD21 highlights tightly arranged concentric FDCs within residual germinal centers sometimes reminiscent of Castleman's disease, whereas FDC in NLPHL form large, expanded spherical meshworks (see Figs. 11.6 and 11.8).

Given these similarities in architecture and B-cell background, the immunophenotype of the H/RS cells is essential in differentiating nodular LR CHL from NLPHL and in establishing the correct diagnosis. As mentioned earlier, the H/RS cells have the immunophenotype of other CHLs ($CD15^{+/-}$, $CD30^+$, $CD20^{-/+}$, $CD45^-$), whereas LP cells strongly express B-cell markers and usually lack CD30 and CD15 antigens (see below).

Genetic Features of Classical Hodgkin Lymphoma

The availability of laser capture techniques for isolating single RS cells from tissue sections and the application of polymerase chain reaction (PCR) to isolated H/RS cells in all subtypes of HL have unequivocally demonstrated that H/RS cells are clonal B cells. This is also true for most of the infrequent cases showing expression of T-cell markers, and the existence of a true T-cell CHL is still controversial. The rearranged IG genes of RS cells in both NLPHL and CHL carry a high load of somatic hypermutations in the variable region of the Ig heavy chain gene, indicating a germinal-center origin. In NLPHL, ongoing mutations are found, in contrast to CHL where ongoing mutations are absent.

Despite the presence of clonally rearranged IG genes, and in contrast to NLPHL, CHL lacks IG transcription and expression on the protein level. In a fraction of CHL, this is due to so-called crippling mutations, which lead to insertion of stop codons or mutations in the IG promotor region disabling IG transcription. Most of these cases are EBV^+, indicating a role of EBV for tumor cell survival. In the remaining 75% of cases of CHL lacking crippling mutations, the absence of IG transcripts possibly results from downregulation of synthesis of transcription factors BOB.1, OCT-2, and PU.1. Immunohistochemistry for the expression of the transcription factors BOB.1, OCT-2, and PU.1 showed decreased or lack of expression in H/RS cells of most cases of CHL.

Despite the clonal B-cell origin of H/RS cells, molecular techniques play only a minor role in the diagnosis of CHL and serve mainly to identify mimics. Conventional PCR techniques such as the BIOMED-2 primer sets for the detection of clonal B-cell rearrangements may or may not detect B-cell clonality, depending on technical sensitivity and the relative number of tumor cells.

Because of the unique properties of CHL with a small minority of tumor cells, the knowledge about genetic alterations of H/RS cells is relatively limited and not relevant for practical diagnostics. H/RS cells are highly aneuploid. Recurrent genetic alterations are amplifications of 2p13 containing the *REL* oncogene, and mutations of nuclear factor κB (NF-κB) inhibitors, which result in constitutive activation of the NF-κB pathway, a hallmark feature of CHL. Another commonly amplified region is 9p24 containing the *JAK2* and the programmed-death ligand genes *PD1L1* and *PD1L2.* PD-L1/2 proteins can be targeted therapeutically by specific antibodies. High-throughput sequencing identified recurrent translocations of the major histocompatibility complex (MHC) class II transactivator *CIITA*, resulting in downregulation of MHC class II expression in 15% of CHL. As mentioned earlier, gene-expression profiling has documented that the neoplastic cells of CHL show a downregulation of most B-cell–associated genes, justifying the separation of CHL from B-cell NHL.

Differential Diagnosis

The differential diagnosis of CHL is broad and includes NLPHL, non-Hodgkin lymphomas, nonhematolymphoid neoplasms, and benign proliferations. Although most cases of CHL can reliably be classified by morphologic examination and with a limited immunohistochemical panel, the distinction from one of the disorders above may be difficult. In addition to mere morphologic and immunophenotypic mimics, true borderline cases between CHL and large B-cell lymphoma exist, so-called gray zone lymphomas. Differential diagnosis may be especially difficult in small biopsies, such as Tru-Cut specimens from the mediastinum or the retroperitoneum. As can be expected from a lymphoma with such a variable morphologic appearance, the range of differential diagnoses to be considered depends in part on the subtype of CHL in question. For NS CHL, the list includes mediastinal large B-cell lymphoma and mediastinal gray zone lymphoma, anaplastic large-cell lymphoma, non-hematopoietic neoplasms such as undifferentiated carcinoma and seminoma, and granulomatous lymphadenitis. TC/HRLBCL, peripheral T-cell lymphoma, EBV-associated lymphoproliferative disorders in the immunosuppressed host, and reactive disorders including

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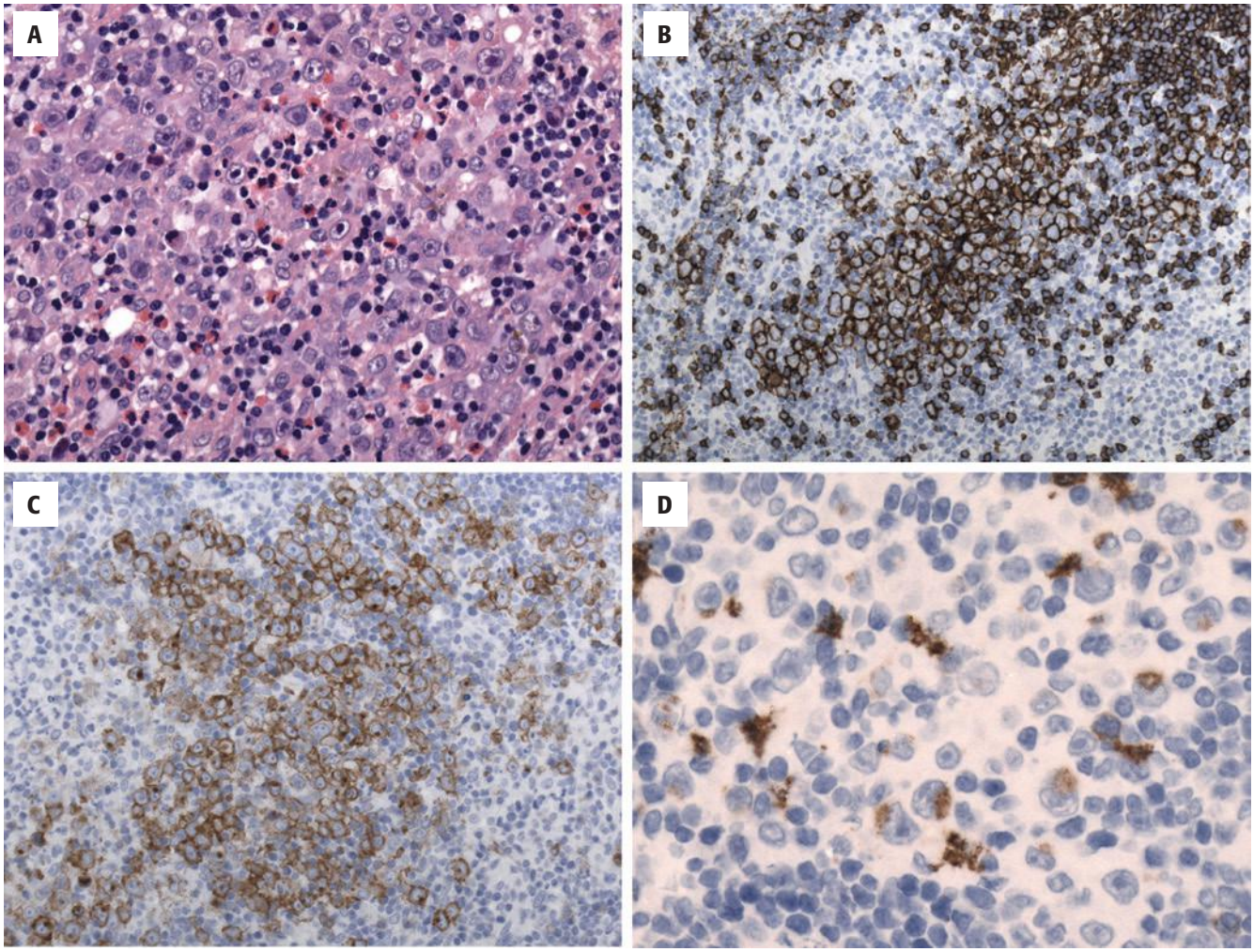


FIG. 11.8

Mediastinal gray zone lymphoma. A, There are sheets of mononuclear cells resembling Hodgkin cells, with occasional RS cells and eosinophils, similar to the syncytial variant of CHL. B, The neoplastic cells show strong and homogeneous CD20 expression and reveal variable expression of CD79a and OCT-2. C, strong expression of CD30 and, D, variable expression of CD15 with a Golgi-staining pattern.

infectious mononucleosis and drug-induced lymphadenitis may simulate MC CHL. Because they both show a follicle-based B-cell background, the main differential diagnosis of LR CHL is NLPHL, and occasionally indolent B-NHL, and is covered in the NLPHL section.

Primary Mediastinal Large B-Cell Lymphoma (PMLBCL) and B-Cell Lymphoma, Unclassifiable, With Features Intermediate Between DLBCL and Classical Hodgkin Lymphoma (BCL-U)

Primary mediastinal (thymic) large B-cell lymphoma (PMLBCL) is a specific subtype of DLBCL thought to arise from thymic B cells. It is recognized as a distinct entity in the WHO classification and accounts for approximately 2% to 4% of non-Hodgkin lymphomas. It shows an overlap with NS CHL in terms of clinical presentation, morphology, immunophenotype, and molecular features. In contrast to non-mediastinal B-cell lymphomas, the male-to-female ratio in PMLBCL is 1:2, and this disorder is found in a younger age group than other large-cell lymphomas (37 vs. 55 years). Patients with PMLBCL frequently present with mediastinal bulk and may have superior vena cava syndrome or dyspnea and cough. PMLBCL is characterized by a diffuse proliferation of medium to large cells with frequently clear cytoplasm sometimes resembling lacunar cells. In addition, H/RS cells may be observed. There is diffuse compartmentalizing background sclerosis rather than nodule formation as in NS CHL. Immunophenotypic similarities include lack of surface immunoglobulin and CD30 positivity. CD15 is occasionally expressed weakly in some large cells in PMLBCL. In contrast to NS CHL, PMLBCL shows consistent, strong expression of B-cell markers such as CD20 and CD79a, B-cell transcription factors BOB.1 and OCT-2, as well as frequent CD23.

In most cases of PMLBCL, the distinction from NS HL is straightforward. However, rare cases of mediastinal lymphoma may show truly overlapping features between PMLBCL and NS CHL, commonly with an asynchrony between morphology

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and immunophenotype. These cases have been designated as gray zone lymphomas and were included in the 2008 WHO classification as B-cell lymphoma unclassifiable (BCL-U), with features intermediate between DLBCL and CHL (Fig. 11.8). BCL-U is most often seen in young males aged 20 to 40 years. Most of such cases exhibit mediastinal disease. BCL-U may have the appearance of diffuse large B-cell lymphoma but with admixed H/RS or lacunar-type cells, whereas other cases resemble syncytial NS HL but may have only a sparse inflammatory background. The immunophenotype of BCL-U also overlaps between the two entities. CD45 is usually expressed by the large cells in both BCL-U and PMLBCL but not by H/RS cells in CHL. Cases morphologically more consistent with NS HL have large cells strongly expressing CD20 and CD79a and transcription factors PAX-5, BOB.1, and OCT-2, whereas cases with features of PMLBCL often only weakly express or are negative for CD20 and other B-cell markers, but are positive for CD15 and CD30, and may be positive for EBV. Rare cases may show typical NS CHL and typical PMLBCL in two different biopsies or in different areas of the same biopsy. These cases should be diagnosed as composite lymphomas, indicating both components, rather than as gray zone lymphomas. In addition, it should be noted for practical purposes that sheets of mononuclear large cells (see Fig. 11.2) or aberrant expression of a single B-cell marker is not sufficient for putting a given case in the BCL-U category.

This overlap between PMLBCL and NS CHL reflects the underlying biological similarities mentioned earlier. The gene-expression profile of PMLBCL is close to the expression profile of NS CHL but quite distinct from regular DLBCL. In addition, recent genetic studies have demonstrated similarities in the genetic profile. Translocations involving the MHC class II transactivator *CIITA* and amplifications of *REL* (2p15) and JAK2 (9p24) oncogenes occur in both disorders, and mediastinal gray zone lymphoma shows a methylation profile intermediate between PMLBCL and NS CHL. Cases of BCL-U usually have a more aggressive clinical course than either CHL or PMLBCL. A consensus regarding therapy for BCL-U has not been determined, although aggressive treatment (chemotherapy plus radiation therapy) appears to be effective.

Diffuse Large B-Cell Lymphoma

Although differentiation between diffuse large B-cell lymphomas (or DLBCLs) and CHL is straightforward in most instances based on morphology and ancillary stains, some variants of DLBCL may present difficulties, especially on a small or needle core biopsy, including DLBCL, anaplastic variant, TC/HRLBCL, and EBV$^+$ DLBCL. TC/HRLBCL may show a morphologic and possibly also biological overlap with NLPHL and therefore is discussed under the differential diagnosis of NLPHL. In some cases of TC/HRLBCL, however, many large cells are binucleate with prominent inclusion-like nucleoli and are indistinguishable from classical H/RS cells. The cells are differentiated from true H/RS cells by their intense staining with CD20, CD79a, and usually CD45. The cells are always CD15$^-$ but may express CD30 (see Table 11.4). Eosinophils, often but not always present in MC CHL, are usually absent in TC/HRLBCL. In addition, EBV may be present in H/RS cells in MC CHL, but it is not found in the large B cells of TC/HRLBCL.

EBV$^+$ DLBCL, extended in the 2016 update of the WHO classification to include also cases occurring in patients younger than 50 years, shows a commonly polymorphic proliferation of large B cells with frequent admixture of H/RS-like cells with common coexpression of CD30 and B-cell markers but CD15 negativity. In contrast to MC CHL, which usually shows a dichotomous distribution of small reactive lymphocytes and admixed H/RS cells, EBV$^+$ DLBCL contains a range of transformed cells including centroblasts and immunoblasts, plasmablasts, and anaplastic large cells. In many instances, these tumors show EBV latency type 3 with expression of all latent EBV proteins, including EBNA-2, whereas EBV$^+$ CHL is invariably of latency type 2, with expression of LMP1 but negativity for EBNA-2.

EBV-Associated Lymphoproliferations in Immunosuppressed Hosts

Lymphoproliferations in the immunosuppressed host, including the posttransplantation and iatrogenic settings, especially methotrexate therapy in rheumatoid arthritis or other autoimmune disease, can show a broad morphologic and phenotypic spectrum, including CHL and CHL-like lymphoproliferations. Most of these cases show association with EBV. If CHL is suspected in an immunosuppressed patient, a spectrum of transformed large cells including H/RS-like cells, coexpression of CD20 and CD30 and EBV latency type 3 with expression of EBNA-2 should prompt a diagnosis of CHL-like lymphoproliferation rather than CHL. Of note, both CHL-like lymphoproliferations and CHL may regress upon reduction of suppression or withdrawal of the causative drug, respectively. Other EBV-related lymphoproliferative disorders with variable clinical presentation and malignant potential such as EBV-associated mucocutaneous ulcer commonly contain H/RS-like cells but can be separated from CHL based on clinical features, the presence of a polymorphic background population, and the coexpression of B-cell antigens.

Anaplastic Large-Cell Lymphoma

In the 2016 update of the fourth edition of the WHO classification, ALCL has finally been separated into two distinct entities—namely, ALK$^+$ and ALK$^-$ ALCL. ALK$^-$ ALCL usually occurs in adults and is defined as a T-cell lymphoma strongly expressing CD30 and consisting of large lymphoid cells with ample cytoplasm and pleomorphic nuclei. Recent molecular studies have identified several recurrent genetic alterations—namely, translocations involving *DUSP22* and *IRF4* at 6p25, and rare rearrangements of *TP63*, which define prognostically and phenotypically distinct groups. ALK$^+$

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ALCL occurs most often in children and young adults and expresses the ALK protein because of translocations of the *ALK* gene.

In most cases, both types of ALCLs are readily distinguished from CHL, especially when either diffuse sheets of neoplastic cells or a sinusoidal pattern of lymph node involvement is present. However, some cases of CHL, especially the so-called syncytial variant of NS CHL, may resemble ALCL, including the presence of cells with reniform or horseshoe-shaped nuclei resembling hallmark cells, which even led to the introduction of the now obsolete provisional entity "ALCL-Hodgkin–like" in the REAL classification. The distinction between these two lymphomas is of utmost importance, because ALCL is treated like an aggressive lymphoma but can be cured with third-generation therapeutic regimens, whereas HL requires a different type of treatment. Some cases of ALCL, both ALK^+ and ALK^-, show similarities with NS CHL, including fibrotic bands and nodule formation (Hodgkin-like pattern). Although the inflammatory cell component is usually less pronounced than in NS CHL, eosinophils, small lymphocytes, histiocytes, plasma cells, and neutrophils may accompany the large cells in ALCL, and these cases can be resolved only with immunophenotyping (see Table 11.4). In addition to ALK protein, T-cell markers, CD15, CD45, PAX-5, EMA, and EBER in situ hybridization are useful for this differential diagnosis. CD2, CD4, CD5, and CD43 are the T-cell markers most often expressed by ALCL cells, although a minority of cases may not express any T-cell markers (null cell ALCL), but these usually belong to the ALK^+ category. PAX-5 is almost always negative in ALCL, although rare positive cases have been described. Clusterin and EMA, which are positive in almost all ALCL and, with rare exceptions, negative in H/RS cells, would favor ALCL, whereas EBV association documented by LMP1, and/or EBER positivity, would be consistent with CHL. The presence of a clonal T-cell receptor gene rearrangement on whole tissue sections is consistent with a diagnosis of ALCL, even for cases that lack expression of T-cell antigens.

Other Types of Peripheral T-Cell Lymphomas

Peripheral T-cell lymphomas (PTCLs) may closely resemble MC CHL and sometimes require molecular studies to demonstrate a clonal T-cell rearrangement in order to avoid misdiagnosis as CHL. Many PTCLs show a polymorphic population of small lymphocytes, eosinophils, histiocytes, and plasma cells resembling the inflammatory background of CHL and frequently contain H/RS-like cells, which either belong to the neoplastic clone and then express T-cell markers or represent EBV-infected transformed B cells. A diagnosis of PTCL can be established with reasonable certainty when most large cells, including H/RS-like cells, stain with T-cell markers (extremely rare in HL) and are also $CD15^-$ and $CD30^{-/+}$. However, classical H/RS cells in CHL may be $CD15^-$, and RS-like cells in PTCL may rarely be $CD15^+$. The expression of T-cell markers in H/RS-like cells may be impossible to determine due to the surrounding small T cells. In such cases, features favoring a diagnosis of PTCL include a higher proliferative rate in the small to medium-sized cells and loss of or significant decrease in the expression of one of the pan T-cell markers and coexpression or double negativity of both CD4 and CD8. This loss or decrease in expression of a T-cell marker is best quantitated with flow cytometry, but it can also be observed with immunostaining. Such aberrant expression of T-cell markers does not, however, indicate clonality, and it is not seen in all peripheral T-cell lymphomas (approximately 75% of cases). The lymphoepithelioid variant of PTCL, or Lennert's lymphoma, characterized by a marked, diffuse proliferation of epithelioid histiocytes may contain H/RS-like cells and therefore imitate the histiocyte-rich variant of MC HL. EBV^+ H/RS-like cells, which frequently coexpress CD30 and CD20, but sometimes may also show CD15 positivity, are especially common in angioimmunoblastic T-cell lymphoma and other PTCLs with T- follicular helper (TFH) cells phenotype. Morphologic features that favor PTCL include irregular nuclear contours of small lymphocytes, cells with clear cytoplasm, and a spectrum of lymphocyte sizes ranging from small to medium to large. Expression of T_FH markers PD-1 (CD279) and CXCL13, CD10, and/or expression of BCL6 in the T-cell population is also useful. Nevertheless, some of these cases are difficult to separate from CHL, even with the use of molecular studies, and sometimes only the disease recurrence reveals the presence of a PTCL.

The clinician must be made aware of this diagnostic dilemma and use his or her clinical judgment in choosing therapy. Peripheral T-cell lymphomas are one of the most aggressive NHLs and must be differentiated from MC CHL, because prognosis and therapy for these two disorders are completely different.

OTHER NON-HODGKIN LYMPHOMAS WITH H/RS-LIKE CELLS AND COMPOSITE LYMPHOMAS

As mentioned earlier, H/RS-like cells can occur in a broad range of NHL. Among the indolent B-NHLs, this can be observed most commonly in chronic lymphocytic leukemia (CLL), and a subset of these patients may develop true CHL. These cases have been considered by some investigators to be a type of Richter's transformation (originally described as a transformation of CLL to a large-cell lymphoma), but it is important to separate this phenomenon from conventional Richter's transformation for treatment purposes. Two different histologic patterns have been reported in CLL. In the first pattern, H/RS cells and variant cells are scattered in a background of typical CLL without the cellular milieu of CHL. The H/RS-like cells, even though they are ringed by T cells, reside in a B-cell background and usually express CD30, occasionally CD15, are variably $CD20^+$, and are commonly infected by EBV. The background B cells have the typical immunophenotype of CLL ($CD20/CD79a^+$ with coexpression of CD5, CD43, and cyclin D1 negative). Such cases should not be diagnosed as

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CHL or Richter's transformation, since this phenomenon does not seem to carry a worse prognosis. More rarely, segregated areas of CHL with the appropriate background and CLL can be observed, thus representing true composite lymphoma. This second pattern, which has been diagnosed as CHL in the setting of CLL or CHL variant of Richter's transformation, may be present within a single node or involve several nodes. The cellular environment in which the H/RS cells ($CD15^+$, $CD30^+$, $CD45^-$) are seen consists of T lymphocytes, eosinophils, plasma cells, and other inflammatory cells in varying proportions. A clonal relationship between the CLL cells and microdissected RS cells (by PCR amplification) has been demonstrated in some, but not all, cases, indicating that classical H/RS cells may be derived from transformed clonal B lymphocytes. In contrast to classical Richter's transformation of DLBCL type, CHL in the setting of CLL arises more commonly in cases with mutated IG genes. EBV positivity of H/RS cells is found in a fraction of cases and appears to be predominantly, but not exclusively, present in clonally unrelated cases.

Because some of the EBV^+ cases have been observed under fludarabine treatment, which can result in severe T-cell lymphocytopenia, it appears that treatment can aggravate the underlying immunodeficiency of CLL and increases the risk of development of an unrelated EBV-associated lymphoma. Clinically, patients with the CHL variant of Richter's transformation often have B symptoms and enlarged lymph nodes that are resistant to CLL types of treatments. Due to the rarity of this type of transformation, no reliable prognostic data are available, but it appears that CHL types of Richter's transformations show a more favorable outcome as compared to classical DLBCL-type transformations.

Transformed B cells with H/RS-like morphology and phenotype, as well as true composite lymphomas with CHL, have also been observed in other B-NHL subtypes.

NON-LYMPHOID NEOPLASMS: METASTATIC CARCINOMAS, MELANOMA, PRIMARY MEDIASTINAL SEMINOMA

Especially in small biopsies, a broad range of nonlymphoid neoplasms may mimic CHL. Undifferentiated nasopharyngeal carcinoma may closely resemble syncytial NS CHL. This neoplasm occurs in young patients, especially males, and often metastasizes to cervical lymph nodes. The metastasis is often the first clinical manifestation, because the primary tumor is usually small and occult. The neoplastic cells may be in small clusters or individually dispersed and may closely resemble H/RS/lacunar-type cells. Sclerosis and a vaguely nodular pattern may accompany the nodal deposits, and eosinophils are commonly found. This tumor is readily differentiated from CHL by staining for cytokeratin, and the malignant cells are also positive for EMA and EBER by in situ hybridization, but they are negative for CD15 and CD30.

Other undifferentiated carcinomas and metastatic melanomas can easily be differentiated from syncytial NS CHL by appropriate immunohistochemical stains. Extragonadal germ cell tumors, especially primary mediastinal seminoma, can also resemble NS CHL. Periodic acid–Schiff reagent, placental alkaline phosphatase, and CD117 are positive in seminoma cells.

Of note, both CD15 and CD30 are expressed by a wide range of non-hematopoietic neoplasms and therefore should not be taken as some evidence for diagnosis of CHL, if the appropriate morphologic findings are lacking.

REACTIVE DISORDERS

Granulomatous Lymphadenitis

Rare cases of tumor cell–rich NS CHL in which there are central areas of suppurative necrosis within sheets of lacunar cells may be misinterpreted as cat-scratch disease or another type of necrotizing granulomatous lymphadenitis, or vice versa. Although lacunar cells are usually abundant and easily recognizable, there are occasional cases in which the cells around the necrosis are predominantly epithelioid histiocytes (see Fig. 11.4). However, careful examination will usually show an occasional cell with a prominent nucleolus not seen in histiocytes. This observation should prompt staining with CD15 and CD30 and other appropriate markers.

Other Reactive Disorders

Drug-induced hypersensitivity reactions, especially to diphenylhydantoin, often subtotally efface the nodal architecture and may provoke a mixed inflammatory infiltrate identical to that in MC CHL, including eosinophils and immunoblasts, with interspersed H/RS-like cells. A vascular proliferation (high endothelial venules) is often present in drug-induced hypersensitivity reactions and is usually not pronounced in MC CHL. The H/RS-like cells are $CD15^-$ but frequently express the activation antigen CD30 at varying intensity and are often mixtures of B and T lymphocytes. A clinical history is of the utmost importance in confirming the diagnosis. Lymphadenopathy generally recedes after cessation of the antiepileptic medication.

The great imitator of CHL, infectious mononucleosis, also needs to be considered in the differential diagnosis. In tonsils, which are frequently removed in infectious mononucleosis, great caution should be exercised in making a diagnosis of CHL. Involvement of Waldeyer's ring by CHL is uncommon, and infectious mononucleosis should be strongly suspected, especially in a young individual. A clinical history, tests for EBV-specific antibodies (viral capsid antigen [VCA]-specific IgM), and examination of a peripheral blood smear are essential in confirming a diagnosis of infectious mononucleosis. The histologic features of infectious mononucleosis depend on the time during the illness that the biopsy is performed. Both in tonsils and lymph nodes, follicular hyperplasia may be present, and there is, at best, subtotal effacement of the nodal architecture by a paracortical proliferation of immunoblasts, resulting in a characteristic mottled appearance.

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Necrosis is frequently present. Some of the immunoblasts are binucleate and morphologically indistinguishable from classical RS cells, and mononuclear immunoblasts may mimic Hodgkin cells. The background cells are different from those seen in CHL and consist of varying proportions of small lymphocytes, plasmacytoid lymphocytes, immunoblasts, plasmablasts, and mature plasma cells. In lymph nodes, the sinuses frequently are distended with monocytoid B cells and some immunoblasts. The H/RS impostor cells express CD30 at varying intensity, but not CD15. Both EBER and LMP1 stains will label cells with a broad size range, and many cells are positive for EBNA-2, in contrast to EBV^+ CHL.

NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA

NLPHL is defined as a monoclonal B-cell neoplasm characterized histologically by a nodular or, less commonly, a nodular and diffuse proliferation consisting of a predominance of small lymphocytes admixed with varying numbers of histiocytes and scattered large neoplastic cells known as LP cells (formerly known as L&H [lymphocytic and histiocytic] variants of H/RS cells). Whether a purely diffuse form of LP HL exists is a matter of controversy, although a diffuse form is not accepted as a definite subtype in the 2008 WHO classification and the 2016 update.

Clinical Findings

The clinical features of NLPHL are well known and are distinct from those of CHL. NLPHL accounts for approximately 5% of HL in Western countries. It occurs in all age groups, with a peak in the fourth decade, and shows a male preponderance of 2.4 : 1. Patients with autoimmune lymphoproliferative syndrome (ALPS), which is caused by germline mutations in apoptosis-associated genes such as FAS and FAS ligand (FASL), show a significantly increased risk for the development of HL, especially NLPHL. Patients with NLPHL usually show isolated lymphadenopathy, most often in the cervical, axillary, or inguinal region (stage I or II) of frequently long duration. In contrast to CHL, nodal spread is not contiguous. NLPHL occasionally involves mesenteric lymph nodes and Waldeyer's ring. Mediastinal involvement is uncommon (7%). Advanced stage is infrequent, occurring in 20% of patients, and bone marrow involvement is rare, occurring in only 2% to 3% of patients, and is associated with a more aggressive course. B symptoms are observed in only 10% of patients.

Prognosis and Therapy

As stated earlier, the prognosis of all types of HLs depends on the stage of the disease. NLPHL is the type of HL most often found in the low stage (I/II); therefore it is associated with the most favorable prognosis. Rarely, patients have advanced disease (stage III or IV). The variant histologic patterns of NLPHL as described by Fan et al. (see below) have been found to be associated with a higher frequency of advanced stage and an increased relapse rate. The course of the low-stage disease is indolent, despite multiple relapses often following prolonged disease-free intervals. Such relapses usually respond well to treatment and are not associated with an adverse prognosis. In contrast to patients with CHL, the relapses often occur after a long interval (10 to 13 years); therefore continued surveillance for late relapses is indicated. As stated earlier, it should be noted that the number of relapses may be overestimated when based on clinical and imaging studies alone, because patients with NLPHL frequently develop lymphadenopathy from follicular hyperplasia with progressive transformation of germinal centers (PTGC); however, no morphologic evidence of HL is detected on microscopic examination. Another reason for a repeated biopsy of enlarged lymph nodes in NLPHL is that transformation to large-cell lymphoma may occur, often many years after the diagnosis of NLPHL.

Currently, therapy consists of involved-field radiotherapy or regional radiotherapy for patients with limited-stage disease and combined modality therapy or chemotherapy for those with advanced-stage disease. A watch-and-wait approach in both adult and pediatric patients has been suggested in this indolent type of HL, because most deaths in patients with NLPHL appear to be treatment related because of coronary artery disease or a second unrelated malignancy. In a 2010 report of 164 patients with NLPHL, the 10-year overall survival for patients who received specific therapy and those followed using the watch-and-wait strategy was 93% and 91%, respectively. In addition, immunotherapy with anti-CD20 in adults has been attempted with some success; therefore it is of utmost importance to make certain that the correct diagnosis of NLPHL is established before initiating immunotherapy.

Pathologic Features

Lymphocyte-Predominant (LP) Cells

LP (lymphocyte-predominant) cells are the neoplastic cell type of NLPHL. Because of its resemblance to an exploded kernel of corn, it is also referred to as a popcorn cell. LP or popcorn cells of NLPHL are large, centroblast-like cells with complex lobulations of their nuclei. The nuclei contain peripherally placed basophilic nucleoli that are smaller than those of classical RS cells (see Fig. 11.8). The cells have a thin rim of cytoplasm. In some cases of NLPHL, occasional LP cells may have more prominent nucleoli and thus resemble classical H/RS cells. On the other hand, cases of lymphocyte-rich CHL may contain cells that morphologically are indistinguishable from LP cells. In these cases, the immunophenotype of the neoplastic cells is paramount for

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NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA—FACT SHEET

Definition
- A monoclonal B-cell neoplasm characterized histologically by a nodular or nodular and diffuse proliferation of small, benign lymphocytes and varying numbers of histiocytes admixed with scattered LP cells

Incidence
- Approximately 5% of all cases of Hodgkin lymphoma

Gender and Age Distribution
- Male predominance (70%)
- Most often seen in 30- to 50-year age group
- Age ranges from children to older adults

Clinical Features
- Indolent disease
- Localized peripheral lymph node enlargement (stage 1 or 2); cervical, axillary, or inguinal nodes most often involved
- B symptoms uncommon
- Multiple relapses, including late relapses after prolonged disease-free intervals
- Advanced stage disease uncommon but more aggressive

Prognosis and Therapy
- Low-stage (I/II) associated with excellent prognosis (10-year survival >80%)
- Relapses respond well to therapy
- Rarely fatal (patients die of unrelated disease: cardiac disease or unrelated malignancy)
- Standard treatment of low-stage disease is involved field radiotherapy or regional radiotherapy
- Treatment of stage I: surgical excision with watch-and-wait approach, or local radiation and/or anti-CD20 treatment; advanced stage, chemotherapy with or without radiotherapy
- Progression to large-cell lymphomas in 3% to 5% of cases; if localized may be associated with good prognosis

establishing the diagnosis. The morphologic, immunophenotypic, and genetic features of LP cells identify them as atypical centroblasts of germinal centers. The probable relationship to progressive transformation of germinal centers (PTGCs; vide infra) suggests that NLPHL is a neoplasm of an abnormal germinal-center reaction.

Microscopic Features

A diagnosis of NLPHL can often be strongly suspected on a low-magnification examination of the lymph node. The nodal architecture, in most instances, is totally effaced by large nodules or occasionally by nodules and diffuse areas. The nodules are often closely apposed and may be molded against one another, or in other instances there may be considerable space between them (see Fig. 11.8). Occasionally, uninvolved parts of the lymph node remain as a rim of compressed tissue at the periphery of the node, usually in a subcapsular location. This uninvolved tissue may show normal or even hyperplastic follicles or, occasionally, progressively transformed germinal centers. Most often, the nodules are distinct and readily identifiable under low magnification, but occasionally they are not well circumscribed and difficult to identify on hematoxylin and eosin (H&E)-stained sections; therefore immunostaining with CD20 may be needed to highlight them. Rarely, diffuse areas may predominate; however, only a single nodule is required to establish a diagnosis of NLPHL. At low power, nodules often show a moth-eaten appearance due to interspersed LP cells and histiocytes. In most cases, LP cells are found within the nodules; however, they may be present in the periphery or outside the nodules in the internodular T-cell areas. The abundance of LP cells in NLPHL varies widely, and in some cases the identification of LP cells and thus the diagnostic separation from progressive transformation of germinal centers (see below) is difficult and may require step sectioning and pertinent immunostaining. When popcorn cells form confluent clusters (a rare occurrence), transition to large-cell lymphoma, which occurs rarely in association with NLPHL, must be considered. Some clusters of LP cells may have the appearance of lacunar cells but have the immunophenotype of LP cells.

In addition to the rare LP cells, the nodules are composed predominantly of small lymphocytes. In addition, they may contain epithelioid histiocytes, sometimes arranged in a ring-like fashion around B-cell nodules. Small epithelioid granulomas may also be present. These granulomas can at times be so prominent that the NLPHL is missed and an erroneous diagnosis of granulomatous lymphadenitis is made. FDCs, usually in the vicinity of the popcorn cells, are a constant feature. FDCs frequently show two nuclei with open chromatin and delicate cell membrane, approximately the size of centroblast nuclei, flattened against each other (so-called kissing nuclei), with single, small, inconspicuous nucleoli.

In addition to the classical pattern of NLPHL with B-cell–rich nodules, other histologic growth patterns may occur. Fan and coworkers have described 6 architectural patterns of NLPHL: (1) classical B-cell–rich nodular with mostly intranodular LP cells, (2) serpiginous/interconnected nodular, (3) nodular with prominent extranodular LP cells, (4) T-cell–rich nodular, (5) diffuse with a T-cell–rich background (T-cell–rich B-cell lymphoma–like), and (6) diffuse moth-eaten B-cell rich.

The presence of variant patterns should be described, since previous work including a recent study of more than 400 cases by Hartmann et al. has shown that they are associated with more advanced disease and a higher relapse rate than classical pattern 1. Concerning the difficult separation between diffuse NLPHL and the T-cell histiocyte–rich large B-cell lymphoma (TC/HRLBCL), see the section on differential diagnosis. Rare cases of NLPHL may show clusters of LP cells, interlobular fibrosis, and an increase in neutrophils reminiscent of NS CHL and have been designated syncytial variant of NLPHL.

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NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA—PATHOLOGIC FEATURES

Microscopic Findings

- Architecture of lymph node effaced by macronodules composed predominantly of small lymphocytes or macronodules with diffuse areas
- Scattered LP cells (popcorn cells) and varying numbers of histiocytes within or surrounding nodules
- Classical H/RS cells absent
- Eosinophils and plasma cells absent or rare
- Necrosis and fibrosis absent
- Benign follicles usually absent between macronodules
- If uninvolved portion of node present, it is confined to periphery, usually as compressed rim in subcapsular region

Immunohistochemical Findings

- LP cells: $CD20^+$, $CD79a^+$, $CD45^+$, $EMA^{+/-}$, $BCL6^+$, $CD15^-$, $CD30^-$, fascin negative
- NLPHL with IgD^+ LP cells shows strong male predominance, more frequent involvement of cervical lymph nodes and more frequent variant histology
- Small lymphocytes within nodules: $CD20^+$, $CD23^+$, $CD79a^+$, IgD^+, IgM^+
- Increased $CD3^+$, $CD57^+$, and $PD\text{-}1^+$ lymphocytes with frequent rosette formation around LP cells
- Expanded $CD21^+/CD35^+$ spherical follicular dendritic meshwork within nodules
- Absence of EBV infection in LP cells

Differential Diagnosis

- Progressive transformation of germinal centers
- Nodular lymphocyte-rich classical HL
- T-cell/histiocyte–rich large B-cell lymphoma
- Follicular lymphoma

Genetics

- LP cells are B cells of germinal-center cell origin
- LP cells have clonally rearranged immunoglobulin (IG) genes
- Clonal rearrangements are usually detectable only in DNA isolated from single LP cells
- Variable region of IG heavy chain genes carries a high load of somatic mutations and show ongoing mutations
- Rearrangements are functional, and IG mRNA transcripts are present in LP cells

Ancillary Studies

Immunophenotype of LP Cells

In contrast to the immunophenotype of RS cells in CHL, LP cells in NLPHL express a completely different set of markers (see Table 11.4). These cells are $CD15^-$, $CD30^-$, and fascin negative, but they consistently express B-cell markers CD20, CD79a (sometimes weaker), CD22, strong PAX-5, and CD45. In addition, LP cells express BCL-6, attesting to their germinal-center (GC) origin, and EMA in approximately 25% to 50% of cases (depending on the clone of the antibody) in a membranous or Golgi pattern, and they also synthesize J chains (a feature of B cells only). LP cells are usually strongly and homogeneously positive for the B-cell transcription factors OCT-2 and its cofactor BOB.1, and variably positive for PU.1. In contrast, H/RS cells of CHL express these markers weakly or lack them altogether. Other markers of GC B cells such as activation-induced cytidine deaminase (AID), human GC-associated lymphoma protein (HGAL), or GC B-cell–expressed transcript 1 (GCET1) are also positive but are not relevant for diagnostic purposes. BCL2 is negative in NLPHL. A subset of cases ranging from 16% to 27% express IgD. IgD expression is associated with strong male predominance, more common involvement of cervical lymph nodes, and dominance of variant histologic patterns, as described earlier (see Fig. 11.8). Expression of IgD can help in the differentiation from TC/HRLBCL.

CD30, CD15, and fascin are negative in most cases of NLPHL, but CD30 frequently stains interspersed immunoblasts not belonging to the malignant clone. However, rare cases may show variable expression of CD30 or CD15 in the neoplastic cells. Immunohistochemical demonstration of a preserved B-cell program is critical for the correct diagnosis of these NLPHL cases. In addition, rare cases of bona fide EBV^+ NLPHL have been described.

The small lymphocytes within the nodules in NLPHL also stain with B-cell markers CD20 and CD79a, whereas the internodular areas are composed predominantly of small $CD3^+$ paracortical T cells (Fig. 11.9). In some of the variant patterns and relapsed cases, the number of T cells within nodules may increase, and occasionally the T cells outnumber the small B cells in some or all of the nodules. CD20 expression on the popcorn cells may be more intense than on the small lymphocytes within the nodule (see Fig. 11.9). The cells immediately surrounding the popcorn cells are $CD20^-$, $CD3^+$ T cells, making it easy to identify LP cells on low magnification (see Fig. 11.9). Therefore, on low magnification of a CD20-stained section, the nodules appear to have little holes of negative areas represented by a corona of T cells around LP cells (see Fig. 11.9). The rosetting T cells express the follicular T-helper-cell markers PD-1 (CD279) and CD57. In addition, small clusters of T cells are found throughout the nodules. It is the presence of these small T-cell clusters that is responsible for the irregular borders or the moth-eaten appearance of the nodules in CD20-stained sections (see Fig. 11.9). This moth-eaten appearance is helpful in the differential diagnosis of NLPHL from progressive transformation of germinal centers, in which T cells are not found in clusters and the contours of nodules are not irregular but rather sharply circumscribed (see Fig. 11.9). CD21/CD35 demonstrates large, expansile, spherical FDC meshworks within the nodules (see Fig. 11.9). These meshworks are absent from diffuse areas. In diffuse areas, the background population consists of $CD4^+$ T cells with frequent CD57 expression. In the absence of $CD57^+$ T cells, a diagnosis of TC/HRLBCL should be considered. Flow cytometry is usually not helpful in the diagnosis of CHL, but in NLPHL, a population of dual $CD4^+/CD8^+$ T cells can sometimes be found and can be a clue to the correct diagnosis when present.

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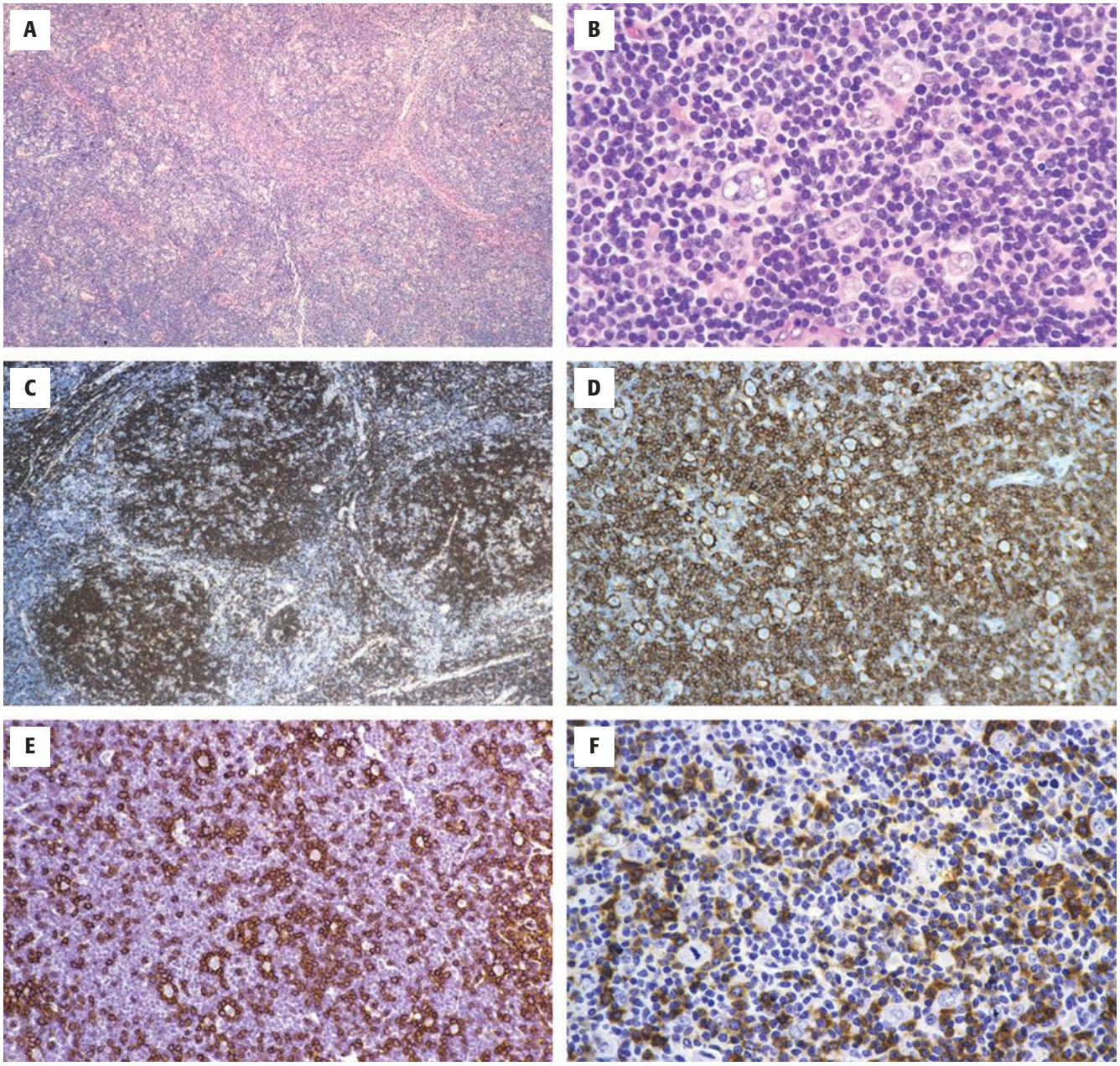


FIG. 11.9

Nodular lymphocyte-predominant Hodgkin lymphoma. A, At low magnification, the lymph node architecture is largely effaced by apposed macronodules (hematoxylin and eosin). B, High magnification of lymphocyte-predominant (LP) variants of RS cells within one of the nodules. C, CD20 stain outlines the large nodules and shows their irregular, moth-eaten borders and the small holes produced by the $CD20^-$ staining T cells (low magnification). D, CD20 outlines the membrane of the larger LP cells, which are stained more intensely than the surrounding B cells of the nodules (high magnification). E, High magnification showing $CD3^+$ rosettes around LP cells. F, $CD57^+$ lymphocytes are randomly scattered within the nodules and occasionally form rosettes around LP cells (high magnification).

Continued

Genetic Features of Nodular Lymphocyte-Predominant Hodgkin Lymphoma

Even before the advent of single-cell microdissection, it was suggested that NLPHL is a GC B-cell neoplasm, and LP cells are regarded as atypical centroblasts. This concept is in keeping with their immunophenotype with expression of pan–B-cell markers, J-chain, the GC marker BCL6, and the B-cell transcription factors OCT-2 and BOB.1. LP cells contain rearranged immunoglobulin genes with high loads of somatic mutations and signs of ongoing hypermutation, as evidence of their GC origin. These transcripts are productive, and IG transcripts can be detected on the mRNA level, although the detection of IG light chains is not relevant for diagnosis. Thus the main difference between H/RS cells of CHL and LP cells is the preservation of the B-cell expression program, a hallmark feature of NLPHL, which should be kept in mind for diagnostic decisions in difficult cases showing

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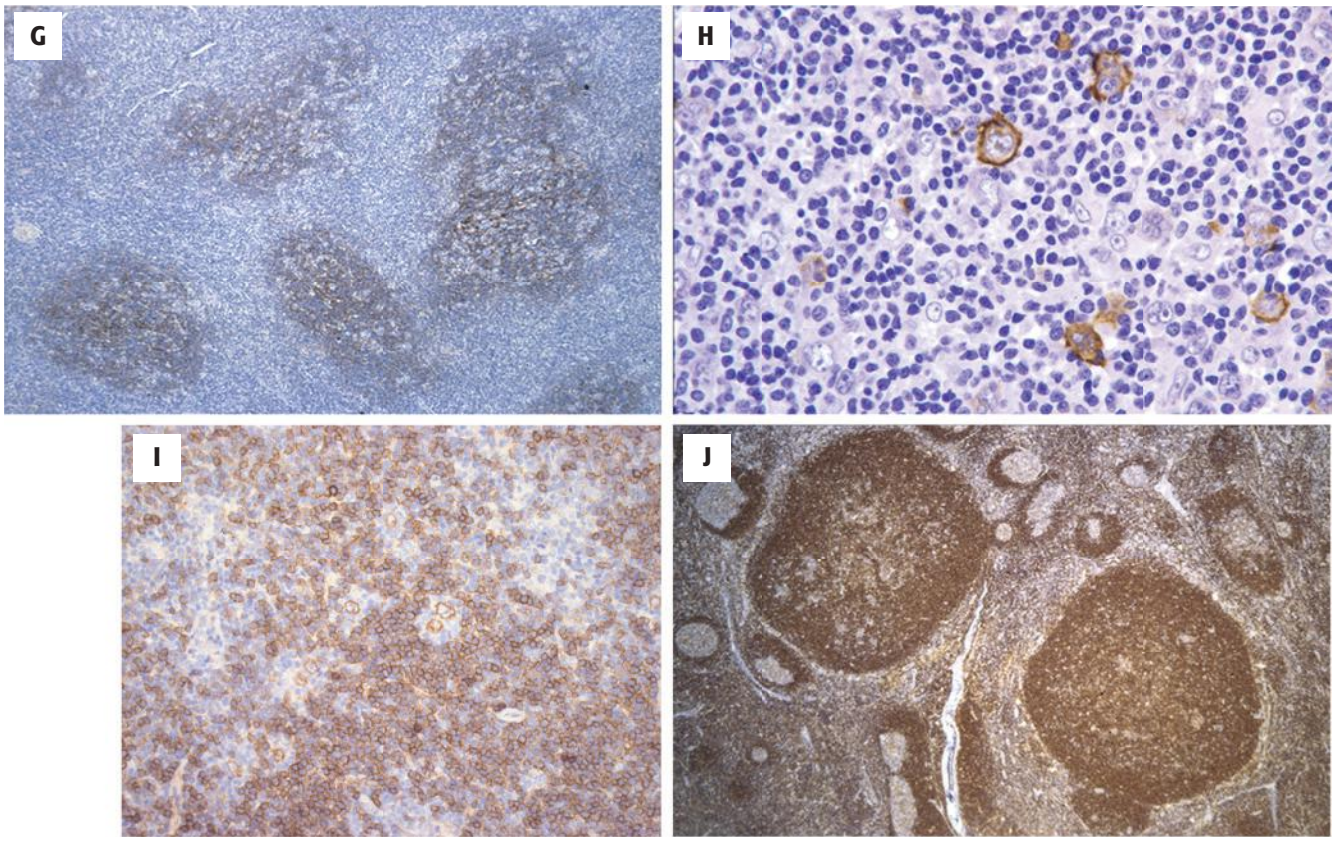


FIG. 11.9, cont'd

G, CD21 shows an expanded follicular dendritic meshwork within nodules. H, EMA-positive LP cells (low magnification). I, IgD staining highlights LP cells in a case of IgD^+ NLPHL and also stains the surrounding mantle zone B cells. IgD^+ LP cells are more common in cases of variant morphology. J, Progressive transformation of germinal centers. Low magnification shows two $CD79a^+$ macronodules composed of small lymphocytes adjacent to reactive germinal centers (low magnification). Germinal centers are not seen among the macronodules of nodular lymphocyte-predominant Hodgkin lymphoma.

an overlap with CHL. Due to the rarity of LP cells, data on genetic aberrations are sparse. LP cells frequently contain breaks of the *BCL6* gene, indicative of *BCL6* translocations, whereas *BCL2* translocations are absent. Recent studies based on microdissected LP cells have demonstrated recurrent genetic alterations, including mutations in *SGK1*, *DUSP2*, and *JUNB,* as well as molecular similarities with TC/HRLBCL. As for CHL, molecular features do not play a role in diagnosing NLPHL.

TRANSFORMATION OF NLPHL TO LARGE-CELL LYMPHOMA

Approximately 3% to 5% of patients with NLPHL develop a large B-cell lymphoma, occurring with, after, or, rarely, prior to development of NLPHL. The large-cell lymphoma can appear in the same lymph node as the HL or in a different node, one unaffected by NLPHL. The presentation of the cells in the large-cell lymphoma is variable, resembling the lobulated appearance of the LP cells or, less frequently, having features of centroblasts or immunoblasts or occasionally exhibiting anaplastic morphology. The neoplastic large cells express B-cell markers CD20 and CD79a and show light chain restriction. When large-cell lymphoma is found in the same node as the NLPHL, the demarcation between the two is usually sharp, although in some cases it is difficult or impossible to clearly separate the HL with clusters of LP cells from the large-cell lymphoma. In fact, sometimes the differentiation between NLPHL with clusters of LP cells and true large-cell lymphoma is not clear. As a rule, diffuse large-cell lymphoma should not be diagnosed unless areas of the node are effaced by a monotonous proliferation of large cells. A clonal relationship between the neoplastic cells in both lymphomas has been demonstrated in most cases, but the large-cell lymphoma may also represent a de novo malignancy.

Differential Diagnosis

The differential diagnosis of NLPHL includes benign lymphoid proliferations as well as CHL and NHLs. Among these disorders, PTGCs, nodular LR CHL, and TC/HRLBCL most commonly present diagnostic difficulties. TC/HRLBCL

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probably shows some biological overlap with NLPHL and may be impossible to discern from cases of NLPHL with variant histology, especially in needle biopsies.

Nodular Lymphocyte-Predominant Hodgkin Lymphoma Versus Progressive Transformation of Germinal Centers

Progressive transformation of germinal centers (PTGC) is a benign disorder of unknown etiology associated with follicular hyperplasia. It occurs predominantly in children and young males, but no age is exempt. Like NLPHL, PTGC tends to recur, especially in the same site. A histogenetic relationship between PTGC and NLPHL has been suggested, and the two conditions may coexist. PTGC may occur before, with, or after a diagnosis of NLPHL. Although individuals with PTGC rarely develop NLPHL, a repeated biopsy is warranted to exclude this possibility if lymphadenopathy recurs. Conversely, biopsy must be repeated in patients with NLPHL when lymphadenopathy recurs to determine which of the two disorders is present. In many instances, low-magnification examination of the lymph node can already resolve the differential diagnosis between PTGC and NLPHL (see Fig. 11.8). The presence of one or more, or even many (florid PTGC) large, expansile nodules randomly distributed in a background of hyperplastic follicles is consistent with the diagnosis of PTGC. In contrast, NLPHL is characterized by the presence of numerous nodules, often back-to-back with no interspersed germinal centers. Cases with partial involvement by NLPHL give the distinct impression of a space-occupying or "node within a node" lesion, with residual follicles appearing in a compressed rim of normal lymphoid tissue, usually in a subcapsular location at the periphery of the node.

The composition of the PTGC nodules depends on the stage of transformation. Fully transformed germinal centers are threefold to fourfold the size of reactive follicles and are composed of small polyclonal lymphocytes of mantle cell origin (IgM^+, IgD^+) with rare remaining centroblasts and some residual follicular dendritic cells. Earlier during the transformation, varying numbers of follicle center cells remain, sometimes together with some tingible body macrophages. It appears that the large nodules are formed by a proliferation of mantle cells in an outward and an inward direction, gradually replacing the germinal center and thus resulting in the formation of the large macronodules. LP cells are absent, by definition, although remaining centroblasts and FDCs (often binucleate cells) can mimic them, requiring close correlation of histologic and immunohistochemical features. In contrast to LP cells, FDCs do not have lobulated nuclei, and the cell membranes are flattened where they abut one another.

Immunohistochemistry also aids in the differential diagnosis, although the background immuno-architecture of the two conditions is similar. In addition to the vast predominance of $CD20^+$, $CD79a^+$ small B cells, in both conditions varying numbers of T cells and $CD57^+$ cells are also present; however, the distribution differs. In PTGC, as in normal germinal centers, the T cells are scattered singly and not in clusters, as is seen in NLPHL, and only rarely T-cell rosettes around remaining centroblasts can be observed. In addition, the prominent PD-1^+ or $CD57^+$ rosettes around LP cells found in NLPHL are absent in PTGC, although numerous follicular T-helper cells are randomly scattered within the nodules of PTGC. As mentioned previously, CD20 staining in PTGC shows a smooth perimeter of nodules in contrast to the frequently irregular, moth-eaten appearance seen in nodules of NLPHL (see Fig. 11.8). The most striking feature of the CD20 stain in NLPHL, large $CD20^+$ LP cells surrounded by unstained coronae of T cells, is absent from PTGC. However, some cases of NLPHL show only rare LP cells within the nodules, and they are encountered predominantly at the periphery of the nodules in areas dominated by T cells. Follicular dendritic meshworks are seen in both conditions. EMA may also be helpful. It may be expressed on LP cells but is not positive on residual centroblasts or follicular dendritic cells of PTGC.

Lymphocyte-Rich Classical Hodgkin Lymphoma Versus Nodular Lymphocyte-Predominant Hodgkin Lymphoma

As mentioned earlier, there are two histologic forms of LR CHL, the more common nodular type and the diffuse type. The nodular type may mimic NLPHL because both have a nodular growth pattern composed of small B-lymphocytes (see Fig. 11.6). In a review of cases of NLPHL performed by the European Task Force on Lymphoma, approximately 30% of cases initially diagnosed as NLPHL were reclassified as nodular LR CHL. The nodules in both types of HLs are composed of expanded mantle-cell B lymphocytes (IgM^+, IgD^+). Like in NLPHL, the nodules usually do not contain eosinophils or plasma cells, and necrosis or fibrosis is absent. Although LR CHL does primarily contain H/RS cells of the classic type, and occasional lacunar-type RS cells, variants morphologically indistinguishable from LP cells may also be present. Therefore, distinction between LR CHL and NLPHL relies on the immunophenotype of the H/RS cells regardless of their cytologic features ($CD15^{+/-}$, $CD30^+$, $CD20^{-/+}$, $LMP1^{+/-}$). These H/RS cells are present within the expanded mantle zone of nodular LR CHL. Another important feature helpful in the differential diagnosis is the presence of residual, most often atrophic, germinal centers in nodular LR CHL that are usually placed eccentrically within some of the nodules. Although epithelioid histiocytes may be present in LR CHL, they are much more common in NLPHL. As in NLPHL, the H/RS cells are prominently rosetted by T cells, as readily seen at low magnification, because they stand out within the B-cell areas. In contrast to NLPHL, PD-1–positive rosettes are infrequently found around classic H/RS cells in LR CHL. CD21 stains show dense concentric rings of the follicular dendritic meshwork in atrophic germinal centers, which extends into the expanded mantle zones in a more loosely woven meshwork, whereas in NLPHL the meshwork appears more uniform

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and considerably larger. EBV LMP-1 and EBER may be positive in the classic H/RS cells of LR CHL; however, they are, with rare exceptions, negative in the popcorn cells of NLPHL. The internodular areas in both conditions are composed of T cells.

Although immunophenotyping reliably distinguishes between NLPHL and LR CHL in most cases, rare cases may also show some immunophenotypical overlap, for example, concerning the expression of B-cell surface markers and transcription factors, pointing to the possible existence of biologic borderline cases.

T-Cell/Histiocyte–Rich Large B-Cell Lymphoma Versus Nodular Lymphocyte-Predominant Hodgkin Lymphoma

The distinction between TC/HRLBCL and NLPHL is very important, because TC/HRLBCL is an aggressive lymphoma that requires aggressive therapy; it often manifests with advanced-stage disease, sometimes involving in addition to lymph nodes, also liver, spleen, and bone marrow. However, recent molecular studies on microdissected tumor cells of the two entities have demonstrated significant overlap of genetic alterations. Currently, it cannot be determined whether NLPHL and TC/HRLBCL represent the two extreme ends of a clinicopathologic spectrum or are two unrelated diseases, or whether two types of TC/HRLBCLs exist, one related to NLPHL, commonly showing LP-type neoplastic cells, and the other a separate disease entity. Genetic studies on microdissected tumor cells have not been able to resolve this question. On one hand, genetic alterations are similar in the two entities, and on the other hand NLPHL showed a higher frequency of genomic imbalances as compared to TC/HRLBCL, making a direct progression from one to the other improbable. Because the main distinctive feature between NLPHL and TC/HRLBCL is the composition of the reactive background, the host response might be of major importance for differences in clinical behavior.

Tissue architecture is the most important feature for separation of these two entities. By definition, TC/HRLBCL shows an entirely diffuse pattern, distinctive from the nodular pattern of NLPHL. However, immunohistologic differentiation between TC/HRLBCL and NLPHL containing extensive, diffuse areas, or in smaller or needle core biopsies containing diffuse areas, may be extremely difficult or at times impossible. TC/HRLBCL is defined as a variant of diffuse large B-cell lymphoma in which there are a limited number of scattered, large, atypical B cells embedded in a background of numerous small non-neoplastic T-cell lymphocytes. Varying numbers of histiocytes (as in LPHL), some in small clusters, are often admixed, but small B cells and follicular dendritic cells should be absent. As can be appreciated from this description, the limited number of large neoplastic cells among a vast predominance of small T cells mimics the histologic features of diffuse, T-cell–rich B-cell lymphoma–like NLPHL. The large cells may be centroblasts or immunoblasts, or they may mimic classic H/RS cells or LP cells, thus suggesting the possibility of either LPHL or CHL. The neoplastic cells will have the same immunophenotype in both diffuse LPHL and TC/HRLBCL ($CD45^+$, $CD20^+$, $CD79a^+$, $EMA^{+/-}$, $CD30^{-/+}$, $CD15^-$; see Table 11.4). However, the T cells in TC/HRLBCL and diffuse areas of NLPHL differ. In TC/HRLBCL, the T cells have a cytotoxic phenotype ($CD8^+$ and $TIA\text{-}1^+$), whereas in NLPHL they are $CD4^+$. Furthermore, $PD\text{-}1^+$ cell rosettes present in NLPHL are absent in TC/HRLBCL. EMA may be positive in the large cells of both disorders, whereas CD15, CD30, and fascin positivity would be consistent with CHL. Detection of EBV in the large cells would also favor CHL because EBV is not typically found in NLPHL or TC/HRLBCL (see Table 11.4). Light chain restriction by immunostaining and clonal immunoglobulin heavy chain gene rearrangement by PCR in whole tissue DNA would favor a diagnosis of TC/HRLBCL. Nodular meshworks of $CD21^+$ FDCs, $CD57^+$ cells, and PD-1–positive cells would favor NLPHL, whereas their absence would be more consistent with TC/HRLBCL.

It is recommended in the fourth edition of the WHO classification that cases of NLPHL with a diffuse T-cell–rich pattern should be interpreted as NLPHL, TC/HRCLBCL-like to distinguish the latter from cases of primary (de novo) TC/HRLBCL. Furthermore, the presence of small B cells and $CD4/CD57^+$ T cells would favor a diagnosis of NLPHL, whereas absence of small B cells and the presence of $CD8^+$ cells and $TIA\text{-}1^+$ cells favor primary TC/HRLBCL. In addition, IgD positivity in LP cells argues against TC/HRLBCL. IgD is not expressed by the large cells in TC/HRLBCL but may be positive in LP cells in a minority of cases of NLPHL, especially cases with diffuse growth pattern.

Follicular Lymphoma Versus Nodular Lymphocyte-Predominant Hodgkin Lymphoma

The differential diagnosis of follicular lymphoma (FL) versus nodular lymphocyte predominant NLPHL is usually not difficult. The follicles in FL are ordinarily considerably smaller than the nodules of NLPHL and are composed of at least a subpopulation of centrocytes that are not found within the macronodules of NLPHL. Extension of neoplastic follicles through the capsule into surrounding tissue, although common in FL, is not seen in NLPHL. CD20 staining usually highlights LP cells surrounded by $CD20^-$ T cells, whereas large cells in FL usually do not stand out. CD3 and PD-1 do not show rosettes, although varying numbers of T cells are seen within neoplastic follicles of FL. BCL2 is expressed by the neoplastic cells of most FL, due to the t(14;18) translocation present in 90% of cases, but is absent in LP cells.

Mantle Cell Lymphoma Versus Nodular Lymphocyte-Predominant Hodgkin Lymphoma

Mantle cell lymphoma (MCL) is usually not confused with NLPHL. It is a B-cell lymphoma composed of small lymphocytes, which coexpress CD5 and CD43 and are characteristically cyclin D1-positive, due to the t(11;14)

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translocation. MCL cases with a nodular pattern may rarely resemble NLPHL. In most, but not all, instances, however, the nodules are not as large as those found in NLPHL. The small lymphocytes in MCL usually, but not always, have irregular nuclear contours in contrast to the round nuclei of small lymphocytes in NLPHL. Most importantly, large cells are absent within nodules except for occasional centroblasts remaining from germinal centers that have been overrun by the lymphoma cells. Pink-staining histiocytes (not epithelioid histiocytes) are often scattered among the nodules, and hyalinized small vessels may be present, two characteristics absent from NLPHL. Immunostaining for CD5 and cyclin D1 easily resolves this differential diagnosis.


The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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SUGGESTED READINGS

Hodgkin Lymphoma

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SECTION 3

Leukemias, Chronic Myeloproliferative Neoplasms, and Myelodysplasia

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12

B-Cell Leukemias of Mature Lymphocytes

■ **Eric D. Hsi, MD**

■ INTRODUCTION

The leukemias of mature B cells are a limited set of diseases in which blood and bone marrow are the primary sites of involvement. Although any lymphoproliferative disorder can eventually enter a leukemic phase, this chapter is limited to B-cell chronic lymphocytic leukemia (CLL), B-cell prolymphocytic leukemia (PLL), hairy cell leukemia (HCL), and HCL variant (HCLv). Burkitt lymphoma, which may present as leukemia, and splenic marginal zone lymphoma (SMZL) are dealt with in other chapters.

■ CHRONIC LYMPHOCYTIC LEUKEMIA

Chronic lymphocytic leukemia is the most common leukemia in the Western world. The age-adjusted estimated annual incidence in the United States is approximately 3.4/100,000. The median age at diagnosis is 65 years, with a 2:1 male-to-female ratio. CLL is an indolent leukemia with a disease course that may span decades. Effective therapies do exist that can induce remissions. However, relapses inevitably occur. Thus, CLL is currently considered incurable in the vast majority of cases.

CHRONIC LYMPHOCYTIC LEUKEMIA—FACT SHEET

Definition

- Mature B-cell leukemia composed of small round lymphocytes
- Small lymphocytic lymphoma represents the tissue equivalent of CLL

Incidence

- Most common leukemia in the Western world (3.4/100,000 in the United States)

Clinical Features

- Older adult (median age 65), with male predominance (2:1)
- Patients may be asymptomatic or present with cytopenias and adenopathy/organomegaly

Prognosis and Therapy

- Indolent, incurable disease with prolonged clinical course
- Treatment varies depending on patient factors and includes watchful waiting; fludarabine, cyclophosphamide, rituximab (FCR); engineered monoclonal antibodies, kinase inhibitors, and a BCL2 inhibitor

Clinical Features

Patients present with lymphocytosis (>5.0 × 10^9/L by definition) and are often asymptomatic. Others may have symptoms relating to organ involvement (splenomegaly, hepatomegaly) or lymphadenopathy. Anemia and other cytopenias are often present due to immune hemolysis related to the leukemia or simple bone marrow replacement by leukemic infiltrates. CLL may progress with increasing numbers of prolymphocytes in the blood, or patients may experience transformation to a large cell lymphoma (Richter's syndrome), heralded by a sudden change in symptoms or rapid localized lymph node enlargement. Staging systems such as that of Rai and that of Binet are used to predict prognosis in CLL patients (Table 12.1). Although these systems are useful in stratifying patients, predicting outcome in intermediate-stage patients is still difficult, and biological predictors are needed.

Pathologic Features

Morphology

The peripheral blood smear of patients with CLL is characterized by a variable lymphocytosis that may uncommonly reach over 500 × 10^9/L. The lymphocytes are typically small and round with condensed chromatin alternating with lighter areas, imparting a "soccer ball" or "cracked" chromatin pattern (Fig. 12.1). The cytoplasm is usually scanty. Variation from this typical form is acceptable within the spectrum of CLL,

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TABLE 12.1
Staging System for Chronic Lymphocytic Leukemia

Staging System	Stage	Clinical Features	Median Survival (yr)
Rai			
Low risk	0	Lymphocytosis	14.5
Intermediate risk	I	Lymphocytosis, lymphadenopathy	7.5
	II	Lymphocytosis, hepatomegaly, and/or splenomegaly	
High risk	III	Lymphocytosis, anemia	2.5
	IV	Lymphocytosis, thrombocytopenia	
Binet	A	Normal hemoglobin, normal platelet count, <3 node-bearing areas	14
	B	Normal hemoglobin, normal platelet count, ≥3 node-bearing areas	5
	C	Anemia (<10 g/dL) and/or thrombocytopenia ($<1 \times 10^9$/L)	2.5

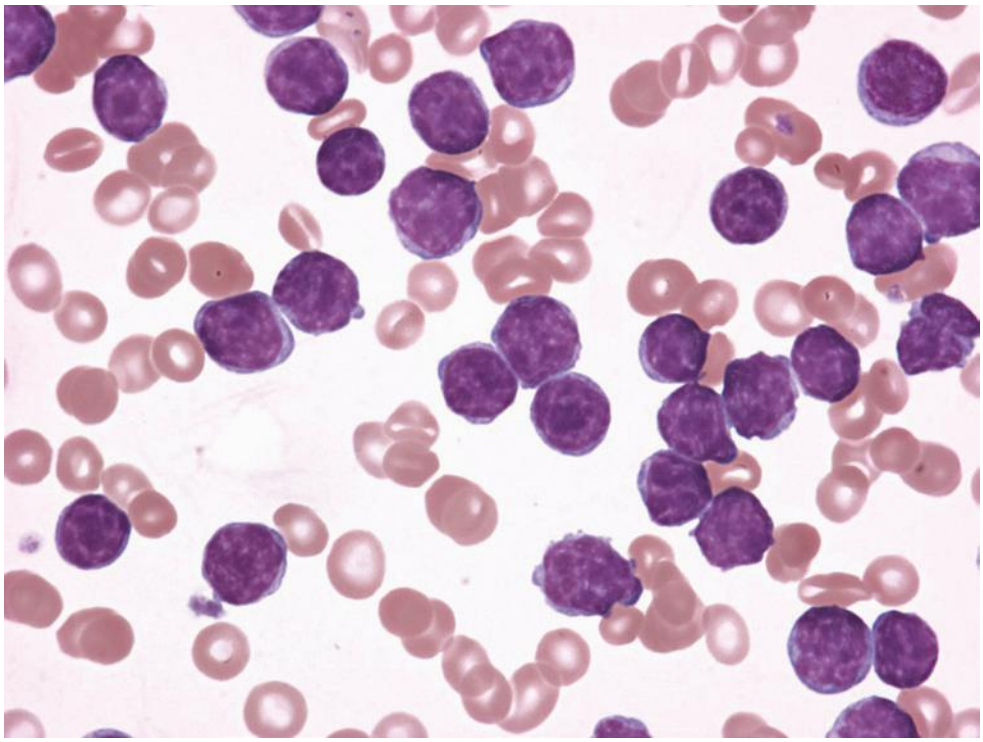


FIG. 12.1
Peripheral blood smear of a case of chronic lymphocytic leukemia (CLL) demonstrating a marked lymphocytosis. The lymphocytes are small with condensed chromatin, imparting a "soccer ball" pattern, and scant cytoplasm (Wright stain).

and some cases may have substantial numbers (>10%) of cells with nuclear irregularity and/or moderate amounts of pale blue cytoplasm (Fig. 12.2). Some studies have associated such variant morphology with worse outcome. Prolymphocytes are present in varying proportions. These cells are 1.5 to 2 times larger than the typical CLL cells with slightly open chromatin and a central nucleolus. Increased numbers of prolymphocytes ($>5 \times 10^9$/L) have been associated with a poor prognosis and certain genetic alterations such as *TP53* abnormalities and trisomy 12. Cases in which prolymphocytes constitute more than 10% but less than 55% of the lymphocytes have been termed *mixed cell type CLL* or CLL/PL in the original French-American-British classification scheme but are not distinguished from CLL in the 2016/17 updated World Health Organization classification. The bone marrow may show variable involvement. Four major patterns are recognized: nodular, interstitial, mixed, and diffuse (Fig. 12.3). The nodules tend to be nonparatrabecular. The cells are similar in appearance to those seen in lymph nodes: small and round with condensed chromatin. Proliferation centers can be seen. In the interstitial pattern, the lymphocytes infiltrate around preserved fat spaces, admixed with varying amounts of residual hematopoietic elements. The diffuse pattern, with areas totally replaced by sheets of leukemia cells, has been associated with a poor prognosis and more advanced disease.

Transformation to PLL is defined by more than 55% of lymphocytes being prolymphocytes and is usually characterized by worsening symptoms, loss of response to therapy, and poor prognosis. Richter's syndrome (transformation to a diffuse aggressive B-cell lymphoma) occurs in approximately 3% to 4% of patients, and survival is short. Unusual transformations/second malignancies such as Hodgkin lymphoma (HL), acute

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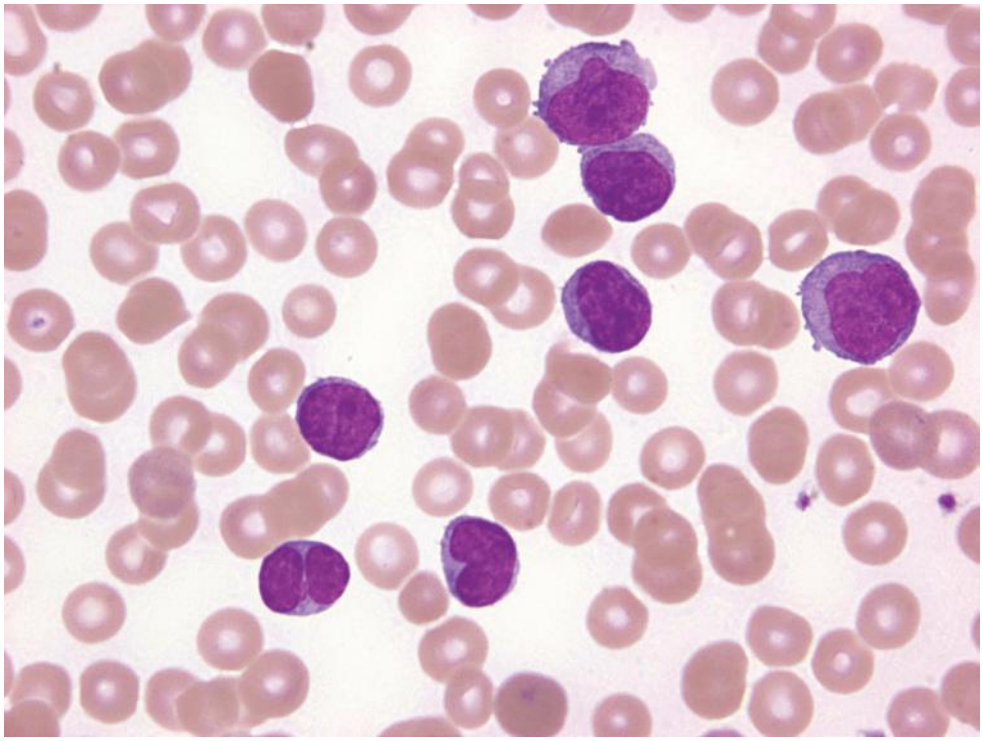


FIG. 12.2
Chronic lymphocytic leukemia (CLL) with variant morphology with irregular nuclei and occasional prolymphocyte (Wright stain). Although unusual, cells with irregular nuclear contours can be seen in CLL. This particular case had an immunophenotype typical of CLL.

lymphoblastic leukemia, and multiple myeloma have been reported. Of note, Reed-Sternberg (RS)-like cells have been shown in some cases to be positive for Epstein-Barr virus and clonally related to the CLL. HL transformation should be reserved for cases with tissue biopsy demonstrating areas with all the histologic features of HL and not used for cases with scattered RS-like cells in a background of SLL.

Immunophenotype

Flow cytometric immunophenotyping is required to confirm the diagnosis of CLL. CLL cells express CD5, CD19, CD20, CD22, CD23, CD200, and restricted surface immunoglobulin light chain. The expression of CD20 and immunoglobulin is usually dim compared to normal B cells (Fig. 12.4). In occasional cases, the immunoglobulin light chain may be so dim as to be virtually undetectable by routine flow cytometry. Heavy chain expression is usually of the IgM and IgD type. FMC7 and CD79b are absent or only dimly expressed. Deviation from this typical immunophenotype occurs, and a scoring system has been proposed to help quantify the likelihood that the diagnosis is CLL (Table 12.2). One caveat to the presence of restricted light chain expression is the existence of rare cases of biclonal CLL in which a kappa-restricted CLL clone and a lambda-restricted CLL clone are seen. Cases might seem polytypic but have the other immunophenotypic features of CLL and, on molecular analysis, show biclonal rearrangement patterns.

Expression of LEF1 is highly specific and sensitive for CLL and is useful in the differential diagnosis of small B-cell leukemias/lymphomas. A nuclear protein involved in WNT/beta-catenin signaling and expressed in normal T cells, LEF1

CHRONIC LYMPHOCYTIC LEUKEMIA—PATHOLOGIC FEATURES

Morphology
- Peripheral blood: small, mature-appearing lymphocytes with condensed chromatin and round nuclei; variable numbers of prolymphocytes (less than 55%)
- Bone marrow: interstitial, nodular, and/or diffuse involvement; proliferation centers can be seen
- Spleen: white pulp predominant or diffuse involvement

Immunophenotype
- CD5+, CD10–, CD19+, CD20+ (dim), CD23+, CD200+, sIg+ (dim), FMC7±, CD79b±, LEF1+
- CD38 and ZAP-70 expression associated with adverse outcome

Genetics
- 13q deletion as sole abnormality associated with good prognosis; del(17p) and del(11q22-23) deletion associated with poor prognosis (see text)
- Unmutated immunoglobulin heavy chain gene variable region (>98% homology to germline) associated with poor prognosis
- Mutations in *SF3B1* (20%), *ATM* (15%), *TP53* (7%), *NOTCH1* (6%), *BIRC3*(4%)

Differential Diagnosis
- Leukemic MCL
- Leukemic FL
- SMZL
- Acute lymphoblastic leukemia (L-1 morphology)
- PLL (B- or T-cell)
- Monoclonal B-cell lymphocytosis

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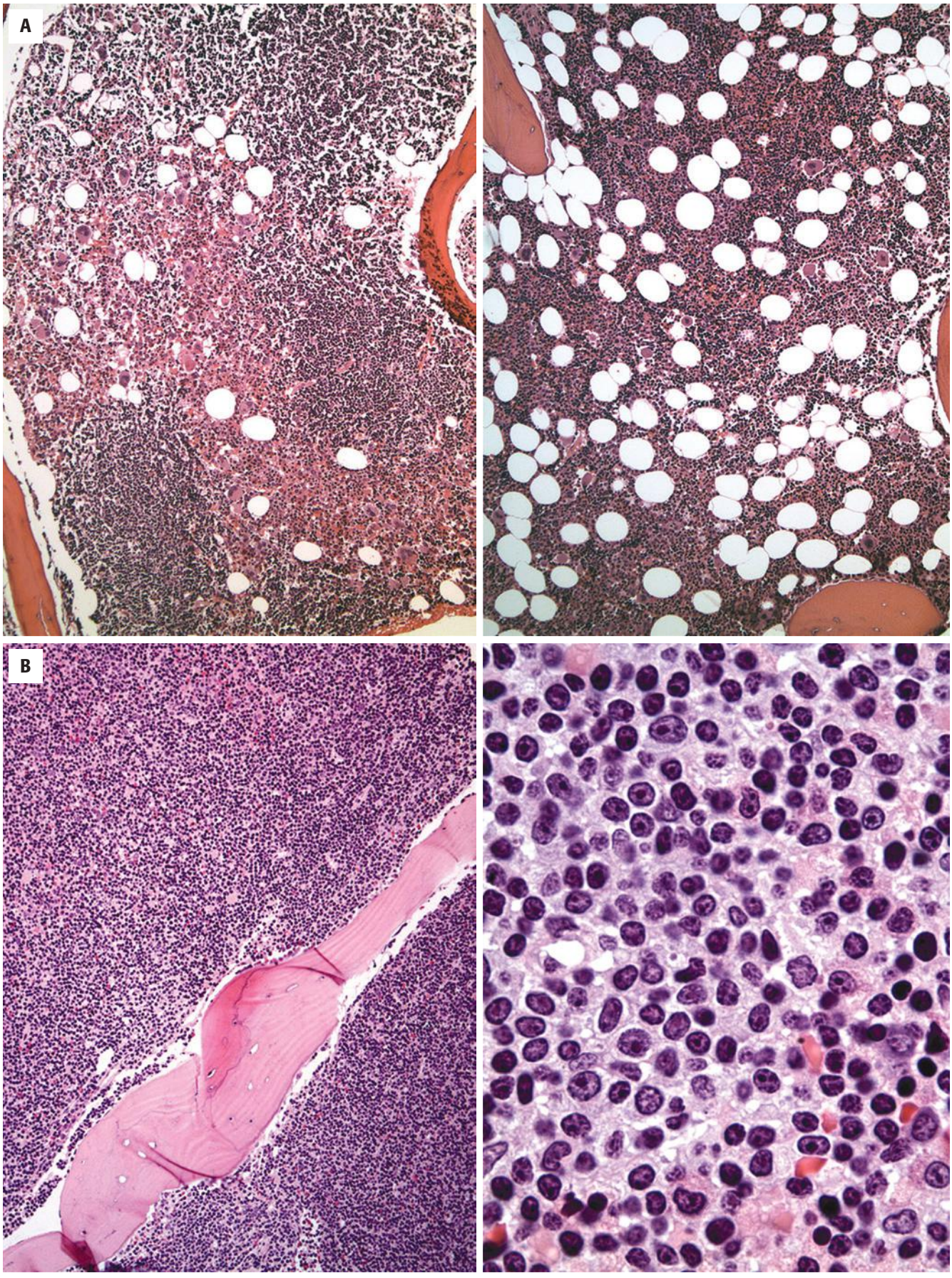


FIG. 12.3

Bone marrow involvement by chronic lymphocytic leukemia (CLL). A, Bone marrow biopsy specimen of patient with chronic lymphocytic leukemia demonstrating nodular *(left)* and interstitial *(right)* patterns. B, Diffuse pattern *(left)* with high magnification showing a collection of nucleolated prolymphocytes characteristic of a proliferation center (hematoxylin and eosin).

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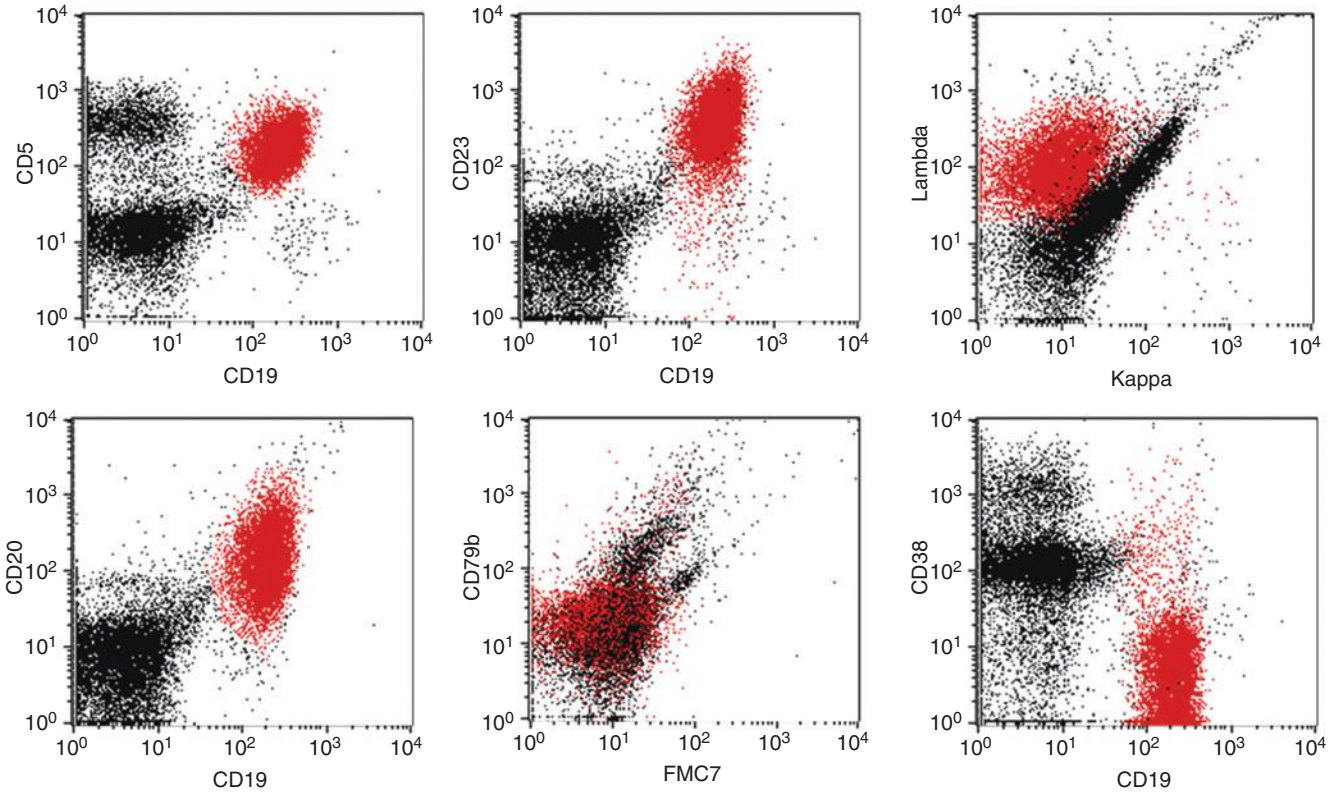


FIG. 12.4

Flow cytometric immunophenotype of chronic lymphocytic leukemia. A prominent B-cell population (red) is present expressing CD5, CD19, CD20 (dim), CD23, and lambda light chains. FMC7 and CD79b are not expressed to any appreciable degree. In this case, CD38 is not expressed, a feature suggesting a more favorable prognosis.

TABLE 12.2

Proposed Scoring System for CLL

	NO. OF CASES (%)		
Score	**CLL**	**Other B-Cell Leukemias**	**B-NHL**
5	209 (52)	0	0
4	139 (35)	0	1 (0.5)
3	39 (10)	1 (1)	7 (4)
2	11 (3)	8 (10)	43 (23)
1	1 (0.2)	25 (33)	75 (39)
0	1 (0.2)	43 (56)	63 (33)

Point system: surface Ig (weak = 1, moderate/strong = 0); CD5 (positive = 1, negative = 0); CD23 (positive = 1, negative = 0); FMC7 (negative = 1, positive = 0); CD22 (weak/negative = 1, moderate/strong = 0).
B-NHL, B-cell non-Hodgkin lymphoma; *CLL,* chronic lymphocytic leukemia.
Data from Matutes E, Owusu-Ankomah K, Morilla R, et al: The immunological profile of B-cell disorders and proposal of a scoring system for the diagnosis of CLL. *Leukemia* 8:1640–1645, 1994.

is most easily assessed by immunohistochemistry in bone marrow trephine or other tissue biopsies, although assessment by flow cytometry has been reported.

Two additional markers have been shown to be important in the prognosis of CLL. CD38 is a marker that initially was thought to correlate with *IGH* variable region *(IGHV)* mutational status (see following). The correlation has subsequently been proved to be imperfect, but it has been shown in numerous studies that CD38 expression (a commonly used cutoff is 30% of CLL cells) is a poor prognostic indicator in CLL. Several studies have also shown it to be independent of other clinical parameters. The intracellular nonreceptor tyrosine kinase ZAP-70 correlates fairly well with *IGHV* gene mutational status and can also be assessed by flow cytometry or immunohistochemistry. Expression in more than 20% of CLL cells is associated with unmutated *IGHV* genes and poor prognosis. Technical challenges, lack of standardization, and advances in molecular genetic markers have prevented widespread clinical use of these markers.

Molecular Genetics

Interphase fluorescence in situ hybridization (FISH) studies have revealed many recurrent genetic abnormalities that also have clinical importance (Table 12.3). Deletion of 13q, the most common abnormality, is associated with typical

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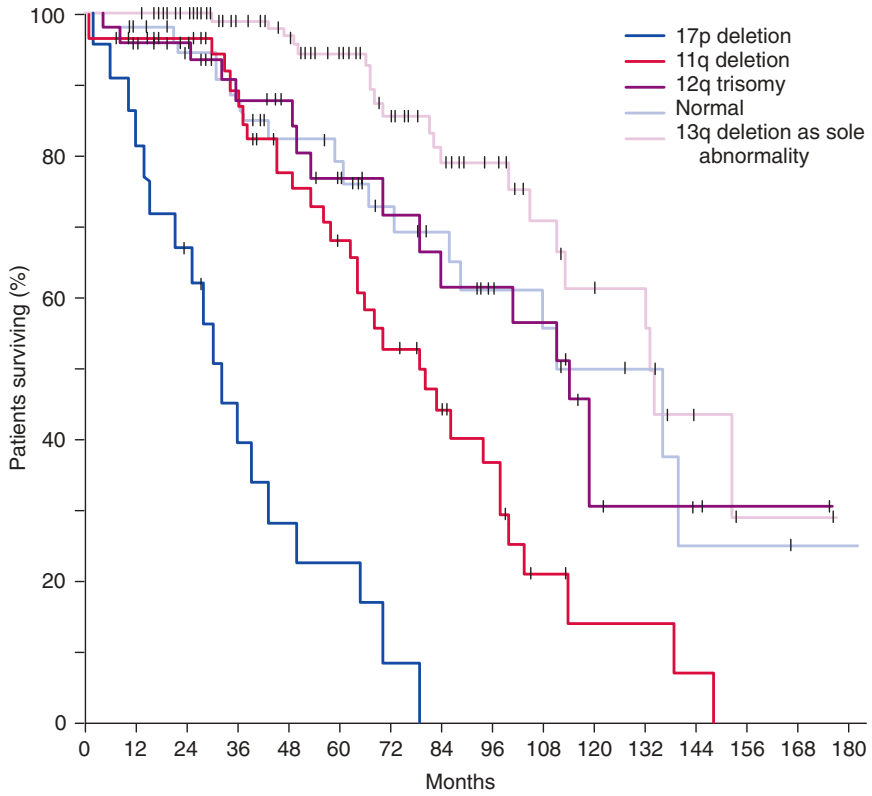
TABLE 12.3
Frequency of Chromosomal Abnormalities[a]

Aberration	Percentage of Cases
13q deletion	55
11q deletion	18
Trisomy 12	16
17p deletion	7
6q deletion	6
Trisomy 8q	5
t(14q32)	4
Trisomy 3q	3
Normal	18

[a]Defined by fluorescence in situ hybridization; some cases may have more than one abnormality.

morphology and good prognosis (Figs. 12.5 and 12.6). Trisomy 12 is associated with atypical morphology and intermediate prognosis. Del6q and del11q22-23 have poor prognosis. Deletion of 17p involving *TP53* is uncommon but associated with poor prognosis and poor response to conventional therapy. Assessment of CLL cases for these markers by FISH panels has become routine. More than any other biomarker in CLL, deletion of 17p is beginning to impact selection of therapy in individual CLL patients outside of clinical trials. Studies evaluating driver mutations, including these, copy number abnormalities in pretreatment and relapse samples show that del13q, trisomy 12, and del11q remain stably clonal over time, indicating that they are early genetic events.

IGHV mutational status has been shown to be an independent predictor of outcome in CLL. *IGHV* can be characterized as unmutated based on 98% or greater homology to the germline sequence. Patients whose CLL cells have unmutated *IGHV* genes generally have a poorer prognosis than patients whose CLL cells have mutated *IGVH* genes. Approximately



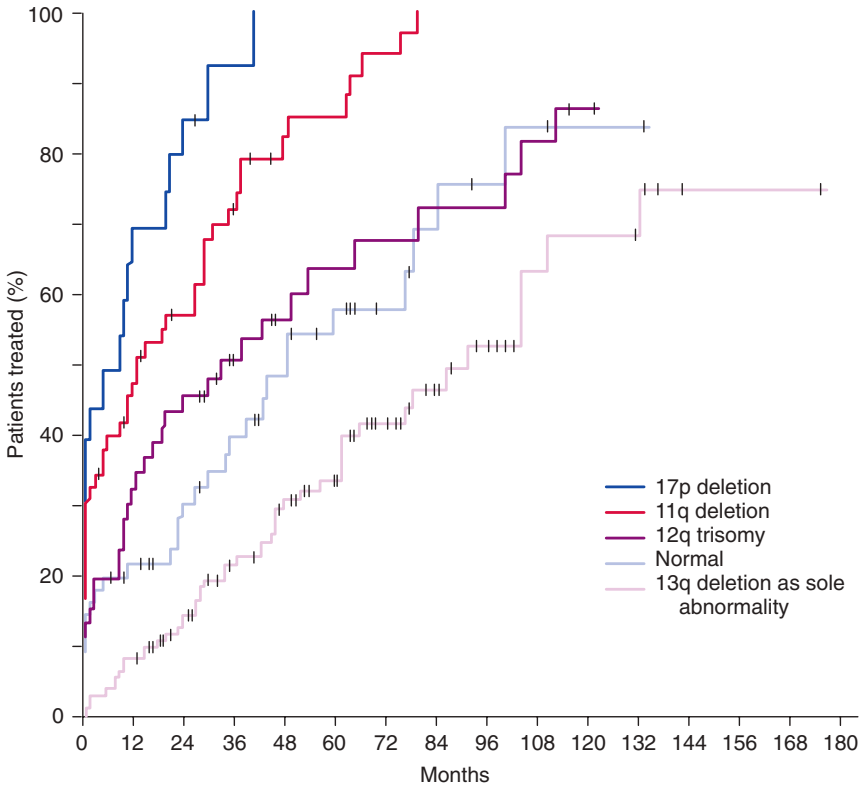
NO. AT RISK

17p deletion	23	18	13	8	5	4	1	0	0	0	0	0	0	0	0	0
11q deletion	56	53	47	43	33	27	20	15	10	4	2	2	1	0	0	0
12q trisomy	47	44	41	29	24	17	14	13	12	11	4	3	2	1	1	0
Normal	57	51	45	37	30	27	20	17	12	11	6	5	2	2	1	1
13q deletion as sole abnormality	117	117	106	91	80	63	45	36	24	16	12	11	3	1	1	0

FIG. 12.5

Outcomes of chronic lymphocytic leukemia patients by FISH abnormality. Karyotypic abnormality predicts survival. *(From Dohner H, Stilgenbauer E, Benner A, et al: Genomic alterations and survival in chronic lymphocytic leukemia.* N Engl J Med *343:1910–1916, 2000, with permission.)*

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NO. UNTREATED

17p deletion	23	7	4	1	0	0	0	0	0	0	0	0	0	0	0	0
11q deletion	56	29	20	13	7	5	2	0	0	0	0	0	0	0	0	0
12q trisomy	47	32	26	18	12	9	7	6	6	4	1	0	0	0	0	0
Normal	57	42	33	24	17	13	8	5	3	2	1	1	0	0	0	0
13q deletion as sole abnormality	117	108	94	72	58	45	28	21	13	7	6	6	1	1	1	0

FIG. 12.6

Outcomes of chronic lymphocytic leukemia patients by FISH abnormality. Karyotypic abnormality predicts requirement for treatment. *(From Dohner H, Stilgenbauer E, Benner A, et al: Genomic alterations and survival in chronic lymphocytic leukemia.* N Engl J Med *343:1910–1916, 2000, with permission.)*

45% of CLL cases will have unmutated *IGVH* genes. As with FISH testing, *IGHV* mutational testing at diagnosis is recommended by some experts to inform clinical management decisions.

MicroRNAs (miRNAs) have become a focus of investigation in CLL. These small noncoding RNAs are important in regulation of gene expression. Two miRNA genes, *miR-16* and *miR-15*, have been mapped to the minimally deleted region in CLL at 13q14.3. These miRNAs control B-cell proliferation and appear to modulate BCL2 expression. Deletion in transgenic mice leads to monoclonal B-cell lymphocytosis and CLL-like disease.

The categorization of CLL cases based on shared B-cell receptor (BCR) structural patterns at the protein level, derived from analysis of complementarity determining region 3 (CDR3) amino acid sequences, is termed *BCR-stereotypy*. Subsetting of CLL cases into one of at least major 19 stereotypic subsets (accounting for perhaps 30% of CLL cases) provides potential information on antigens recognized by these CLL clones and on the possibility of antigen drive. Correlations between stereotypes and other genetic abnormalities, biological features such as signaling pathways, and prognosis are being identified. How these may inform prognosis and treatment decisions remains an open question.

Massively parallel sequencing studies evaluating whole exomes and genomes for CLL samples have begun to define the mutational landscape and patterns of clonal evolution in this disease. Although integrative analysis of these complex data and determination of the clinical relevance in daily practice are ongoing challenges, there are emerging themes. First, there is a limited set of mutations in CLL, and studies have suggested a mean of 15 nonsynonymous mutations per patient. Among the more commonly mutated genes are *SF3B1*, *ATM*, *TP53*, *BIRC3*, and *NOTCH1*. Second, although there is a large list of mutated genes, driver mutations (those that are above background mutation rates and felt to be biologically relevant to leukemogenesis and progression) may be present in only 30 or so genes. These genes can be organized into a limited set of pathways or functional groups. Most driver mutations associate with the unmutated *IGHV* subtype (poor prognosis) of CLL, with the exception of del(13q), *MYD88*, and *CHD2*, which appear to segregate more with mutated

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IGHV CLL. Third, clonal evolution can occur at relapse and is often influenced by treatment. When sensitive methods are used, relapse driver mutations can be detected in the pretreatment samples in approximately 50% of cases. Fourth, presence of subclonal driver mutations in pretreatment samples is associated with shorter progression-free survival independent of other risk factors. Table 12.4 shows some of the more commonly mutated genes, functional pathways, and approximate frequencies. This is a rapidly changing area, and the interested reader may refer to key references for this chapter.

Differential Diagnosis

The differential diagnosis of CLL includes other B-cell leukemias and peripheralized lymphomas, particularly mantle cell lymphoma (MCL), marginal zone lymphoma, and follicular lymphoma (FL). Given the phenotypic and morphologic similarities, leukemic MCL is an important consideration. Morphologically, MCL cells may show slight nuclear irregularities and occasional intermediate-sized cells (Fig. 12.7). Flow cytometry is extremely helpful. Although both CLL and MCL express CD5, CD23 is expressed in most cases of CLL and is absent in MCL. Other useful features include bright CD20 and surface immunoglobulin (sIg) expression in MCL and expression of CD79b and FMC7. CD200 is brightly expressed in CLL by flow cytometry but is dim or negative in MCL. The t(11;14)(q13;q32) involving *IGH* and *CCND1* can confirm a diagnosis of leukemic MCL with the caveat that some cases of myeloma may also have this translocation. However, a plasma cell leukemia would not

TABLE 12.4
CLL Mutations

Common Recurrent Somatically Mutated Genes and Associated Pathways in CLL/SLL

Pathway	*Genes and approximate frequency*[a]
NOTCH signaling	*NOTCH1, FBXW7*
Inflammatory pathways	*MYD88, RIPK1, DDX3X, BIRC3, TRAF3, TRAF2, SAMHD1*
B-cell receptor signaling and differentiation	*ITPKB, CARD11, GNB1, BCOR, EGR2, IRF4*
WNT signaling	*MED12*
DNA damage and cell cycle control	*ATM, POT1, TP53, CHEK2, DYRK1A, ELF4, BRCC3*
Chromatin modification	*CHD2, HIST1H1B, HIST1H1E, ASXL1, ZMYM3, IKZF3, BAZ2A*
RNA and ribosomal processing	*SF3B1, XPO1, FUBP1, EWSR1, NXF1, XPO4, DDX3X*
mRNA translation	*RPS15*
MAPK-ERK	*KRAS, NRAS, BRAF, MAP2K1*
MYC-related	*FUBP1, PTPN11, MGA, FBXW7*

[a]Approximate frequency.
Red 20% to 24%; gold 15% to 19%; purple 5% to 9%; green 1% to 4%.
Adapted from Puente XS, Pinyol M, Quesada V, et al: Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. *Nature* 475:101-105, 2011.

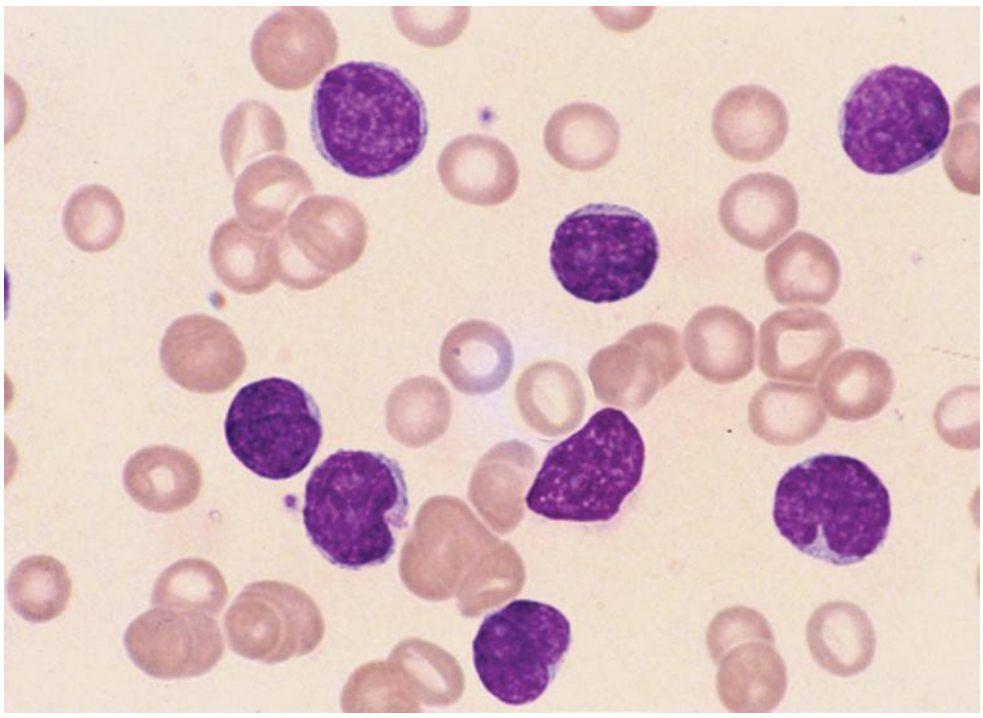


FIG. 12.7
Leukemic mantle cell lymphoma. Note the small to intermediate-sized cells with nuclear irregularities. Some of the cells have small inconspicuous nucleoli that are not seen in the small lymphocytes of chronic lymphocytic leukemia (Wright stain).

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TABLE 12.5
Immunophenotype and Molecular Genetic Features of B-Cell Lymphoproliferative Disorders

	CD5	CD10	CD20	CD23	CD25	CD79b	FMC7	CD103	CD123	sIg	t(14;18)	t(11;14)	*BRAF*	V600E
CLL	+	–	+(dim)	+	–	±(dim)	±(dim)	–	–	+(dim)/–		–	–	–
MCL	+	–	+	–	–	+	+	–	–	+		–	+	–
MZL	–	–	+	±	-	+	+	–	–	+		–	–	–
HCL	–	±	+	–	+	+	+	+	+	+		–	–	+
–	–	+	–	–	+	+	+	–	+		–	–	–	
FL	–	+	+	±	–	+	+	–	–	±		+	–	–

CLL, Chronic lymphocytic leukemia; *FL,* follicular lymphoma; *HCL,* hairy cell leukemia; *HCLv,* hairy cell leukemia variant; *MCL,* mantle cell lymphoma; *MZL,* marginal zone lymphoma.

co-express CD20 and CD5. Table 12.5 shows the phenotypic and genetic features of the various B-cell lymphoproliferative disorders. Peripheralized FL may also have individual cells that resemble CLL cells but always has at least occasional, and often many, deeply clefted cells. Expression of CD10 and lack of CD5 is the rule in FL. The presence of a t(14;18)(q32;q21) is strong evidence against CLL and favors FL. However, rare cases of CLL may harbor this translocation, but the phenotype, morphology, and clinical behavior are those of CLL. Splenic marginal zone lymphoma will typically have occasional cells that have abundant cytoplasm and/or cytoplasmic villous projections. The immunophenotype is that of an immunoglobulin light chain–restricted, CD5– lymphoproliferative disorder. Rare cases of marginal zone lymphoma may express CD5. Some cases of CLL do show atypical features such as irregular nuclei or mild deviation from the normal immunophenotype. However, elimination of other serious considerations using combined morphology, flow cytometry, and, when needed, molecular studies allows a confident diagnosis in most cases.

Occasional cases of T-cell PLL can mimic CLL morphologically, but attention to the morphologic features (small nucleoli) and flow cytometric immunophenotype makes distinction straightforward. Likewise, some cells of acute lymphoblastic leukemia (with a French-American-British classification L-1 morphologic subtype) might have small cells resembling CLL, but close attention to morphologic details such as chromatin pattern and immunophenotyping allows easy distinction from CLL.

Persistent polyclonal B-cell lymphocytosis might be mistaken for a B-cell leukemia. This disorder presents most commonly in female smokers with a modest lymphocytosis composed of polyclonal B cells, which may coexpress CD5. Deeply clefted lymphocytes are present, which makes confusion with CLL less likely than for other entities such as leukemic FL. In fact, *BCL2/IGH* translocations have been detected in these cases but are also polyclonal. Additional reported abnormalities include supernumerary +i(3)(q10) with recurrent gains of 3q, in which the minimally amplified region includes the *MECOM* gene. CLL-phenotype monoclonal B-cell lymphocytosis should be distinguished from CLL based on absolute monoclonal B-cell count (see below).

Prognosis and Therapy

As noted previously, the prognosis varies depending on stage and biological marker studies. Still, the disease course is measured in years to decades. A conservative approach to therapy is often taken (watch and wait), withholding treatment until the patient becomes symptomatic. Currently approved therapies are generally aimed at symptomatic disease and are not curative. Fludarabine, combined with cyclophosphamide, and rituximab (FCR) have become prominent as first-line therapy. Newer therapies, such as second-generation anti-CD20 antibodies, ibrutinib, idelalisib, and venetoclax, targeting cell-surface molecules, signaling pathways, and apoptosis resistance have been approved for the treatment of CLL.

There is great interest in incorporating molecular markers for prognosis and prediction, and detailed review of these is beyond the scope of this chapter. Large series consistently appear to find that mutations in *SF3B1* and abnormalities of *TP53* (mutations/deletions) are poor prognostic factors, independent of other risk factors, including *IGHV* mutational status. In a multicenter trial of CLL patients treated with FCR, thymidine kinase (≥10 U/L), unmutated *IGHV*, 11q deletion, 17p deletion, *TP53* mutation, and *SF3B1* mutation were independent prognostic factors for progression-free survival. *IGHV* mutational status, 17p deletion, and *TP53* mutation were also independent factors in overall survival.

Detection of minimal residual disease (MRD) is also of interest since it can predict long-term outcomes after chemoimmunotherapy. MRD-negativity has also been suggested as a clinical endpoint in CLL treatment. Methods including clone-specific polymerase chain reaction (PCR), multicolor flow cytometry, and next-generation sequencing for clonotypic detection of the *IGH* gene rearrangement are capable of detecting MRD at the level of 10^{-4} or better (for molecular methods such as *IGH* sequencing). Lack of technical

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standardization, agreement on timing of MRD analysis, optimal sample type, and clinical actionability remain barriers to application outside of the clinical research setting.

MONOCLONAL B-CELL LYMPHOCYTOSIS (MBL)

Monoclonal B-cell lymphocytosis is a low-level ($<5 \times 10^9$/L) asymptomatic proliferation of monoclonal B cells. Diagnostic criteria are in Box 12.1. MBL has been recognized in the past, and application of advanced phenotyping, genetic tools, and clinical follow-up are better characterizing it. Most data exist for CLL phenotype MBL. We now know that 2% to 3% of healthy subjects have an MBL using sensitive multiparameter flow cytometry (FC), most (but not all) of which are CD5+ CLL phenotype MBL. The incidence of MBL increases with age, reaching up to 5% in adults over age 60. Application of extraordinarily sensitive methods shows that minute clones may be detected in much higher proportions of normal individuals. Most patients remain asymptomatic, but there is low incidence of progression to CLL. In fact, recent studies show that nearly all CLL is preceded by MBL.

For patients with MBL who are discovered for clinical reasons such as modest leukocytosis, the rate of progression to CLL that requires therapy is 1% to 2%/year. Of note, the *IGHV* and genetic features are that of good prognosis CLL. However, MBL found in population screening studies using very high sensitivity methods show a different *IGHV* gene repertoire than that found in CLL, and these patients only rarely progress to overt CLL. Thus, for CLL-phenotype MBL, investigators and clinicians now define low-count versus high-count MBL with a commonly used cutoff of 0.5×10^9/L. Clinical follow-up is recommended only for high-count MBL since risk of progression to CLL exists for this group.

Genomic analysis of MBL cases demonstrate similar genetic events present in MBL as in CLL. Indeed, MBL cases seem to be indistinguishable at the genomic, transcriptomic, and epigenomic levels from CLL cases of the same *IGHV* mutational subgroup. The number of driver genetic alterations is lower than overt CLL, reflecting an earlier state of clonal evolution.

Bone marrow biopsy is not routinely done for MBL, but if performed may show low-level infiltration by small lymphocytes, usually less than 5% to 10% of the bone marrow cellularity. Interstitial infiltrates, rounded lymphoid aggregates, or subtle interstitial infiltrates visible only upon immunostaining have been described. Although patients with high-count MBL should be followed periodically to monitor for development of CLL or other lymphoma, active treatment is not required.

Non-CLL phenotype MBL is usually CD5-negative and data are emerging that at least some of these cases are related to splenic marginal zone lymphoma.

Box 12.1 Diagnostic Criteria for MBL

Diagnostic Criteria

- Documentation of clonal B-cell population by one or more of the following:
  - Light chain restriction: Overall kappa:lambda ratio >3:1 or <0.3:1 *or*
  - >25% of B cells lacking or expressing low-level surface immunoglobulin *or*
  - Heavy chain monoclonal *IGHV* rearrangements *or*
  - Presence of a disease-specific immunophenotype
- Absolute B-cell count $<5 \times 10^9$ cells/L
- No extramedullary disease

Subclassification

- CLL-like phenotype
  - Co-expression of CD5 with CD19; CD20 (dim); and CD23
  - Light chain restriction with dim surface immunoglobulin expression (very small MBL clones may be oligoclonal and thus not light chain restricted)
  - Low vs. high count MBL (0.5×10^9 cells/L)
- Atypical-CLL phenotype
  - Co-expression of CD5 with CD19 but CD23 negative or CD20 (bright)
  - Light chain restriction with moderate to bright surface immunoglobulin expression
  - Exclude t(11;14) to rule out mantle cell lymphoma
- Non-CLL phenotype
  - CD5 usually negative (may be related to splenic marginal zone lymphoma)
  - CD20 positive
  - Light chain restriction with moderate to bright surface immunoglobulin expression

(Adapted from Marti GE, Rawstron AC, Ghia P, et al: Diagnostic criteria for monoclonal B-cell lymphocytosis. International Familial CLL Consortium, *Br J Haematol* 130(3):325–332, 2005 and Swerdlow SH, Campo E, Pileri SA, et al: The 2016 revision of the World Health Organization classification of lymphoid neoplasms, *Blood* 127(20):2375–2390, 2016.)

B-CELL PROLYMPHOCYTIC LEUKEMIA

Prolymphocytic leukemia is defined as a B-cell leukemia that is composed of more than 55% prolymphocytes in the peripheral blood. PLL can arise from preexisting CLL (prolymphocytic transformation of CLL), in which case this should be noted in the diagnosis. Our concept of PLL is evolving because cases of leukemia with more than 55% prolymphocytes appear to be a heterogeneous group of diseases encompassing transformed CLL, nucleolated variants of MCL, and de novo PLL. We consider the de novo form in this section. De novo PLL is an uncommon disorder, accounting for less than 2% of lymphoid leukemias.

Clinical Features

Patients with PLL are usually older (median age, 70) with a male predominance. Patients most commonly present with splenomegaly and high leukocyte count (often, $>100 \times 10^9$/L)

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and usually lack significant lymphadenopathy. Cytopenias are common, usually due to marrow replacement. A small subset of patients may be asymptomatic with an indolent phase followed by progression to an aggressive phase.

Pathologic Features

Morphology

The peripheral blood smear shows numerous (>55% of lymphocytes) prolymphocytes, characterized by intermediate size, slightly dispersed chromatin, and a prominent central nucleolus. The cytoplasm is pale blue and moderate to abundant in amount. The nuclear contours are classically round, but indentations can be seen (Fig. 12.8). The bone marrow often shows extensive involvement with intermediate-sized cells containing small nucleoli. The spleen may show white pulp involvement with or without red pulp involvement. Again, the cells are round and intermediate in size and show small central nucleoli in tissue section.

PROLYMPHOCYTIC LEUKEMIA—FACT SHEET

Definition
- Mature B-cell leukemia composed of prolymphocytes (>55% of lymphocytes)

Incidence
- Rare (<2% of lymphoid leukemias)

Clinical Features
- Older adults (median age 70)
- Splenomegaly and marked leukocytosis, without lymphadenopathy

Prognosis and Therapy
- Median survival, 3 to 4 years
- Therapy is variable, consisting of immunochemotherapy, alemtuzumab, kinase inhibitors (ibrutinib, idelalisib)

PROLYMPHOCYTIC LEUKEMIA: PATHOLOGIC FEATURES

Morphology
- Peripheral blood: more than 55% of lymphocytes are prolymphocytes; usually a high white blood cell count
- Bone marrow: extensive involvement by prolymphocytes
- Spleen: white pulp predominant or diffuse involvement

Immunophenotype
- CD5–, CD10–, CD20+ (bright), CD23–, sIg+ (bright), FMC7+, CD79b+

Genetics
- No specific abnormality
- Abnormalities of *TP53* and *MYC* common

Differential Diagnosis
- CLL with increased prolymphocytes
- MCL, nucleated variant
- Other non-Hodgkin lymphoma

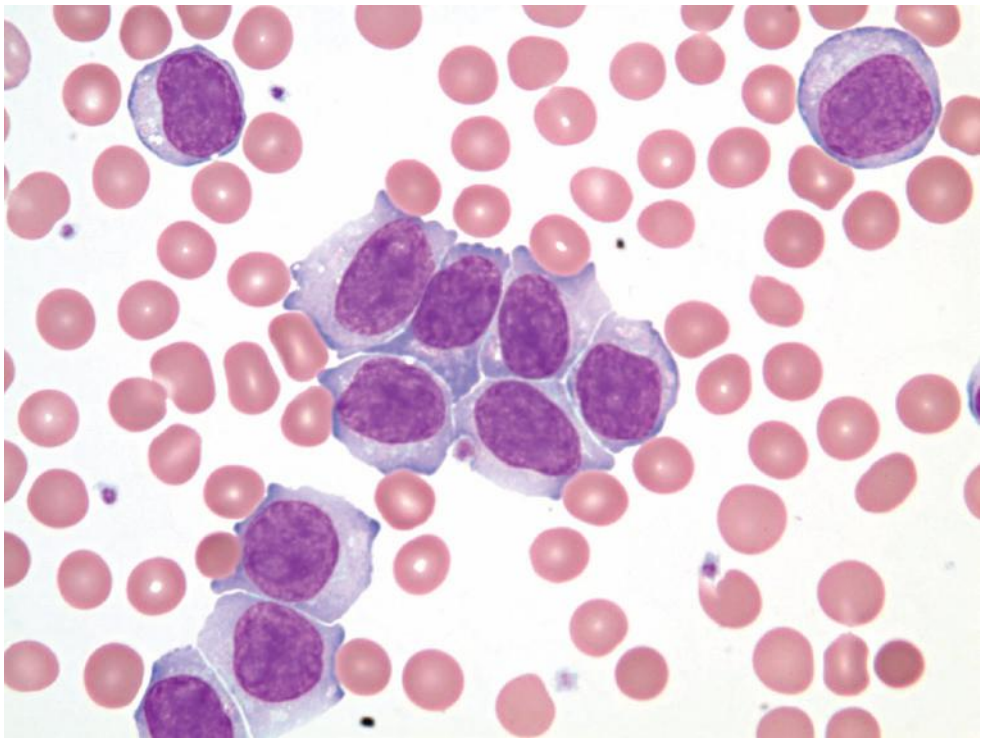


FIG. 12.8
B-cell prolymphocytic leukemia. Numerous intermediate-sized prolymphocytes are present with prominent central nucleoli (Wright stain).

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Immunophenotype

The reported immunophenotype of PLL is variable, likely because previous reports have included several entities (listed previously). PLL expresses CD19, CD20 (bright), CD79b, FMC7, and sIg. CD5 may be expressed in some cases; however, prior data concerning CD5 expression may be inaccurate due to differences in inclusion criteria. Expression of cyclin D1, associated with an *IGH-CCND1* fusion, strongly suggests a nucleolated variant of MCL and essentially excludes PLL.

Molecular Genetics

Rearranged immunoglobulin genes are present. Given the confusion in the precise definition related to MCL, the molecular genetic features of PLL are not yet precisely defined. The t(11;14)(q13;q32) resulting in an *IGH/CCND1* fusion is present in a minority (20% of some series) of previously reported PLL. However, these cases are best considered a variant of MCL. Other nonspecific abnormalities reported in PLL include *TP53* abnormalities (deletion or mutation in 75% of cases), and *MYC* abnormalities (amplification or translocation) are now recognized to occur in over 50% of cases.

Differential Diagnosis

The differential diagnosis of a leukemia composed of prolymphocytes includes transformed CLL, MCL variant, T-cell PLL, and HCLv. A history of CLL allows one to reasonably diagnose a case of B-cell leukemia with more than 55% prolymphocytes as prolymphocytic transformation of CLL. Presence of t(11;14)(q13;q32) allows one to confirm a diagnosis of MCL. T-cell PLL cells may resemble B-cell PLL cells morphologically because of the presence of nucleoli, although the T-PLL cells tend to have more nuclear irregularities and the chromatin may be more condensed. Immunophenotyping easily identifies a T-cell process in T-cell PLL. HCLv cells may have small nucleoli but still have some cytoplasmic features of HCL that are not seen in PLL. The presence of CD103 or *MAP2K1* mutation also favors HCLv.

Prognosis and Therapy

Prolymphocytic leukemia is usually an aggressive disease, although a subset of cases will not follow a rapidly progressive course. Older age, anemia, and presence of *TP53* abnormalities may indicate more aggressive disease. Interestingly, *MYC* abnormalities including *IGH-MYC* translocation are not necessarily indicative of an aggressive course.

B-PLL is difficult to treat, and median survival is 3 to 4 years. Therapy has not been uniform for this uncommon disease. Current recommendations depend on phase of disease and genetic features. Watchful waiting for stable indolent B-PLL may be appropriate while chemoimmunotherapy is used as first-line treatment for aggressive phase cases with normal *TP53*. Those with *TP53* abnormalities may benefit from alemtuzumab and other targeted therapies such as ibrutinib and idelalisib. Remissions are often short, and allogeneic stem cell transplantation consolidation may be considered, particularly for cases with abnormal *TP53*.

HAIRY CELL LEUKEMIA

Hairy cell leukemia is an uncommon mature B-cell leukemia composed of cells with cytoplasmic projections when examined on Wright-stained smear. It represents 2% of lymphoid leukemias.

Clinical Features

Hairy cell leukemia presents in middle-aged to elderly patients (median age, 50) with a 4:1 male-to-female predominance. Patients present typically with cytopenias including monocytopenia and splenomegaly. Infectious complications also occur, particularly with bacterial organisms.

Pathologic Features

Morphology

On peripheral blood smear, HCL cells have a characteristic appearance. The chromatin is slightly less condensed than a

HAIRY CELL LEUKEMIA—FACT SHEET

Definition
- Mature B-cell leukemia composed of cells with characteristic cytoplasmic "hairy" projections

Incidence
- Uncommon (<2% of lymphoid leukemias)

Clinical Features
- Presents in middle age (median age 50 years), with male predominance
- Cytopenias, including monocytopenia and splenomegaly, are common.

Prognosis and Therapy
- High rate of durable remission with purine nucleoside analogue therapy
- *BRAF* inhibitors being studied with success

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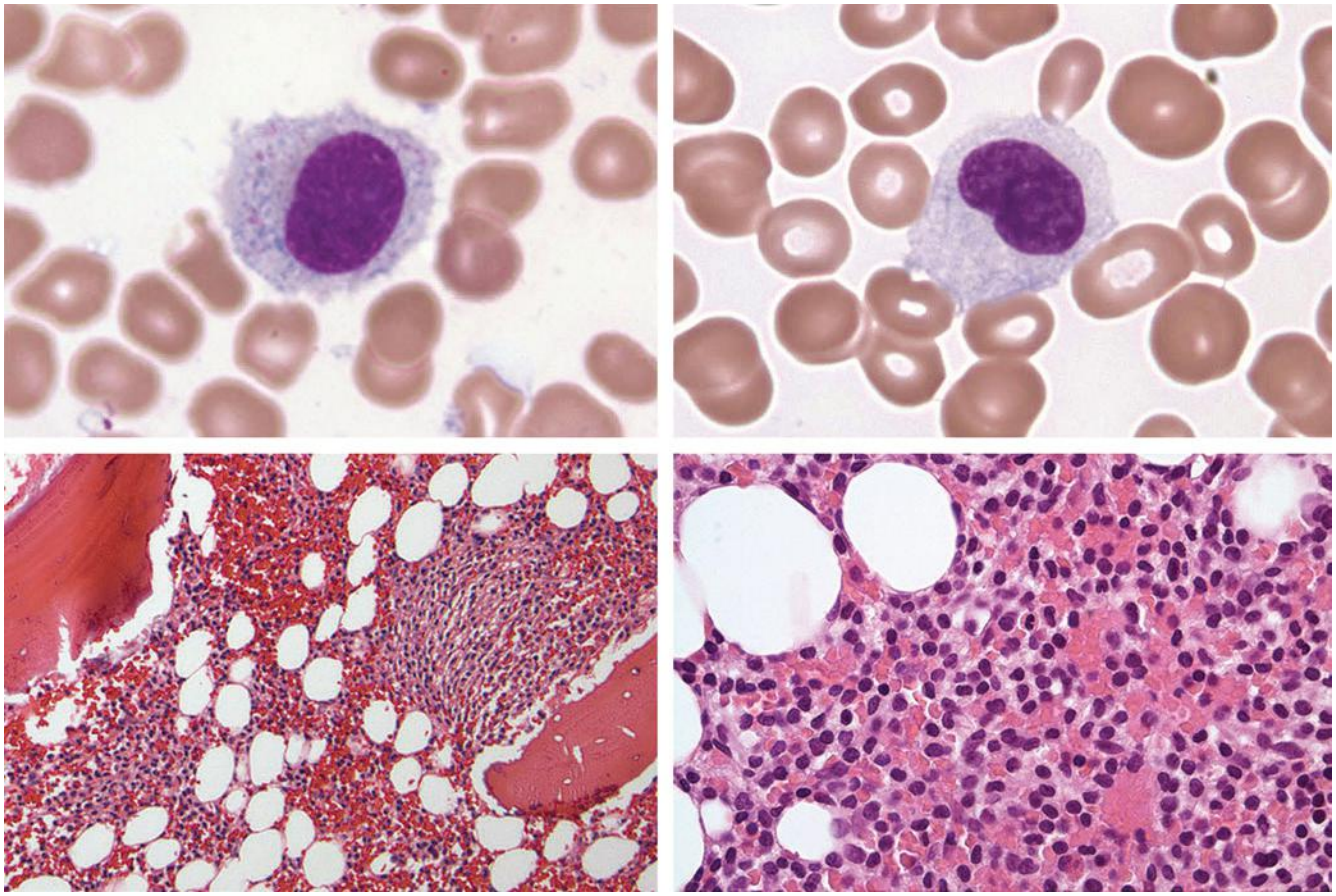


FIG. 12.9

Hairy cell leukemia. *Top,* Typical morphology. Small cells with round to bean-shaped nuclei with reticulated chromatin pattern and abundant "lacy" cytoplasm. Some cells may have ragged circumferential cytoplasmic borders imparting a "hairy" appearance (Wright stain). *Bottom*, Aggregates of hairy cells that at higher magnification show the characteristic morphology with cells spaced apart from one another due to the abundant cytoplasm (hematoxylin and eosin).

mature non-neoplastic lymphocyte with a reticulated pattern. The cytoplasm is moderate in amount and stains pale gray-blue. It has a textured or "lacy" quality to it. Fine cytoplasmic projections can be seen around the circumference of the cell (Fig. 12.9). The bone marrow aspirate may be hemodiluted because reticulin fibrosis is usually present and accounts for frequent "dry taps" in patients with HCL. Trephine biopsy is therefore very important. The infiltrate can be nodular, interstitial, or paratrabecular (see Fig. 12.9). The collections of lymphocytes may have a "fried egg" appearance, imparted by the moderate amounts of cytoplasm. Nuclei are usually round to oval, but the cells may also have a spindled quality. Involvement may be subtle, and immunostains for B-cell markers, such as CD20 or DBA.44, can highlight the cells, often showing many more cells than suspected on hematoxylin and eosin–stained sections. Careful examination of a CD20-stained section may show the cytoplasmic projections. Mast cells are increased in bone marrow of HCL patients and may be seen on aspirate smear and in tissue section. In the spleen, HCL involves the red pulp preferentially (Fig. 12.10). This is in contrast to the white pulp involvement seen in other low-grade B-cell lymphoproliferative disorders. Blood lakes are seen. These are nonendothelium-lined spaces filled with

HAIRY CELL LEUKEMIA—PATHOLOGIC FEATURES

Morphology
- Peripheral blood: circulating hairy cells
- Bone marrow: interstitial, nodular, or diffuse infiltrate of cells ("fried egg" appearance or spindling); reticulin fibrosis
- Spleen: red pulp involvement

Immunophenotype
- CD5–, CD10–, CD11c+ (bright), CD19+, CD20+, CD22+ (bright), CD25+, CD103+, CD123+, annexin A1+, sIg+

Genetics
- *BRAF* V600E in almost all non-*IGHV4-34* cases
- *MAP2K1* activating mutations in *IGHV4-34* cases

Differential Diagnosis
- SMZL
- HCLv

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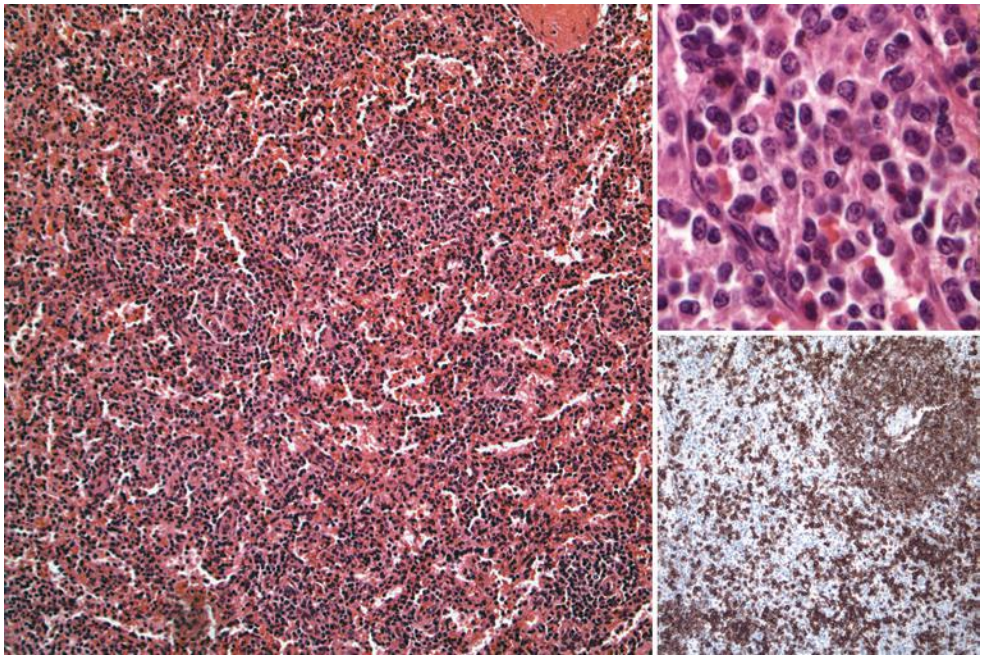


FIG. 12.10
Hairy cell leukemia involving spleen with red pulp. *Left,* low magnification; *top right,* high magnification; CD20 immunostain *(bottom right)* highlights the B-cell red pulp infiltrate.

erythrocytes and surrounded by leukemia cells. A tartrate-resistant acid phosphatase stain is strongly positive in HCL cells but is not required for diagnosis in the era of multiparameter flow cytometry.

Immunophenotype

Flow cytometric analysis shows the cells express CD19, CD11c, CD20, CD22, CD25, CD103, CD123, FMC7, annexin A1, and monotypic immunoglobulin light chains. Strong co-expression of CD11c, CD22, CD25, and CD103 is quite characteristic of this leukemia. CD103, CD123, and annexin A1 are fairly sensitive and specific. Cyclin D1 can be weakly expressed in HCL, and CD10 can be seen in approximately 10% of cases. Based on the presence of the *BRAF* V600E mutation in almost all cases of HCL (see below), immunohistochemistry using the *BRAF* V600E mutation-specific antibody is also diagnostically useful. Phospho-ERK, whose expression is a consequence of this mutation, can also be demonstrated in bone marrow trephine biopsies by immunohistochemistry.

Molecular Genetics

Immunoglobulin genes are monoclonally rearranged. There is no specific karyotypic abnormality; however, gain of 5q13-31 and loss of 7q32 occur in a minority of cases and that the region at 7q32 is the same area also deleted in some cases of SMZL. Expression array studies have shown that HCL cells are related to postgerminal center memory B cells but that they differ from memory cells in expression of chemokine and adhesion molecules. Although cyclin D1 is overexpressed in some cases, the t(11;14)(q13;q32) seen in MCL is absent.

Next-generation sequencing in HCL has led to the discovery of the activating *BRAF* V600E mutation in almost all cases of HCL, with the exception of HCL cases using the *IGVH4-34* gene segment. *IGVH4-34* usage in classic HCL is estimated to be used in less than 5% of cases. Interestingly, the majority of these cases harbor activating mutations in *MAP2K1*, encoding MEK1. The *BRAF* V600E is only rarely present in other mature B-cell leukemias and lymphomas; thus it is a fairly specific mutation. It can be detected in blood samples from HCL patients by standard molecular tests. The mutation is also the target for BRAF inhibitor therapy.

Differential Diagnosis

The characteristic morphologic and immunophenotypic features usually allow easy separation from other B-cell lymphoproliferative disorders such as CLL, PLL, and the leukemic phase of FL or MCL. The main diagnostic considerations are SMZL and HCLv. Morphologically, HCL cells have cytoplasmic projections around the circumference of the cell, whereas SMZL cells usually have their cytoplasmic projections only around part of the cells, often at opposite ends of the cells. The cells in SMZL are often more heterogeneous appearing, with some containing nucleoli and others more closely resembling CLL cells. HCLv cells have a more condensed chromatin pattern than typical HCL cells and also have a small visible nucleolus. Immunophenotypically, the expression of bright CD11c, bright CD22, CD25, and CD103 is quite characteristic of HCL. CD25 is absent in HCLv. SMZL usually lacks these markers but may express them in a minority of cases. For example, CD103 can be expressed in approximately 15% of cases. When dealing with

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lymphocytes with villous/hairy projections, CD123 appears useful in distinguishing HCL (positive) from HCLv and SMZL (negative). This latter marker is not specific because it can be seen in some cases of CLL, acute myeloid leukemia (AML), and B-cell precursor acute lymphoblastic leukemia. Annexin A1 is also reported to be seen in essentially all cases of HCL and is quite specific (approaching 100%). From the molecular genetic standpoint, the presence of the *BRAF* V600E is quite specific for HCL and is uncommonly seen in SMZL (≤2%) and not seen in HCLv.

Prognosis and Therapy

With recent therapies using nucleoside analogues such as pentostatin or cladribine, the prognosis of HCL is excellent, with high complete response rates and excellent long-term survival. However, relapses do occur, and complications such as infection can be a source of morbidity. The discovery of the *BFAF* V600E has led to the use of BRAF inhibitors with success, and trials are under way to determine best use of targeted agents in this disease.

HAIRY CELL LEUKEMIA VARIANT—FACT SHEET

Definition
- Unusual B-cell leukemia with features resembling typical HCL but having higher WBC than HCL, cytologic variation with more condensed chromatin and nucleoli

Incidence
- Rare (<1% of lymphoid leukemias, estimated to be 10% of HCL)

Clinical Features
- Presents (median age 71 years) with leukocytosis and lacks monocytopenia
- Splenomegaly and cytopenias usually are present
- A monoclonal serum paraprotein is often found

Prognosis and Therapy
- Treatment varies according to patient features; HCL variant will not respond to typical HCL treatment
- Splenectomy results in durable partial responses and is of value in palliation
- Median survival, 9 years

HAIRY CELL LEUKEMIA VARIANT

Hairy cell leukemia variant is quite uncommon, representing 0.4% of chronic lymphoproliferative disorders. It differs from HCL in several clinical, morphologic, and immunophenotypic features. Currently, HCLv is a provisional WHO entity considered under the rubric of splenic B-cell lymphoma/leukemia, unclassifiable in order to reflect our incomplete understanding of the definition and uncertain relationship to other ill-defined splenic lymphomas/leukemias including so-called splenic diffuse small B-cell lymphoma.

Clinical Features

The median age at onset is 71 years, older than in typical HCL, and there is only a slight male predominance (1.6). Splenomegaly and cytopenias are common, as in typical HCL, but monocytopenia is not. There is usually a leukocytosis instead of leukopenia.

HAIRY CELL LEUKEMIA VARIANT—PATHOLOGIC FEATURES

Morphology
- Peripheral blood: cells resembling HCL cells, but with more condensed chromatin pattern and small nucleoli (prolymphocytoid variant of HCL)
- Bone marrow: sinusoidal or interstitial
- Spleen: red pulp involvement

Immunophenotype
- CD5–, CD10–, CD19+, CD20+, CD22+, CD25–, CD103+, CD123–, annexin A1–, sIg+

Genetics
- *MAP2K1* mutations in 50% of cases

Differential Diagnosis
- SMZL
- HCL
- Splenic diffuse red pulp small B-cell lymphoma (relationship between this and HCLv under investigation)

Pathologic Features

Morphology

Morphologically, the cells resemble typical HCL cells in blood but have a more condensed chromatin pattern and a nucleolus (Fig. 12.11). The cytoplasmic projections are less prominent than in typical HCL. Bone marrow infiltration appears similar in pattern to typical HCL, with an interstitial or sinusoidal pattern commonly seen. Some cases may show a "fried egg" appearance. In the spleen, as in typical HCL, there is a predilection for diffuse red pulp involvement (Fig. 12.12). Portal and sinusoidal infiltrates are seen in the liver.

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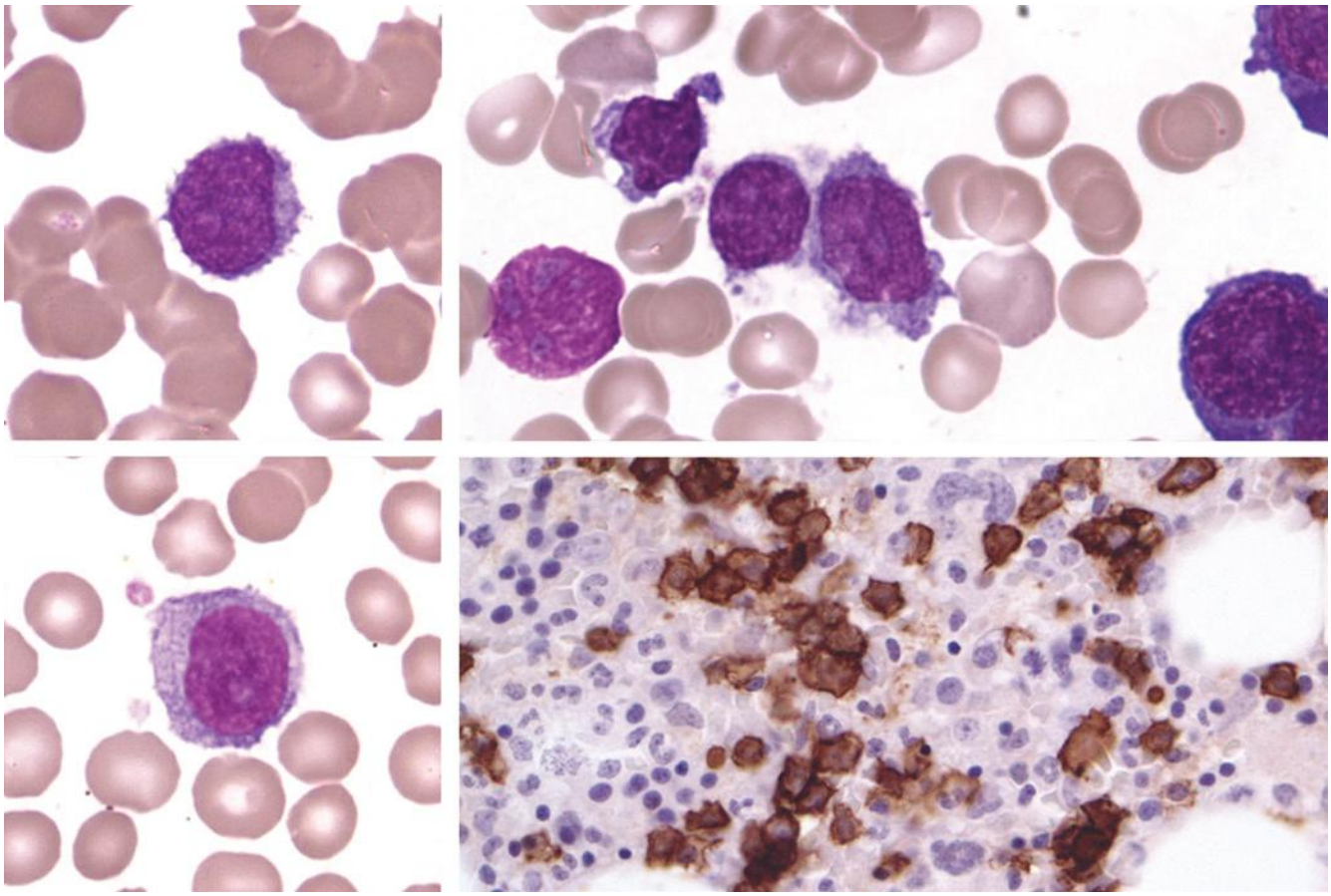


FIG. 12.11

Hairy cell leukemia variant. *Left,* Cells with scant to moderate amounts of cytoplasm with variable cytoplasmic projections and small nucleoli (Wright stain). Bone marrow aspirate shows similar cells *(top right).* Immunostains in the bone marrow show that a subtle CD20+ B-cell infiltrate is present. Flow cytometry showed an immunophenotype of CD19+, CD20+, CD5–, CD10–, CD25–, CD103+. Tartrate-resistant acid phosphatase stain was negative.

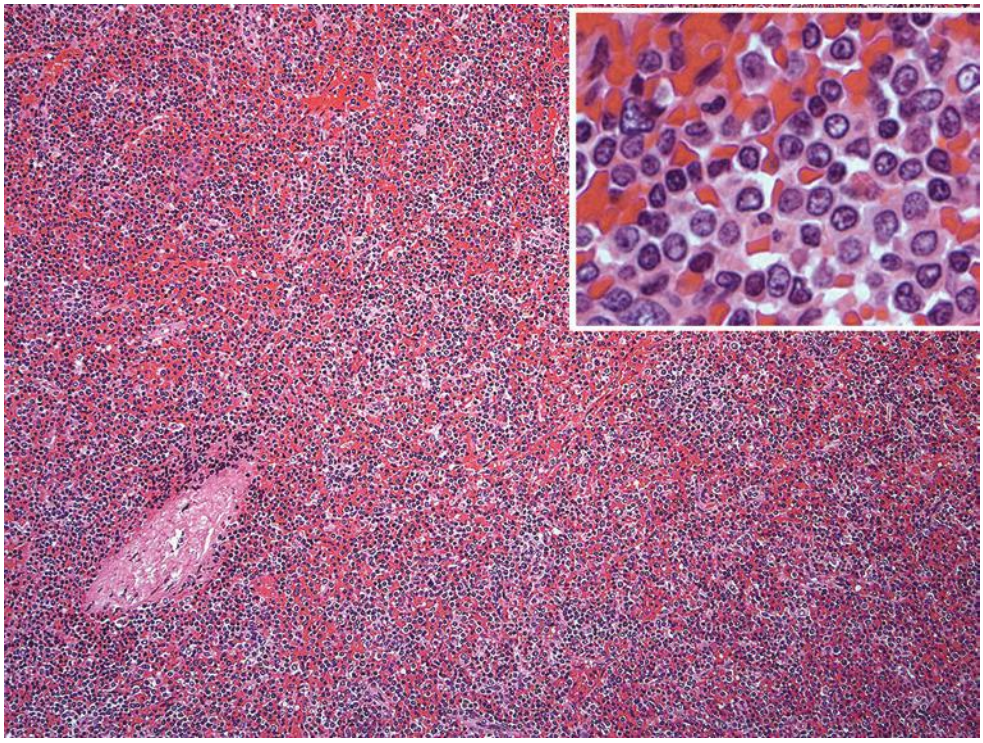


FIG. 12.12

The same patient as in Fig. 12.11 underwent splenectomy, which demonstrated a marked red pulp leukemic infiltrate similar to typical hairy cell leukemia (hematoxylin and eosin).

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Immunophenotype

Immunophenotyping shows some similarities to typical HCL. HCLv cells express CD19, CD20, CD22, and CD11c. CD103 is expressed in the majority of cases (60%), but CD25, CD123, and annexin A1 (ANXA1) are absent. Unlike typical HCL, HCLv cells are negative for tartrate-resistant acid phosphatase.

Molecular Genetics

Until recently, no characteristic genetic abnormality was known for HCLV. However, whole exome sequencing studies have identified activating mutations in *MAP2K1* in approximately 50% of cases. The *BRAF* V600E seen in HCL is not present in HCLv. Thus, as with classic HCL, activation of the RAF-MEK-ERK pathway appears important to the biology of this leukemia. Whether this discovery will be seen as evidence supporting recognition as a discrete entity in the WHO is a matter of debate.

Differential Diagnosis

The main differential diagnostic considerations are HCL and SMZL. In addition to the classic morphologic features described earlier, flow cytometry can be very helpful. HCL expresses CD11c, CD25, CD103, CD123, and ANXA1, whereas the variant form usually lacks CD25, CD123, and ANXA1. SMZL cells also lack CD103 (in most cases). CD123 and ANXA1 are also absent in SMZL. It has been suggested that expression of preswitched immunoglobulin (IgM/IgD) or both preswitched and postswitched immunoglobulin (IgG/IgA) heavy chains are characteristic of HCLv. Demonstration of the *BRAF* V600E is highly suggestive of HCL, although uncommon cases (≤2%) of SMZL may harbor this mutation. *MAP2K1* mutation favors HCLV, although it can also be seen in IGHV4-34 bearing classical HCL.

Prognosis and Therapy

Distinguishing HCLv from HCL is of clinical importance because of the lack of response to standard HCL therapy. Monoclonal antibody therapy against CD20 and CD22 shows promise. Splenectomy has been suggested as an alternative therapy in these patients.


The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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Mature T-Cell and Natural Killer–Cell Leukemias

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INTRODUCTION

The mature T-cell and natural killer (NK)-cell leukemias form a heterogeneous group of diseases with diverse etiologies and markedly varied clinical behavior. When applied to T cells, the term *mature* or *peripheral* is often used to describe nonblastic T-cell neoplasms marked by their resemblance to post-thymic T lymphocytes and characterized by the absence of terminal deoxynucleotidyl transferase (TdT) expression and usually by the presence of surface CD3 expression (except in those cases in which CD3 is aberrantly deleted). CD3 is a structure of several polypeptides that is intimately associated with the surface T-cell receptor complex. It is present in the cytoplasm of early T-cell precursors but is present on the cell surface only in the post-thymic stage of T-cell maturation. The diagnosis and appropriate classification of T-cell and NK-cell leukemias rely heavily on the correlation of morphologic, immunophenotypic, genetic, and clinical data.

EVALUATION OF PERIPHERAL BLOOD IN MATURE T-CELL LEUKEMIAS

MORPHOLOGIC EVALUATION

Thorough morphologic assessment of a carefully prepared peripheral blood smear can yield valuable clues in the diagnosis of mature T-cell leukemias. As with other lymphoproliferative disorders, the peripheral blood morphology of various T-cell leukemias is often not specific as to subtype; however, specific morphologic findings can be of use in the differential diagnosis and subclassification of these disorders. For example, the distinct morphology of large granular lymphocyte (LGL) leukemias helps to set them apart from other T-cell leukemias or lymphomas (e.g., hepatosplenic T-cell lymphoma) that can manifest with similar clinical patterns of anatomic distribution. Likewise, the bizarre, flower-like appearance of the neoplastic cells in human T-lymphotropic virus type 1 (HTLV-1)–associated adult T-cell leukemia–lymphoma (ATLL) can be contrasted with the more subtle, delicate nuclear folding of cerebriform lymphocytes in Sézary syndrome (SS)–mycosis fungoides (MF).

Although peripheral blood smear examination is an indispensable part of the diagnostic evaluation in T-cell leukemias, it is important not to get dogmatic regarding morphologic correlates in these diseases. The abnormal lymphocytes in a variety of T-cell lymphomas can mimic the cells of more specific entities such as SS or ATLL. Likewise, the LGLs synonymous with LGL leukemia can be present in a variety of reactive lymphocytoses. As with other diagnostic modalities, examination of the blood smear should be considered necessary but not sufficient for the specific diagnosis and classification of mature T-cell leukemias.

FLOW CYTOMETRIC IMMUNOPHENOTYPING

The value of flow cytometric immunophenotyping in the diagnosis and monitoring of T-cell leukemias and lymphomas is generally underappreciated, in part due to long-heralded truisms. The common wisdom states that because T cells do not have an easily assessable phenotypic measure of clonality (analogous to immunoglobulin light chain expression on B cells), flow cytometry is of limited value in the diagnosis and management of T-cell leukemias and lymphomas. This concept would be true if clonality were the only important determinant in diagnosis. In fact, flow cytometry is highly useful in the workup of T-cell neoplasms for two main reasons. First, a majority (>90% in one study) of T-cell neoplasms show at least one demonstrable immunophenotypic aberrance on flow cytometric analysis (e.g., deletion of normally expressed antigens, differences in intensity of antigen expression compared to normal T cells), providing immunophenotypic evidence of malignancy. Second, the specific pattern of aberrance is often characteristic of a particular type of T-cell lymphoma or leukemia and therefore can be of help in subclassification. Most recently, the use of large T-cell receptor Vβ family–specific antibody panels that cover most of the T-cell receptor family repertoire has made immunophenotypic analysis for restricted T-cell receptor expression (as a surrogate for T-cell monoclonality) possible. However, antibodies to

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some families are not yet commercially available, and interpretation of a clonal population requires marked skewing of the normal T-cell repertoire distribution. Nonetheless, a high percentage of T-cell neoplasms show some immunophenotypic aberrancy. With these concepts and the fact that peripheral blood and bone marrow are excellent substrates for flow cytometry, there is a strong case for the routine flow cytometric analysis of either blood or bone marrow in the diagnostic evaluation of T-cell leukemias.

NK cells and some cytotoxic T-cell subsets also express a class of receptors known as killer-associated immunoglobulin-like receptors (KIRs). Recently, KIR isoforms have been used in the assessment of clonality of NK or T-LGL proliferations, analogous to the use of κ and λ light chain for clonality detection in B cells or the use of Vβ isoforms for clonality in T cells.

The effective application of flow cytometry to the diagnosis of T-cell leukemias also requires knowledge of the array of normal T- and NK-lymphocyte subsets that may be found in peripheral blood and bone marrow. A lack of knowledge of physiologic immunophenotypes of these subsets may lead to overdiagnosis of abnormal T-cell populations. For example, the normal (usually minor) subset of T cells that expresses the T-γδ receptor complex manifests as a population of T cells with modally brighter CD3 than other T cells and the absence of CD4, CD5, and CD8. Physiologic expansions of this normal subset may be interpreted erroneously as immunophenotypically aberrant T-cell populations. Similarly, dermal-derived T lymphocytes may show down-regulation of CD7 that could be erroneously interpreted as aberrant.

ANTIGEN RECEPTOR GENE REARRANGEMENT STUDIES

In the case of B-cell leukemias and lymphomas, clonality can be inferred by the restriction of B-cell populations to the expression of a single class of immunoglobulin light chain (either κ or λ). Unfortunately, the molecular components of the T-cell receptor complex come not in two types but in dozens, making their routine assessment challenging for diagnostic laboratories. As a result, the DNA-based assessment for clonal patterns of T-cell antigen receptor gene rearrangement has become the standard for assessment of T-cell clonality.

As noted in the previous discussion of flow cytometry, assessment of clonality per se is not always necessary in the diagnosis of either B- or T-cell neoplasms. However, because T-cell leukemias and lymphomas often present diagnostic challenges, and non-neoplastic conditions may enter the differential diagnosis, gene rearrangement studies are a relatively common part of their workup. With advances in polymerase chain reaction–based T-cell gene rearrangement studies, this once cumbersome analysis has evolved to become relatively simple, while preserving high levels of sensitivity and specificity.

CYTOGENETIC ANALYSIS

The diagnostic contribution of karyotypic analysis to mature T-cell leukemias varies depending on the specific disease entity, as noted in the individual descriptions that follow. In general, the cytogenetic analysis of mature, nonblastic leukemias is challenging because the cells in these disorders often do not have high proliferative rates, and standard cytogenetic analysis relies on the isolation of dividing cells. As a result, cells in such disorders can be analyzed using either cultures stimulated by mitogens or interphase methods (i.e., fluorescence in situ hybridization) that do not require the presence of dividing cells. In some instances, such as T-cell prolymphocytic leukemia (T-PLL), cells have a high proliferative rate and yield characteristic karyotypic abnormalities, making cytogenetic analysis an important aspect of diagnosis and classification.

SPECIFIC TYPES OF MATURE T-CELL AND NATURAL KILLER–CELL LEUKEMIAS

LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

Clinical Features

In the mid-1970s, there were reports of a distinct type of lymphoproliferative disorder marked by the leukemic proliferation of T cells containing prominent azurophilic cytoplasmic granules and expressing Fc receptors for the γ heavy chain of the immunoglobulin G molecule. This disorder has since been known by many names, including T-gamma lymphocytosis and large granular lymphocytosis, and it is now classified as T-cell LGL leukemia (T-LGL leukemia; Fig. 13.1). Although generally a leukemia of cytotoxic T lymphocytes, a subset of LGL leukemias may show a true NK-cell immunophenotype, as discussed in more detail later in this section.

LGL leukemia in its typical form is a clinically indolent disorder and usually manifests with a distinct array of clinical and laboratory findings. Median age at diagnosis is 60 years, with a relatively even prevalence in men and women. Although many reports stress that absolute peripheral blood lymphocyte counts may be normal at diagnosis, the median lymphocyte count in one large series was 8×10^9/L (approximately twice the upper limit of normal for adults), and most patients exhibit peripheral blood absolute lymphocytosis. Splenomegaly in the absence of lymphadenopathy is common at clinical presentation. Although approximately 60% of patients with LGL leukemia have a positive rheumatoid factor, frank rheumatoid arthritis is present in approximately one fourth of cases. Neutropenia is also common at presentation in LGL leukemia and is a major cause of morbidity in this disorder. Of interest, bone marrow biopsy specimens from patients with LGL leukemia generally show adequate granulocyte

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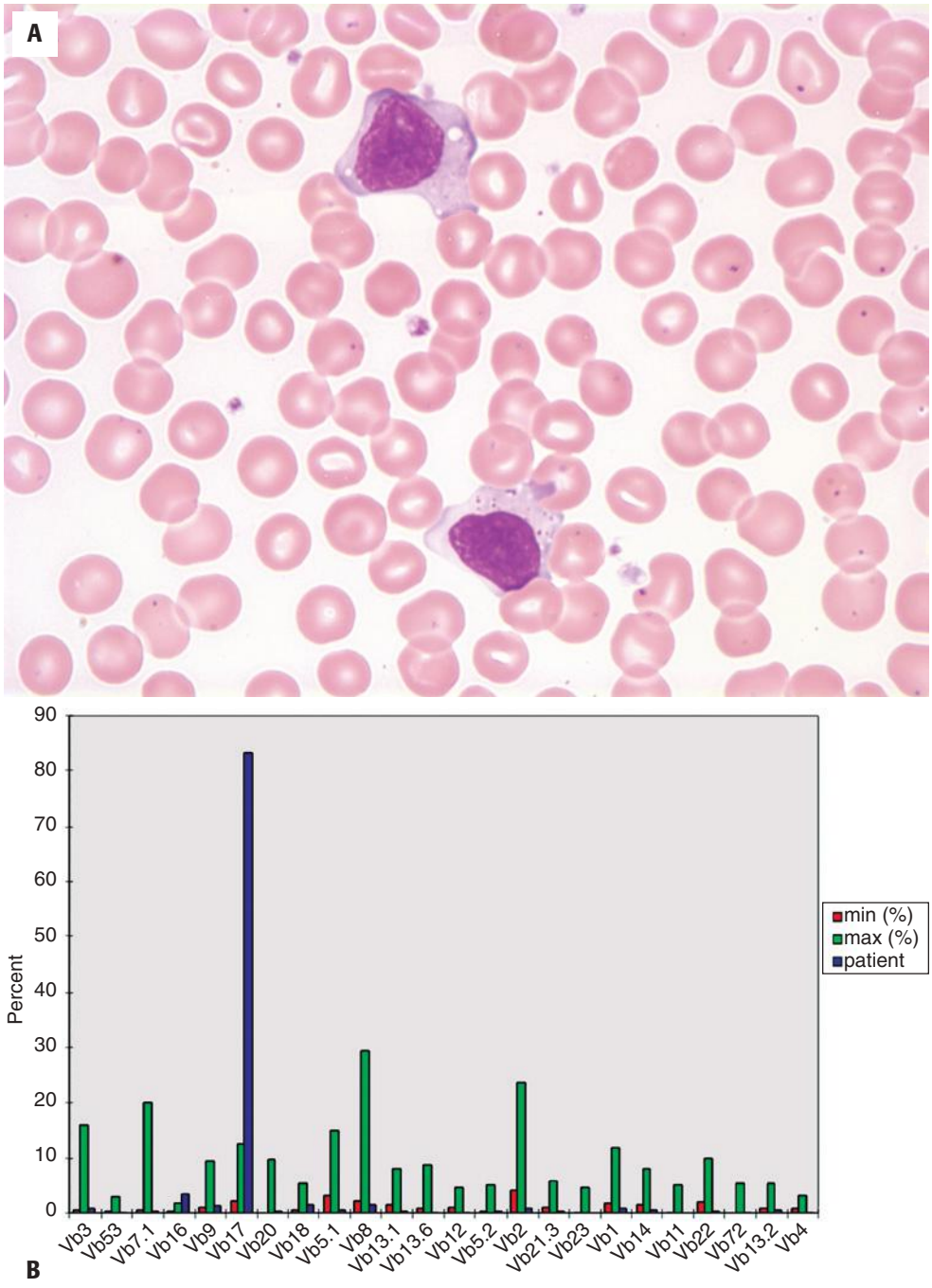


FIG. 13.1

T-cell large granular lymphocyte leukemia. A, Circulating large granular lymphocytes. B, Preferential V-β expression as a marker of clonality in circulating T cells. *(B, Courtesy Eric Hsi.)*

precursors. The mechanism of neutropenia in this disorder has not been fully characterized, but recent data suggest increased peripheral neutrophil apoptosis, possibly linked to increased levels of circulating Fas ligand (part of the Fas apoptotic pathway) in these patients. The complications of chronic neutropenia are commonly the reason for eventual therapeutic intervention in this otherwise generally nonaggressive neoplasm. LGL leukemia also has been linked to pure red cell aplasia in some patients. In fact, some studies indicate that large granular lymphocytic leukemia may be the most common underlying disease in patients with noncongenital pure red cell aplasia.

Following the initial descriptions of this disorder, LGL leukemia was often referred to as large granular lymphocytosis, because the disease typically follows an indolent clinical course (median survival >13 years in one series), and clonality was, at the time, difficult to determine in T-cell or NK-cell populations. Since then, however, the detection of clonal T-cell receptor gene rearrangements and clonal cytogenetic abnormalities in patients with the classic clinical and laboratory features of large granular lymphocytosis has established that this syndrome generally represents a clonal neoplastic disorder. Recent reports have indicated a potential association between LGL leukemia and subclinical detectable populations of clonal

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LARGE GRANULAR LYMPHOCYTIC LEUKEMIA—FACT SHEET

Clinical Features
- Splenomegaly (common)
- Rheumatoid factor positive in most patients, with frank rheumatoid arthritis in approximately one fourth of patients
- Infectious complications related to neutropenia

Peripheral Blood Morphology
- Variable absolute lymphocyte count (but usually >4 × 10^9/L)
- Predominance of LGLs
- Neutropenia (common)
- May be associated with pure red cell aplasia

Bone Marrow Morphology
- Lymphoid infiltrates may be scant or subtle
- Despite peripheral neutropenia, marrow generally shows normal granulopoiesis

Immunophenotype
- Usually the neoplastic cells are cytotoxic T cells that are positive for CD2, CD3, CD5, CD7, CD8, CD16, and CD57 and negative for CD4 and CD56
- Sometimes it is $CD56^+$; this has been associated with more aggressive behavior in some studies
- Occasionally patients with this specific clinical syndrome will show a true NK-cell phenotype ($CD2^+$, $CD3^-$, $CD5^-$, $CD7^{-/+}$, $CD8^{+/-}$, $CD16^+$, $CD56^+$, and $CD57^{-/+}$)

Genetics
- Clonal cytogenetic abnormalities may be present, but no single abnormality is characteristic
- Molecular studies show clonal T-cell antigen receptor gene rearrangement pattern in T-LGL leukemia. Cases with a true NK immunophenotype will not show clonal T-cell gene rearrangement pattern
- Activating signal transducer and activator of transcription (STAT3) mutations in up to 75% of cases of T-LGL and 48% of NK-LGL lymphocytosis

B cells (so-called monoclonal B-cell lymphocytosis) or monoclonal gammopathies.

Morphologic Findings

The leukemic cells of T-LGL are large lymphocytes with mature chromatin and moderate amounts of pale cytoplasm. Some cases may show mild nuclear indentation, but deep clefts or lobulations are not seen. The cytoplasm contains variably prominent azurophilic granules. Ultrastructurally, the azurophilic granules consist of parallel tubular arrays containing cytotoxic enzymes such as T-cell intracellular antigen (TIA-1), perforin, and granzyme. Bone marrow biopsy and immunostaining for CD3 or cytotoxic molecules characteristically shows a sinusoidal infiltrate.

Immunophenotype

A majority of LGL leukemias consist of cytotoxic T lymphocytes that express pan T-cell–associated antigens (e.g., CD2, CD3, CD5) and generally express the cytotoxic T-cell marker CD8 but are negative for the T-helper antigen CD4. Expression of CD5 is often decreased compared with normal T-cell populations, and in some cases CD5 may be absent. T-LGL leukemia cells also typically express the NK-cell–associated antigens CD16 (the Fc receptor-γ noted in original descriptions of LGL leukemia) and CD57 but are usually negative for the NK-cell–associated antigen CD56 (the neural cell adhesion molecule). Expression patterns of other markers not typically used in diagnostic immunophenotyping—specifically the absence of CD27, CD28, CD45RO, and CD62L (L-selectin) and the expression of CD45RA—are consistent with a constitutively activated terminal-effector memory T-cell phenotype, which may explain at least in part some of the autoimmune phenomena observed in many cases of LGL leukemia. The immunophenotype in LGL leukemia is characteristic but may not show frank antigenic aberrance compared with physiologic subsets of cytotoxic T lymphocytes. For that reason, T-cell antigen receptor gene rearrangement analysis should be used to confirm the diagnosis.

A minority of LGL leukemia cases consist of true NK lymphocytes. Indolent forms of NK-LGL leukemia may appear as a clinicopathologic syndrome indistinguishable from T-LGL leukemia. However, there are also forms of indolent NK LGL lymphocytosis that lack the full spectrum of LGL leukemia features such as organomegaly. True NK-LGL leukemias do not express surface CD3 but do express the NK-cell–associated antigens CD16 and CD56. In contrast to aggressive NK-cell leukemia, indolent NK-cell LGL proliferations are Epstein-Barr virus negative. Clonality has been demonstrated in a few cases by human androgen receptor assay, and more recently by demonstration of monotypic restriction of KIR subtype expression (Fig. 13.2). In addition, aberrant expression patterns of NK-associated antigens such as CD94 or CD161 have been cited as evidence of neoplasia in NK-cell populations in lieu of markers of clonality. Some investigators believe that at least some cases of chronic NK-cell lymphocytosis represent a chronic reactive or perhaps a clonal nonmalignant condition.

Genetic and Cytogenetic Findings

T-LGL leukemia is a monoclonal disorder, and, therefore, T-cell receptor gene rearrangement studies show clonal rearrangement patterns. Both the TCR-β and TCR-γ loci can usually be shown to be rearranged. The neoplastic nature of T-LGL leukemia was first revealed by the discovery of clonal cytogenetic abnormalities in this disorder. The initial report of clonal cytogenetic abnormalities in T-LGL leukemia described two cases: one with clonal trisomy 8 and one with clonal trisomy 14. Since then, numerous cytogenetic abnormalities have been described in T-LGL leukemia, but none is considered a unique or defining abnormality. These abnormalities include, among others, structural abnormalities of chromosomes 7 and 14 that are common to other T-cell neoplasms.

NK-LGL leukemia does not show clonal T-cell receptor gene rearrangements, but clonality or aberrancy can be

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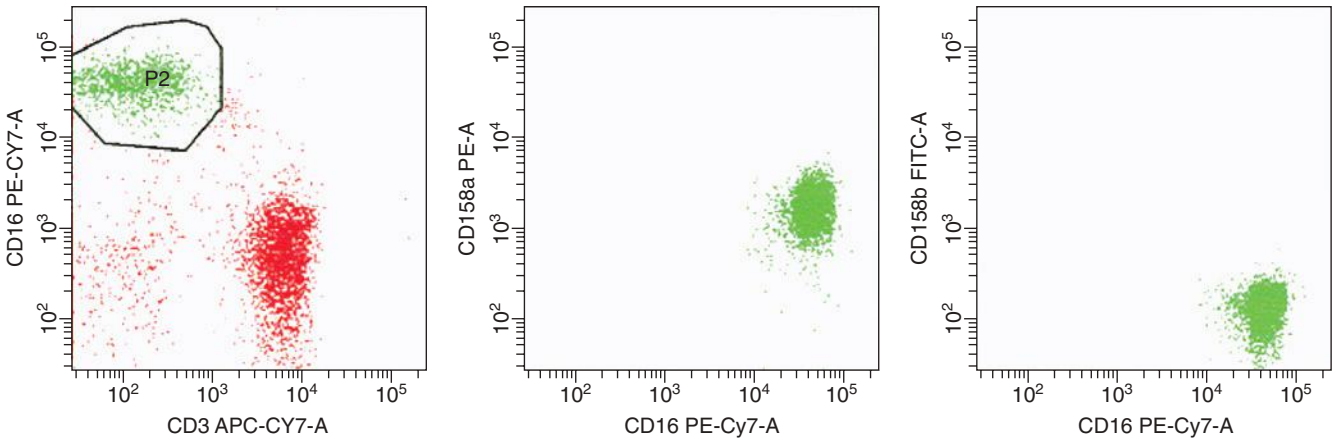


FIG. 13.2

Large granular lymphocyte leukemia of NK cells. Flow cytometry histogram of a peripheral blood sample shows that all NK cells in the sample ($CD3^-$, $CD16^+$, highlighted in green) are restricted to the expression of a single killer immunoglobulin-like receptor (KIR), in this case CD158a. *(Courtesy Curtis Hanson.)*

demonstrated in many cases by immunophenotypic assessment of KIR expression or abnormal deletion of NK-associated antigens.

Activating STAT3 mutations predominantly in exons 21 and 22, encoding the Src homology 2 domain, occur in 28% to 75% of T-LGL and 30% to 48% of NK-LGL cases and support the neoplastic nature of these indolent proliferations. The clinical significance of these mutations is uncertain.

Differential Diagnosis

Reactive Lymphocytosis

Some types of transient or non-neoplastic lymphocyte proliferations may be marked by a relative or absolute increase in circulating LGLs. The characteristic reactive T lymphocytes of infectious mononucleosis may occasionally harbor cytoplasmic granules; however, infectious mononucleosis typically is marked by striking morphologic heterogeneity among circulating lymphocytes, in contrast to the monotonous LGL proliferations typical of T-LGL leukemia.

A common but underrecognized form of reactive peripheral blood lymphocytosis, known as stress lymphocytosis, may occur following marked tissue injury (e.g., myocardial infarct, cerebrovascular accident, pulmonary embolus, and physical trauma). Stress lymphocytosis generally consists of a mixture of lymphocyte types, but circulating LGLs may be increased. However, stress lymphocytosis is typically transient, subsiding within hours or days and giving way to absolute peripheral neutrophilia. Some patients with HIV infection may demonstrate a transient $CD8^+$ T-cell lymphocytosis. A subset of these patients may develop persistent T-cell clones that phenotypically mimic T-LGL leukemia but lack typical clinical features of T-LGL leukemia. These appear to represent immune response to viral infection, and one should avoid overinterpretation of T-cell clones in the blood of such patients. If CD56 is expressed in a potential case of T-LGL leukemia, the leukemic phase of hepatosplenic T-cell lymphoma should be excluded. Features supporting the latter include hepatosplenomegaly, aggressive clinical course, hemophagocytosis, and presence of isochromosome 7q.

Posttransplantation Large Granular Lymphocyte Proliferations

Persistent T-cell LGL proliferations have been described in recipients of allogeneic hematopoietic stem-cell transplants. These proliferations may be polyclonal, oligoclonal, or monoclonal. Some data indicate that a relatively common immunophenotype in such cases includes expression of CD57 in the absence of either CD16 or CD56, in contrast to the $CD16^+$,$CD57^+$ phenotype typical of de novo T-LGL leukemias. Rare reports suggest that some of these posttransplantation LGL proliferations represent true neoplasms; however, the bulk of evidence seems to support that, despite the clonal nature of some of these cases, they likely represent non-neoplastic graft-versus-host phenomena in bone marrow or stem-cell transplant recipients. Recent data suggest lower relapse rates of primary diseases in patients with more robust LGL responses after hematopoietic stem-cell transplant, further supporting the notion that this represents a graft-versus-host phenomenon. Longer term follow-up, even in cases with substantial lymphocytosis and documented clonality, supports classification of this phenomenon as reactive rather than neoplastic. Similar LGL proliferations have also been described in some solid organ transplant recipients.

SÉZARY SYNDROME AND MYCOSIS FUNGOIDES

Clinical Features

Mycosis fungoides (MF) is a clinicopathologically distinct form of primary cutaneous T-cell lymphoma. A minority of

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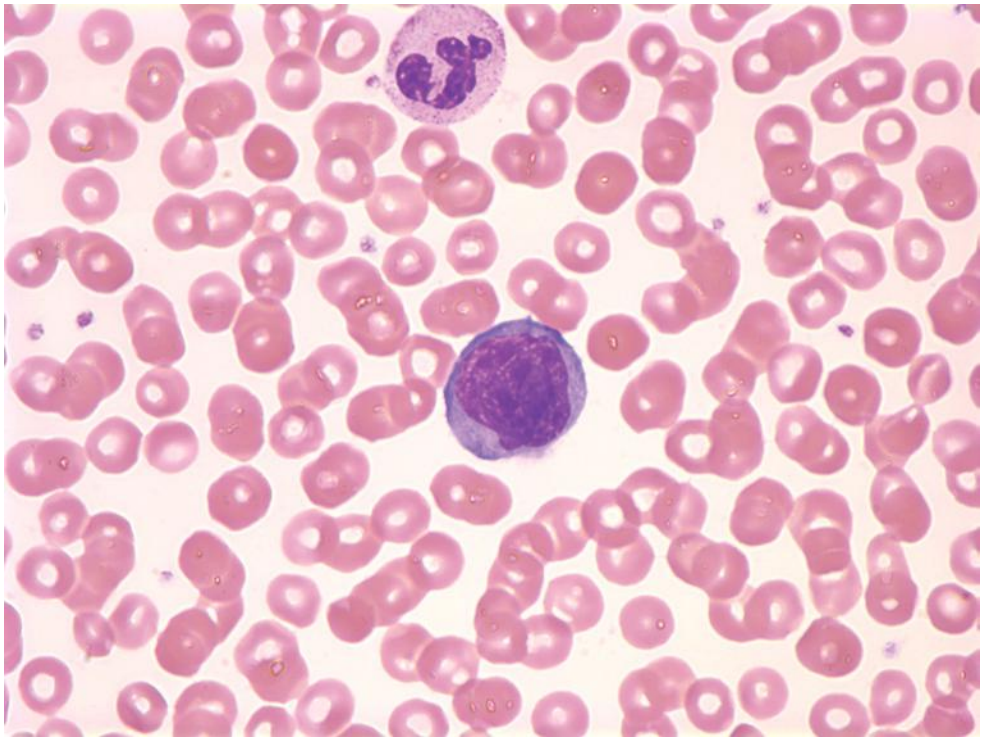


FIG. 13.3
Characteristic cerebriform nuclear morphology of circulating Sézary cells in a patient with Sézary syndrome–mycosis fungoides.

patients with MF develop a syndrome of diffuse erythroderma, diffuse lymphadenopathy, and leukemic involvement by the lymphoma. This constellation of findings is termed *Sézary syndrome* (SS), and the circulating lymphoma cells are termed *Sézary cells* (Fig. 13.3). The International Society for Cutaneous Lymphomas has set forth a proposed hematologic definition of Sézary syndrome that includes erythroderma plus one or more of the following:

- Minimum of 1000/mm^3 Sézary cells
- CD4-to-CD8 ratio greater than 10, owing to an increase in CD4$^+$ or CD3$^+$ cells by flow cytometry
- Aberrant expression of pan–T-cell antigens by flow cytometry
- Increased lymphocyte count with T-cell monoclonality by molecular techniques
- Abnormal clonal karyotype

MF has a variable clinical course, with numerous emerging therapeutic options. True SS is clinically aggressive. A recent study evaluating the International Society of Cutaneous Lymphomas/European Organization for Research and Treatment of Cancer (ISCL/EORTC) staging criteria observed a median survival of true SS of 3 years, with 26% survival at 5 years and 12% at 10 years.

SÉZARY SYNDROME—FACT SHEET

Clinical Features
- Leukemic phase of MF (cutaneous T-cell lymphoma)
- Marked by diffuse erythroderma, lymphadenopathy, and circulating lymphoma cells (Sézary cells)

Peripheral Blood Morphology
- Circulating Sézary cells: morphologically distinct, convoluted (cerebriform) cells

Immunophenotype
- Typical immunophenotype is T-helper cell with deletion of expression of CD7 (CD2$^+$, CD3$^+$, CD4$^+$, CD5$^+$, CD7$^-$, CD8$^-$), but other T-cell antigen deletions may be seen
- Expression of CD25 (interleukin 2 [IL-2] receptor) is variable or absent despite this being a therapeutic target for denileukin diftitox

Genetics
- Clonal cytogenetic abnormalities are common, but no single abnormality is characteristic

Morphologic Findings

The diagnosis of circulating Sézary cells relies heavily on careful microscopic examination of a routinely stained peripheral blood film. Classic Sézary cells present as distinctly abnormal, delicately convoluted cells that are generally larger than background benign lymphocytes (see Fig. 13.3). Smaller variants of Sézary cells are sometimes referred to as *Lutzner cells*. There may be histologic differences in skin biopsy specimens of patients exhibiting primary SS versus those who develop SS after an established phase of MF limited to the skin. Primary SS may lack the characteristic epidermotropic skin infiltrates of MF and instead may show subtler perivascular infiltrates. Some consider primary SS to be a

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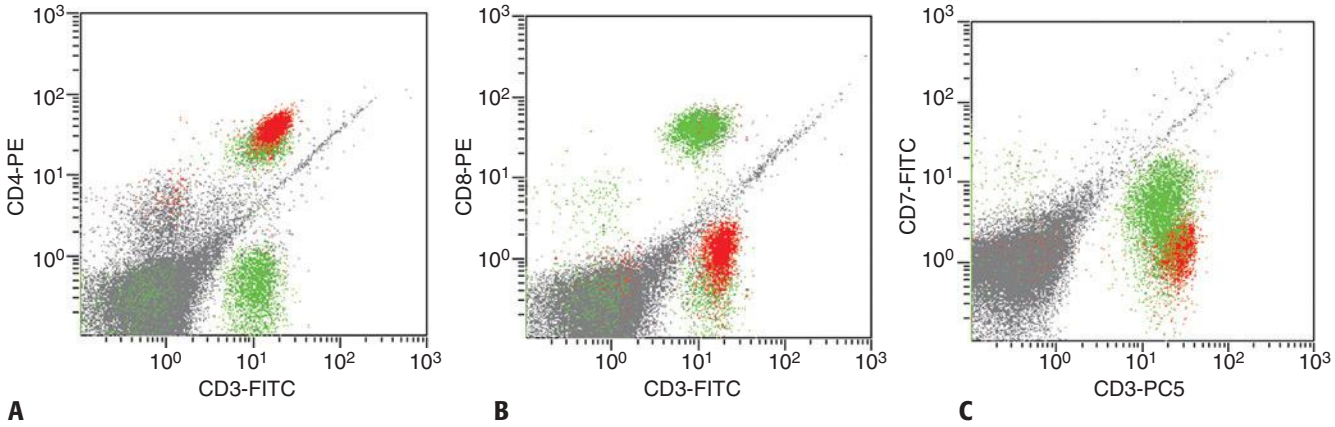


FIG. 13.4

Flow cytometric histograms displaying typical immunophenotypic findings in Sézary syndrome–mycosis fungoides. Background normal T cells *(green)* are contrasted with Sézary cells *(red),* which exclusively express CD4 (A) but not CD8 (B) and show deletion of the CD7 antigen (C). *FITC,* Fluorescein isothiocyanate; *PE,* phycoerythrin; *PC5,* phycoerythrin Cy5.

clinicopathologic entity distinct from secondary SS evolving in patients with established MF.

Immunophenotypic Findings

Although peripheral blood morphology remains a staple of assessment and staging in SS-MF, flow cytometric immunophenotyping can be helpful. Classically, Sézary cells are T-helper cells and will therefore express CD4 in the absence of CD8. Furthermore, deletion of expression of the pan–T-cell–associated antigen CD7 is noted in the Sézary cells of a large majority of patients (Fig. 13.4). Note, however, that physiologic (i.e., non-neoplastic) dermal T lymphocytes can display this same immunophenotype (including absence of CD7 expression), and that these cells can occasionally be detectable in circulation. Therefore, the combined evaluation of peripheral blood morphology and immunophenotype in difficult cases may be more useful than either evaluation alone.

Many clinical laboratories rely on the detection of the basic $CD3^+$, $CD4^+$, $CD7^-$, $CD8^-$ immunophenotype in the documentation of circulating Sézary cells; however, there are additional immunophenotypic studies that can be of help. Previous studies indicated that Sézary cells tend to express the immunophenotype of memory T-helper cells (also called *helper-inducer* or *inducer of help cells*)—that is, T-helper cells that assist in B-cell activation. These cells express CD29 and CD45RO but do not express CD45RA. This immunophenotype contrasts with the $CD45RA^+$, $CD29^-$, $CD45RO^-$ immunophenotype characteristic of naive T cells (also called *suppressor-inducer* or *inducer of suppression cells*) and may be of help diagnostically. More recently, there is evidence that assessment of CD26 (dipeptidyl aminopeptidase IV) may be of diagnostic use in SS-MF. Previous studies have indicated a $CD4^+$,$CD26^-$ immunophenotype in a large majority of samples with morphologic evidence of circulating Sézary cells and that this absence of CD26 expression can be useful in distinguishing Sézary cells from background T lymphocytes.

The assessment of CD25 expression in SS/MF has gained attention in recent years because CD25 is an IL-2 receptor molecule that is a target of denileukin diftitox, a diphtheria-toxin conjugated form of IL-2 approved for use in patients with MF whose malignant cells express CD25. However, most cases of MF do not express high levels of CD25 and many appear $CD25^-$ on routine flow cytometric evaluation. Furthermore, there is evidence that the efficacy of denileukin diftitox may not necessarily depend on CD25 expression on the neoplastic cells of SS-MF. To date, there has not been a clear consensus on the need for routine assessment of CD25 expression status in patients with SS-MF. It may be useful in the differential diagnosis with adult T-cell leukemia–lymphoma, which expressed CD25.

Cytogenetic Findings

Cytogenetic analysis is not a mainstay of diagnosis in SS-MF. Nonetheless, studies have shown high rates of clonal cytogenetic abnormalities in these disorders. Numerous recurring abnormalities have been identified, involving numerous different chromosomes. These karyotypic abnormalities may be complex, and no single abnormality is consistently associated with SS-MF.

Molecular Genetic Findings

High throughput sequencing and copy number studies of SS have varied findings but commonly mutated genes (on the order of 10% of cases or more) including epigenetic regulators *(ARD1A),* JAK/STAT pathway genes, T-cell signaling pathway genes *PLCG1* and *CARD11, TP53,* and *FAS.* Deletion of *TP53* and *CDKN2A* are also seen in over 50% of cases.

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Differential Diagnosis

SS-MF may show considerable overlap with HTLV-1–associated ATLL, and this differential diagnosis is discussed in the next section of this chapter. T-PLL may also show clinical and morphologic overlap with SS-MF, including skin involvement and circulating convoluted lymphocytes. However, marked nuclear convolution, although sometimes present, is less common in the cells of T-PLL than in the cells of SS-MF, and the characteristic (albeit not entirely specific) immunophenotype of SS/MF may help to distinguish it from T-PLL. Furthermore, the inv(14)(q11q32) on cytogenetic analysis is more characteristic of T-PLL than of SS-MF. Ultimately, however, careful correlation with the clinical pattern may be necessary for reliable distinction between these two disorders. In actual practice, a diagnosis of MF has often been made by skin biopsy and clinical assessment before the evaluation of peripheral blood, aiding considerably in differential diagnosis.

ADULT T-CELL LEUKEMIA–LYMPHOMA ASSOCIATED WITH HUMAN T-LYMPHOTROPIC VIRUS 1

Clinical Features

HTLV-1 is a retrovirus, endemic in many areas worldwide, particularly southwestern Japan, Central Africa, and the Caribbean. HTLV-1 has been linked to the development of a specific clinicopathologic syndrome known as ATLL. Although the link is well established, epidemiologic studies from high-prevalence regions indicate a long latency period for HTLV-1, with a lifetime ATLL risk of approximately 2.5% in infected individuals.

ATLL occurs in adults (median age, 56 years; range, 27 to 82 years) and has four major clinical presentations: acute, lymphomatous, chronic, and smoldering. The acute subtype (55% to 60% of cases) is marked by severe peripheral blood lymphocytosis (often greater than 100×10^9/L), numerous "flower" cells in the peripheral blood (Fig. 13.5), hypercalcemia, hepatosplenomegaly, elevated serum lactate dehydrogenase, and a rapidly progressive course with a median survival of 6 months despite multiagent chemotherapy. The acute variant may also present with lytic bone lesions, likely because of a release of cytokines that accelerate bone resorption—the same process that results in hypercalcemia at presentation. The chronic subtype (15% to 20% of cases) can manifest with a peripheral blood absolute lymphocytosis and circulating flower cells, but generally the lymphocyte count is much lower than that seen in the acute variant. Exfoliative skin rashes may be seen in chronic ATLL, but hypercalcemia is generally absent. The median survival for chronic ATLL is more than 2 years, but most patients die within 5 years because of progression to acute or lymphomatous ATLL. Smoldering ATLL (approximately 5% of cases) is marked by a normal peripheral blood leukocyte count, with circulating neoplastic cells constituting less than 5% of total leukocytes. Although skin rashes can develop in this variant, patients with smoldering ATLL do not have hepatosplenomegaly, hypercalcemia, or lymphadenopathy. The median survival for smoldering ATLL is also more than 2 years but less than 10 years because of the progression to acute ATLL or to infectious complications. Finally, presentation as lymphoma without leukemia occurs in approximately 20% to 25% of patients and has an aggressive clinical course with a median survival of approximately 1 year. These patients have generalized lymphadenopathy but generally do not have hepatosplenomegaly, skin involvement, or hypercalcemia; some patients may develop leukemia during the course of disease.

Lymphomagenesis in HTLV-1 infection is a complex, multiple-step process. This concept is manifest in the long latency period between HTLV-1 infection and onset of ATLL, and in the low lifetime risk of ATLL in HTLV-1–infected individuals. HTLV-1 randomly integrates into the genome of infected cells; in other words, the pattern of HTLV-1 integration is distinct in every infected cell, except in cells that are clonally related. The virus encodes for two oncoproteins, p40 Tax protein and HTLV-1 basic leucine zipper factor (HBZ), which are thought to have a key role in the development of ATLL in chronically infected individuals. Tax activates the viral promoter and the cyclic AMP and nuclear factor κB pathways. Anti-apoptotic proteins are upregulated and p53 is suppressed. HBZ, which is consistently expressed in all cases of ATLL, is thought to contribute to cellular proliferation and survival of the clone. Epigenetic alterations are also involved in the

HUMAN T-LYMPHOTROPIC VIRUS 1–ASSOCIATED ADULT T-CELL LEUKEMIA–LYMPHOMA—FACT SHEET

Clinical Features
- Often divided into four clinical subtypes: acute, chronic, smoldering, and lymphomatous
- Long latency period between HTLV-1 infection and development of ATLL
- Low overall lifetime risk of ATLL in chronically HTLV-1–infected individuals
- Virally encoded p40 Tax and HBZ oncoproteins are important in leukemogenesis
- May show substantial clinical overlap with MF-SS
- Confirmation of HTLV-1 infection (by serology or otherwise) required for diagnosis

Peripheral Blood Morphology
- Bizarre flower-like lymphocytes

Immunophenotype
- Usually T-helper phenotype, $CD3^+$, $CD4^+$, $CD8^-$; cells generally $CD7^-$, $CD25^+$
- FOXP3, a feature of T-regulatory cells, is often expressed

Genetics
- Clonal cytogenetic abnormalities may be seen, but no single abnormality is linked to this disease
- Clonal integration of HTLV-1 genome can be demonstrated

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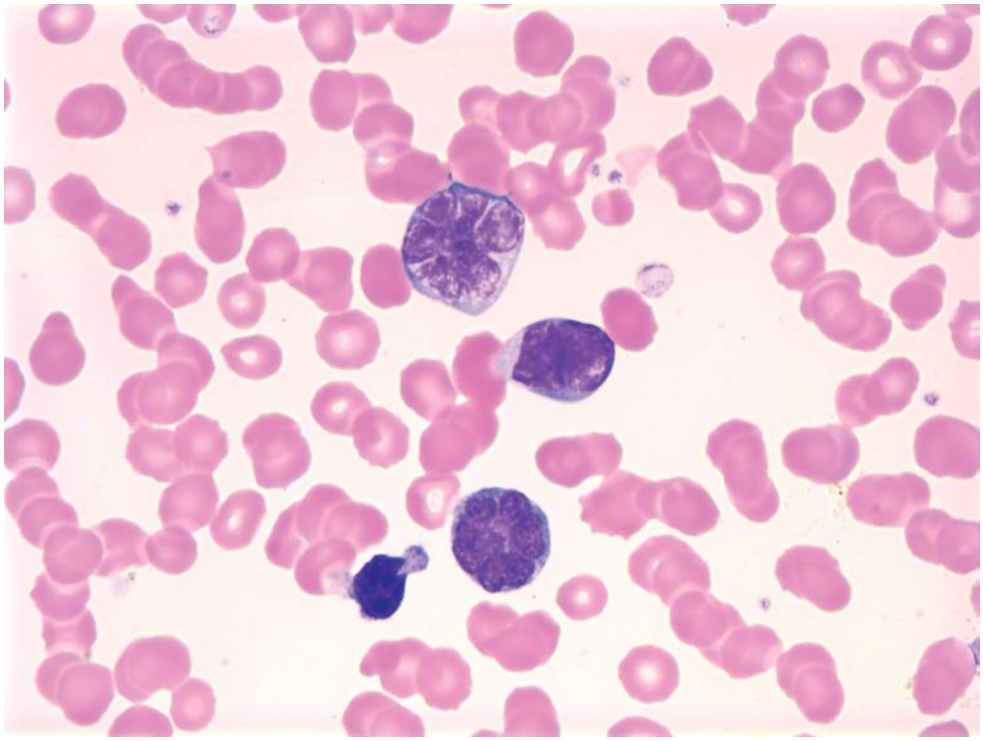


FIG. 13.5

Adult T-cell leukemia–lymphoma (ATLL) associated with the human T-lymphotropic virus type 1. Flower-cell morphology of circulating neoplastic cells in a patient with ATLL associated with the human T-lymphotropic virus type 1.

development of ATLL, and hypermethylation is associated with disease progression.

Morphologic Findings

The most characteristic morphologic feature of ATLL is the presence of medium to large multilobate lymphocytes or flower cells in the peripheral blood. These cells are larger than normal peripheral blood lymphocytes and have moderately condensed chromatin, absent or small nucleoli, scant slightly basophilic cytoplasm, and nuclear multilobation (see Fig. 13.5). The leukemic cells are distinguished from Sézary cells by nuclear outpouching rather than complex nuclear infolding. The white blood cell count ranges from 25,000 to 450,000 × 10(9)/L in acute ATLL. In contrast, chronic ATLL tends to have only mildly to moderately elevated white blood cell counts, with more than 10% leukemic cells. Leukemic cells may be seen in the peripheral blood in smoldering ATLL but do not give rise to peripheral lymphocytosis (<3% leukemic cells).

Lymph nodes in acute ATLL and lymphomatous ATLL are virtually always involved. The low-magnification growth pattern is always diffuse, usually with totally effaced architecture except for occasional dilated sinuses containing individually scattered tumor cells. At high magnification, the prominent multilobation so typical of leukemic cells in the peripheral blood may be inconspicuous. However, tumor cells in lymph nodes are invariably pleomorphic cells, and most cases have a mixture of small to large pleomorphic cells with medium to large cells predominating. Occasional lymph nodes may have a predominance of small pleomorphic cells. Nuclear contours usually appear irregular, cleaved, or lobated but can occasionally appear cerebriform. Occasional lymphomas may have Reed-Sternberg–like cells.

Bone marrow biopsy specimens are usually involved by ATLL, but the degree of marrow involvement is often sparse or mild in comparison to the degree of peripheral blood involvement. Occasionally marrow involvement may be extensive, but marrow infiltration usually does not lead to hematopoietic abnormalities. The pattern of marrow involvement is diffuse and interstitial or sinusoidal and may be subtle.

Cutaneous involvement by ATLL occurs in 25% to 60% of patients with acute ATLL. Other sites commonly involved by ATLL include the liver, spleen, lungs, and central nervous system.

Immunophenotypic Findings

Immunophenotyping by flow cytometry may be helpful in further evaluating the abnormal cells in ATLL. By definition, ATLL cells are post-thymic T cells and will therefore express surface CD3. They are also generally positive for various pan–T antigens such as CD2 and CD5 and display a T-helper phenotype (positive for CD4 but negative for CD8); however, aberrant loss of the normally expressed T-cell antigen CD7 is a consistent finding in ATLL. Finally, ATLL cells generally express the α-chain of the interleukin-2 receptor CD25. FOXP3, which is a marker of T-regulatory cells, is expressed in many cases of ATLL.

Cytogenetic Findings

As with SS-MF, cytogenetic analysis of cases of ATLL have revealed numerous and complex chromosomal

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abnormalities; however, no single karyotypic abnormality is diagnostically linked to HTLV-1–associated ATLL. Therefore cytogenetic analysis is not essential in the workup of this disorder.

Molecular Genetic Findings

Molecular genetic studies of ATLL have identified recurrent alterations such as gain-of-function mutations in the T-cell receptor/NF-κB signaling pathway *(PLCG1, PRKCB, CARD11,* and *VAV1)* and *CTLA4-CD28* and *ICOS-CD28* gene fusions. Molecules involved in immune surveillance, including HLA-A/B, CD58, PDL1, and FAS/CD95, are also recurrently affected.

Differential Diagnosis

To the diagnostic pathologist, the differential diagnosis of ATLL includes several other T-cell neoplasms that can involve the blood at presentation, including T-PLL, SS-MF, and the leukemic phase of other peripheral T-cell lymphomas (including the small-cell variant of ALCL that can present in leukemic phase). The cells of T-PLL can often show some degree of nuclear irregularity, but the frank flower-cell morphology is somewhat unusual for T-PLL. Because ATLL often involves the skin at presentation, it can mimic SS-MF closely in both its morphologic and clinical features. Both ATLL and SS-MF are marked by the presence of circulating convoluted lymphocytes. However, the convolutions seen in SS-MF often impart an inwardly folded, creased, cerebriform look to the cells (see Fig. 13.3), whereas ATLL cells tend to have more outward nuclear projections (see Fig. 13.5). ATLL and SS-MF also frequently share the immunophenotypic finding of loss of the CD7 antigen. However, CD25 expression is almost always seen in ATLL, whereas it may be variable or absent in SS-MF and can therefore be a useful marker in the differential diagnosis of these entities. Although these differences can be helpful, there remains significant overlap clinically and diagnostically between ATLL and SS-MF. Therefore, ultimately, the distinction of ATLL from other T-cell neoplasms depends on documenting infection of the patient by HTLV-1, by either serologic analysis or detection of clonal integration of the HTLV-1 genome into neoplastic cells by molecular techniques.

T-CELL PROLYMPHOCYTIC LEUKEMIA

Clinical Features

Prolymphocytic leukemia (PLL) was originally described in 1973 and was thought at the time to be a variant of chronic lymphocytic leukemia. Because the neoplastic cells in this disorder displayed prominent single nucleoli, the term *prolymphocytic* was used to describe them, despite subsequent studies that confirmed these cells were mature and did not arise from lymphoid precursor cells. Subsequent studies showed that a subset of cases classifiable as PLL by morphologic criteria were of the T-cell type (with the majority being of the B-cell type). Since then, there has been a substantial reappraisal of B-cell PLL as a distinct diagnostic entity, with many reported cases of B-cell PLL appearing to represent mantle cell lymphoma or other distinct B-cell lymphoma subtypes. However, T-PLL remains a well-defined disease entity, with distinct clinical, morphologic, immunophenotypic, and genetic features.

Clinically, T-PLL manifests with profound leukocytosis, often in excess of 100×10^9/L, because of the proliferation of circulating prolymphocytes (Fig. 13.6). T-PLL typically also appears with splenomegaly, which is often marked. T-PLL may also appear with diffuse lymphadenopathy, a finding that is rare in its B-cell counterpart. T-PLL may occur more commonly in patients with ataxia-telangiectasia, possibly because of abnormalities in the *ATM* gene associated with that disorder. The disease course of T-PLL has generally been considered aggressive, with median survivals of 12 months or less. A subgroup of patients with an initial indolent course

T-CELL PROLYMPHOCYTIC LEUKEMIA—FACT SHEET

Clinical Features
- T-PLL often develops with blood leukocyte counts >100 × 10^9/L
- Splenomegaly is common; lymphadenopathy may also be present
- Median survival <1 year, even with treatment
- Some cases are associated clinically with ataxia-telangiectasia; these cases may involve the ATM gene on chromosome 11q23

Peripheral Blood Morphology
- Larger cells with distinct central nucleoli (prolymphocytes)
- Small cells with indistinct or absent nucleoli (small cell variant of T-PLL, sometimes reported as T-cell chronic lymphocytic leukemia)

Immunophenotype
- Usually (>60%) a T-helper phenotype: $CD3^+$, $CD4^+$, $CD8^-$
- May show aberrant deletion of one or more T antigens
- Minority of cases (approximately 20%) show CD4/CD8 coexpression (more common in the small-cell variant, one of the rare instances in which mature T cells coexpress CD4 and CD8)
- An even smaller minority (10% to 15%) showing cytotoxic–suppressor phenotype: $CD3^+$, $CD4^-$, $CD8^+$
- TCL-1 expressed in 70% of cases

Genetics
- T-PLL is often associated with a specific cytogenetic abnormality: inv(14)(q11;q32), which juxtaposes the T-cell α/δ gene complex at 14q11 with the TCL-1 gene at 14q32. (Note: This is not to be confused with the 14q32 breakpoint common in B-cell lymphomas, which involves the immunoglobulin heavy chain joining region)
- Other cytogenetic abnormalities may be seen, including trisomy for the long arm of chromosome 8

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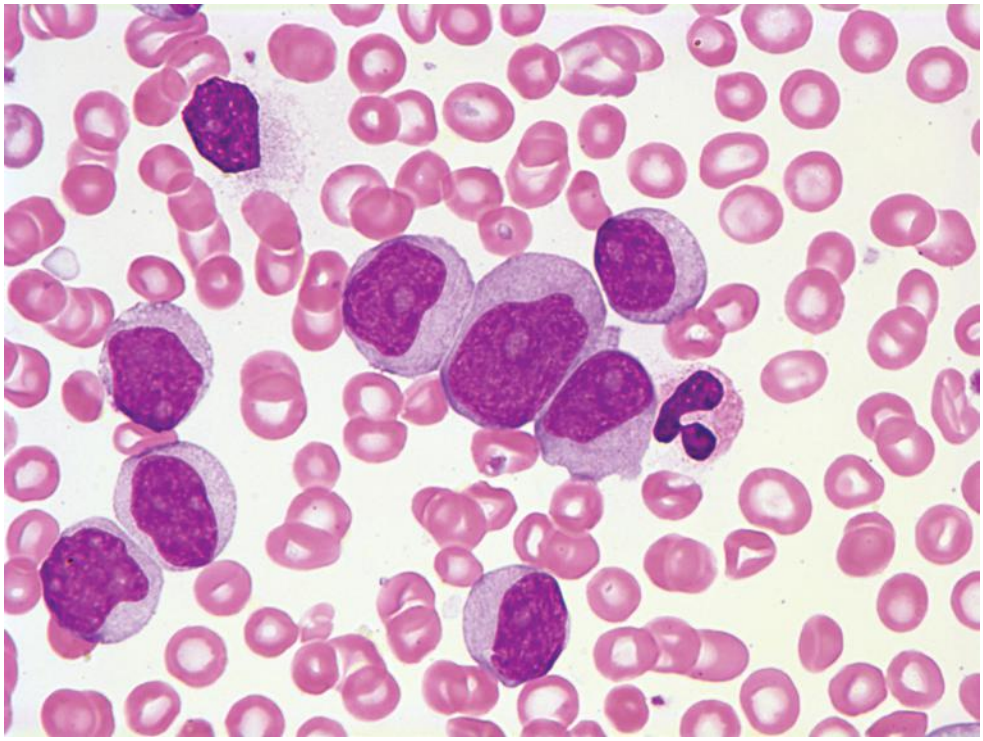


FIG. 13.6
Circulating neoplastic prolymphocytes in a patient with T-cell prolymphocytic leukemia.

has been described. This form had a median survival of 51 months in one study; however, many patients progressed to an aggressive phase with short survival, similar to that classically recognized. Treatment with a monoclonal antibody to CD52 (alemtuzumab) has been reported to induce remission and shows promise in improving survival.

Morphologic Findings

Morphologically, T-PLL comes in two forms. In its more typical form, the circulating neoplastic cells display single prominent central nucleoli. These cells are often round or oval but may show irregular nuclear contours (see Fig. 13.6). A second morphologic variant, referred to as the *small-cell variant of T-PLL,* is marked by the proliferation of small round lymphocytes lacking visible nucleoli on light microscopic evaluation, although distinct single nucleoli are visible in these cells by electron microscopy. The small-cell variant does not appear to be a clinically or biologically distinct disease, but it is important to the diagnostic hematopathologist for purposes of differential diagnosis and classification. Involvement of extramedullary sites occurs with skin, lymph nodes, and liver being common sites. The morphologic findings are not specific, and clinical history is often needed to arrive at the correct interpretation. Fortunately, the diagnosis of T-PLL has usually been established in blood or bone marrow.

Immunophenotypic Findings

The immunophenotype of T-PLL is somewhat variable. In addition to expressing major pan–T-cell–associated antigens, most cases (>60%) express CD4 in the absence of CD8. Approximately 20% coexpress CD4 with CD8, and this subgroup of dual $CD4^+/CD8^+$ cases is weighted toward the small-cell variant of T-PLL. Finally, a smaller minority (approximately 10% to 15%) are positive for CD8 in the absence of CD4. Although aberrance in the level of expression of pan–T-cell antigens may be seen, there is no single pattern of antigen deletion that characterizes T-PLL. However, the finding of dual CD4 and CD8 expression in approximately 20% of cases is important because this is one of the rare instances in which a nonblastic (post-thymic) T-cell neoplasm expresses this immunophenotype. TCL-1 expression may be diagnostically useful. It is expressed in most cases of T-PLL (70% to 80% of cases) but not other T-cell malignancies; however, it is also expressed in some B-cell neoplasms. Therefore, in the setting of T-cell malignancy, it appears to be a relatively specific but not completely sensitive marker for T-PLL.

Cytogenetic Findings

Specific cytogenetic abnormalities are characteristic of T-PLL, although not exclusive to it. The most commonly cited recurring abnormality is a paracentric inversion of chromosome 14, inv(14)(q11q32), that involves juxtaposition of the T-cell receptor α gene (*TRA@*) complex and the *TCL1* oncogene. It is important to note that the band (q32) on the long arm of chromosome 14 associated with the *TCL1* gene is the same as that harboring the immunoglobulin heavy chain joining region that is involved in many different types of B-cell lymphomas. Therefore, it is important not to infer lineage based solely on the presence of a chromosome 14q32 abnormality on karyotypic analysis. Other abnormalities,

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including trisomy for the long arm of chromosome 8, have also been associated with T-PLL.

Molecular Genetic Findings

Molecular genetic studies have shown that activating mutations in *IL2RG, JAK1, JAK3,* and *STAT5B* are common (70% of cases) and mutually exclusive. These mutations conferred growth advantage in cell culture model systems in vitro. Studies have also found mutations in epigenetic regulators enhancer of zeste 2 (EZH2) (13%), ten-eleven-translocation-2 (TET2) (17%), and BCOR (9%). These data inform us on the biology of T-PLL and provide rationale for treatment aimed at blocking the JAK/STAT pathway and possible use of epigenetic modifiers.

Differential Diagnosis

The differential diagnosis of T-PLL is discussed in the sections describing other T-cell leukemias. One semantic point, however, warrants discussion. The term *T-cell chronic lymphocytic leukemia* (T-CLL) is sometimes used to describe the entity now generally classified as the small-cell variant of T-PLL. However, the term *T-CLL* is discouraged for two important reasons. First, clinical and cytogenetic data, including documentation of the inv(14)(q11q32) in many reported T-CLL cases, support the concept that small-cell T-PLL is in fact the same biologic entity as T-PLL. Second, the term *T-CLL* has been used somewhat loosely in the literature, and some reports of T-CLL describe what is known as *T-LGL leukemia*. Because T-LGL leukemia is an indolent condition and T-PLL is highly aggressive, confusion of these two entities could have profound clinical ramifications.

AGGRESSIVE NATURAL KILLER–CELL LEUKEMIA

Clinical Features

Some cases of LGL leukemia that show a true NK phenotype are associated with a highly aggressive clinical course. Aggressive NK-cell leukemia is rare in North America but is prevalent in Japan and other East Asian nations. This disease entity generally follows a fulminant clinical course and is therefore classified in the World Health Organization (WHO) classification of hematopoietic neoplasms as aggressive NK-cell leukemia.

Because of its rarity, most of the clinical information about this disorder is based on fairly small case series. However, typical reported signs and symptoms at presentation include fever, lymphadenopathy, and hepatosplenomegaly. Peripheral blood counts vary widely at presentation, ranging from frank leukopenia to leukocyte counts in excess of 200×10^9/L. As is the case with certain peripheral T-cell lymphomas, patients may have a concomitant hemophagocytic syndrome and coagulopathy, but these findings are not reported consistently across all series. Regardless of therapeutic intervention, clinical progression in aggressive NK-cell leukemia is usually rapid, with median survival in larger retrospective series reported as less than 2 months from the time of diagnosis.

AGGRESSIVE NATURAL KILLER–CELL LEUKEMIA—FACT SHEET

Clinical Features
- Rare disorder, more prevalent in the Eastern Hemisphere
- Aggressive clinical course with survival often measured in weeks to months
- Develops with fever, hepatosplenomegaly, lymphadenopathy
- May represent the leukemic form of extranodal NK-cell–T-cell lymphoma of nasal type, but some clinical differences reported (e.g., prevalence of organomegaly and lymphadenopathy in aggressive NK-cell leukemia but not commonly in the nasal type NK-cell–T-cell lymphoma)
- Strong association with Epstein-Barr virus infection

Morphology
- Atypical lymphocytes (slightly larger than LGLs), variably prominent nucleoli, cytoplasmic granules

Immunophenotype
- Can be distinguished from mimics based on clinical presentation and on true NK immunophenotype (surface $CD3^-$, cytoplasmic $CD3\varepsilon^+$, $CD2^+$, $CD4^-$, $CD5^-$, $CD7^{-/+}$, $CD8^{-/+}$, $CD16^+$, $CD56^+$, $CD57^{-/+}$, cytotoxic molecules [TIA-1, granzyme B, perforin] positive)

Genetics
- Clonal T-cell antigen receptor gene rearrangements are absent
- Epstein-Barr–encoded RNA positive

Like the nasal-type T-cell–NK-cell lymphomas prevalent in the Eastern hemisphere, the cells of aggressive NK-cell leukemia are almost always positive for the Epstein-Barr virus; therefore in situ hybridization for the Epstein-Barr–encoded RNA may be of diagnostic utility. Indeed, aggressive NK-cell leukemia is regarded by some investigators as representing the leukemic phase of extranodal T-cell–NK-cell lymphoma of the nasal type, based on similar epidemiologic distribution and Epstein-Barr virus association. Rare cases of EBV-negative aggressive NK-cell leukemia have been described but appear to be similar to EBV-positive cases in pathologic and clinical features, including a very poor prognosis. Differences in clinical presentation (including higher prevalence of lymphadenopathy and organomegaly in aggressive NK-cell leukemia) and recent data suggesting differences in genomic alteration patterns based on comparative genomic hybridization have left open the possibility that aggressive NK-cell leukemia and T-cell–NK-cell lymphoma are distinct entities.

Morphologic Findings

Aggressive NK-cell lymphoma generally manifests as a leukemia of LGLs. Examination of the peripheral blood smears of patients with NK-cell–LGL leukemia often reveals markedly

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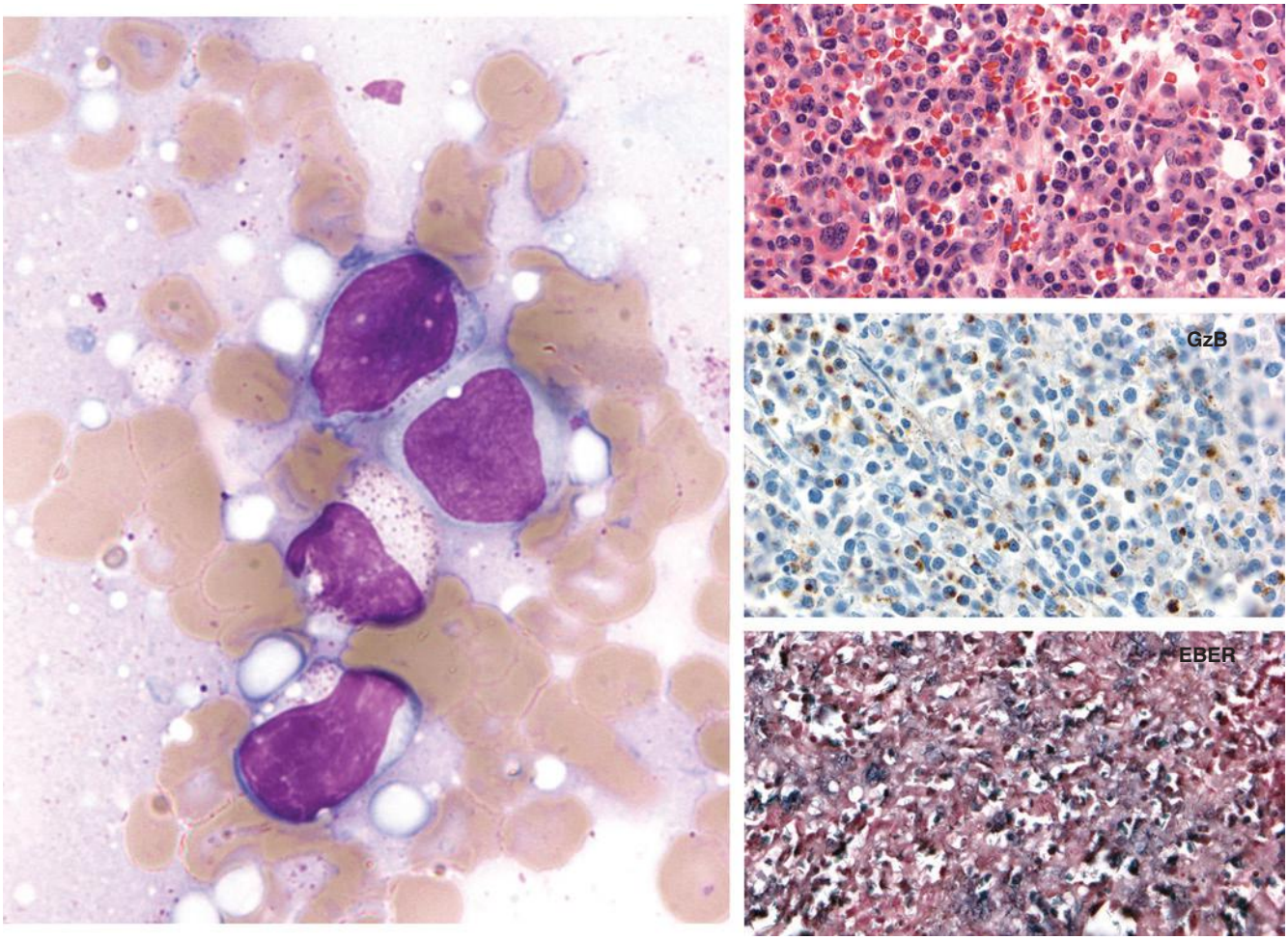


FIG. 13.7

Aggressive natural killer–cell leukemia. The disease manifested in this patient with blood, bone marrow, and liver involvement. Flow cytometry showed an immunophenotype of $CD2^+$, cytoplasmic $CD3^+$, $CD5^-$, $CD7^+$, $CD16/56^+$, $CD57^-$. The bone marrow aspirate smear shows atypical lymphoid cells with cytoplasmic granules *(left)*. The trephine biopsy specimen showed an interstitial infiltrate of neoplastic lymphocytes that also expressed cytotoxic proteins such as granzyme B and were positive for Epstein-Barr–encoded RNA. *(Courtesy Eric Hsi.)*

pleomorphic lymphoid cells in contrast to the relatively monotonous, mature-appearing cells of T-LGL leukemia. Bone marrow involvement can be a subtle interstitial infiltrate or obvious diffuse involvement (Fig. 13.7).

Immunophenotypic Findings

As noted, aggressive NK-cell leukemias generally display a true NK-cell immunophenotype: surface CD3-negative (but positive for the cytoplasmic CD3ε), positive for CD2, CD16, and CD56, variably positive for CD7 and CD8, and negative for CD4. CD57 may be expressed in a minority of cases but is usually negative. Other pan–T-cell–restricted antigens are generally negative. Epstein-Barr virus infection can be demonstrated by immunohistochemistry or Epstein-Barr–encoded RNA in situ hybridization.

Cytogenetic Findings

There is no consistent cytogenetic abnormality associated with aggressive NK-cell leukemia, although abnormalities of chromosome 6 (both the long arm and the short arm) appear relatively common. Numerous clonal abnormalities, including some also described in other T-cell or NK-cell disorders, have been reported. T-cell antigen receptor gene rearrangement studies will not show evidence of clonal rearrangement in this disorder.

Differential Diagnosis

The presenting signs and symptoms of aggressive NK-cell leukemia may overlap with other T-cell and NK-cell neoplasms. As noted previously, this disorder may simply represent the leukemic phase of extranodal NK/T-cell lymphoma of the nasal type and therefore may show substantial clinical overlap. Aggressive NK-cell leukemia, however, generally shows a greater propensity for lymph node involvement and organomegaly. Furthermore, the skin involvement commonly reported in nasal type NK-cell–T-cell lymphomas is unusual in aggressive NK-cell leukemia.

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The organomegaly and constitutional symptoms at presentation overlap considerably with hepatosplenic T-cell lymphoma. However, hepatosplenic T-cell lymphoma will usually not have the lymphadenopathy associated with aggressive NK-cell leukemia. Furthermore, the neoplastic cells of hepatosplenic T-cell lymphoma have a distinct T-cell immunophenotype, including expression of surface CD3 (not seen in aggressive NK-cell leukemia) and deletion of numerous T-cell lineage antigens. Furthermore, although neoplastic cells may be seen in the peripheral blood in cases of hepatosplenic T-cell lymphoma, a true leukemic phase of this disorder is rare.

LEUKEMIC PHASE OF OTHER PERIPHERAL T-CELL LYMPHOMAS

This chapter has highlighted T-cell neoplasms that tend to manifest clinically as leukemia. However, many different types of peripheral T-cell lymphomas can involve the peripheral blood at some point in their clinical course. Because some of these lymphomas may show considerable morphologic and immunophenotypic overlap with the entities described in this chapter, it is important for diagnostic pathologists to interpret the presence of abnormal circulating T cells in the appropriate clinical context. Precise classification of peripheral T-cell lymphomas that do not commonly manifest in the leukemic phase generally depends on examination of an enlarged lymph node or other primary site of tissue involvement.

LYMPHOCYTIC VARIANT HYPEREOSINOPHILIC SYNDROMES

Clinical Features

The term *hypereosinophilic syndrome* was used historically to describe a diverse set of clinicopathologic conditions marked by persistent unexplained peripheral blood eosinophilia and evidence of end-organ damage by eosinophilic infiltration (see Chapter 17). More recently, many processes previously classifiable as hypereosinophilic syndrome have been identified as true eosinophilic neoplasms linked to genetic abnormalities affecting specific loci encoding for growth factor receptors, particularly platelet-derived growth factor receptor, α subunit (PDGFRA); platelet-derived growth factor receptor, β subunit (PDGFRB); and fibroblast-derived growth factor 1 (FGFR1). Eosinophilic neoplasms linked to these growth factor receptor genes are currently classified under the WHO category of "myeloid/lymphoid neoplasms with eosinophilia and rearrangements of PDGFRA, PDGFRB, or FGFR1," and many hypereosinophilic syndromes that lack these abnormalities remain idiopathic. A subset of clinical hypereosinophilic syndromes, however, appear to involve the non-neoplastic proliferation of eosinophils in response to cytokine release from abnormal (and usually clonal) T-cell subsets. Such processes have been termed *lymphocytic variant hypereosinophilic syndromes* (L-HESs), and their study has been focused on the nature of the abnormal T-cell proliferations that underlie these disorders.

LYMPHOCYTIC VARIANT HYPEREOSINOPHILIC SYNDROMES—FACT SHEET

Clinical Features
- Skin manifestations (e.g., eczema, erythroderma, urticaria)
- Increased absolute eosinophil count
- Absolute lymphocyte count is usually normal or mildly elevated
- Increased serum IgE levels

Morphology
- Peripheral blood eosinophils appear mature, typical
- No specific peripheral blood lymphocyte morphology

Immunophenotype
- Most commonly associated with abnormal $CD3^-$, $CD4^+$ T cells detectable in peripheral blood
- Minority of cases may show variant T-cell immunophenotypes such as $CD3^+/CD7^-$ or $CD3^+/CD4^-/CD8^-$

Genetics
- Clonal T-cell antigen receptor gene rearrangement detectable in most patients
- No specific or defining cytogenetic abnormalities

L-HES appears fundamentally to represent an abnormal T-lymphoid proliferation. However, there is no clear consensus as to whether L-HES represents a truly neoplastic process, a preneoplastic clonal proliferation, or a distinct pattern of immune dysregulation. Regardless, the clinical manifestations appear to be linked to the secretion of interleukin (IL) 5 (a potent mediator of eosinophil activation) by the abnormal T-cell clone, with resultant non-neoplastic stimulation of eosinophil proliferation. Some have proposed that the propensity of these abnormal T cells to overproduce IL-5 may be related to their generally $CD3^-$ immunophenotype, with activation of alternative T-cell activation pathways (independent of the CD3-anchored T-cell receptor) resulting in overproduction of Th-2–type cytokines, including IL-5.

Although the exact pathogenesis of the abnormal T-cell proliferation in L-HES is unknown, these disorders are marked by a common pattern of clinicopathologic signs and symptoms. Males and females appear to be affected in equal proportion (in contrast to the marked male predominance of eosinophilic myeloproliferative neoplasms). Patients tend to exhibit chronic skin manifestations (e.g., pruritus, erythroderma, eczema, urticaria) and, in contrast to eosinophilic myeloproliferative neoplasms, elevated levels of serum IgE. Complete blood count in these patients uniformly reveals absolute eosinophilia (eosinophils $>5 \times 10^9$/L). Most patients easily exceed the minimum diagnostic criterion of a sustained eosinophil count of at least 1.5×10^9/L, but eosinophil counts vary widely and some patients might not exceed the 1.5×10^9/L threshold. The peripheral absolute lymphocyte count is often normal, but some patients demonstrate high-normal or frankly elevated absolute lymphocyte counts ($>4 \times 10^9$/L). The diagnosis

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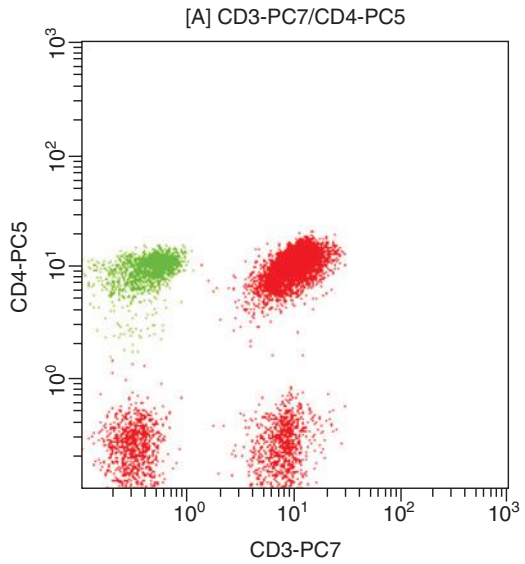


FIG. 13.8

Flow cytometric histogram of peripheral blood lymphocytes showing the $CD3^-$, $CD4^+$ abnormal T-cell immunophenotype *(green)* typical of lymphocytic variant hypereosinophilic syndrome (L-HES).

requires confirmation of an abnormal T-cell population with characteristic immunophenotypic aberrancies as detailed below in Immunophenotypic Findings.

The clinical behavior of L-HES is generally indolent. Some patients do not require specific therapy, but corticosteroids are generally the first-line approach for those who require therapy. There are several individual reports of patients with L-HES developing overt peripheral T-cell lymphomas over time (often after many years); however, the actuarial risk of the development of frank T-cell lymphoma in L-HES patients is unknown.

Morphologic Findings

The main morphologic finding in L-HES is a peripheral blood eosinophilia. Eosinophils are generally mature, without other morphologic stigmata of myeloproliferative neoplasms (e.g., circulating blasts, immature granulocytes, leukoerythroblastic changes). There is not a specific lymphocyte morphology described in association with L-HES and, as noted previously, many patients exhibit nonelevated absolute lymphocyte counts.

Immunophenotypic Findings

In most patients with L-HES, flow cytometric immunophenotyping of the peripheral blood reveals a distinct pattern of $CD4^+$ T-helper cells with deletion of the pan–T-restricted surface antigen CD3 ($CD3^-$, $CD4^+$; Fig. 13.8). A minor subset of patients with L-HES show variant immunophenotypes, including a double-negative $CD3^+$, $CD4^-$, $CD8^-$ pattern, or deletion of the pan–T-associated antigen CD7. The double-negative immunophenotype resembles that seen in the physiologic subset of γδ T cells, but it has been shown that in association with L-HES, the double-negative cells express the T-αβ receptor complex. Whether these two less common immunophenotypic variants represent a truly pathogenic aberrant T-cell population is not entirely clear in all cases because these immunophenotypes resemble those seen in normal subsets of dermal-derived T lymphocytes and because many reported cases with these less common variant immunophenotypes have not included documentation of T-cell clonality.

Genetic and Cytogenetic Findings

Sporadic cytogenetic abnormalities, including abnormalities of chromosome 6q, have been reported in association with L-HES. However, most cases are not associated with clonal cytogenetic abnormalities, and there is no cytogenetic abnormality specific or predictive for L-HES.

In general, clonality of T cells can be demonstrated in L-HES by T-cell antigen receptor gene rearrangement studies. T-cell clonality is not universal, however, and there are reported cases of L-HES that do not have demonstrable T-cell clones. It is possible that at least a subset of the nonclonal cases reported do not represent the true clinicopathologic syndrome of L-HES.

Differential Diagnosis

A key differential diagnosis of L-HES is the true eosinophilic myeloproliferative neoplasms associated with rearrangements of *PDGFRA, PDGFRB,* or *FGFR1*. In general, the workup for suspected HES should include assessment for these abnormalities. Details regarding the cytogenetic and molecular genetic workup for these abnormalities can be found in Chapter 17.

Many peripheral T-cell lymphomas or leukemias can develop with the aberrant T-cell immunophenotypes detectable in L-HES (including aberrant deletion of CD3 or CD7, or double-negative $CD4^-$,$CD8^-$ immunophenotypes), and these lymphomas can sometimes develop with skin manifestations and marked peripheral blood eosinophilia. L-HES generally does not manifest with substantial lymphadenopathy or organomegaly. If these abnormalities are present, then biopsy of a lymph node or other primary site may be helpful in this differential diagnosis. L-HES is not an appropriate diagnosis if another well-defined PTCL subtype can be diagnosed. Biopsy of skin lesions may also be helpful in certain cases because the clinical presentation may mimic primary cutaneous T-cell lymphoma and MF. Finally, a markedly elevated peripheral absolute lymphocyte count should evoke a workup for a peripheral T-cell neoplasm other than L-HES. Although absolute lymphocyte counts may be modestly increased in some L-HES patients, most have normal absolute lymphocyte counts, and lymphocyte counts in excess of 10×10^9/L are rare in cases reported in the medical literature.

In the flow cytometric evaluation of suspected L-HES, it is important to note that other hematologic cell types, particularly monocytes, express CD4 in the absence of CD3, and careful steps should be taken to assure that analysis of CD3 expression is restricted to the T-lymphocyte population in the sample.

The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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14

Acute Myeloid Leukemia

■ **Daniel A. Arber, MD**

■ INTRODUCTION AND CLASSIFICATION

Expanding knowledge of acute myeloid leukemia (AML) over the past four decades has resulted in major advances in the understanding and classification of this disease. AML is known to represent a number of distinct but related diseases, some of which require unique therapeutic approaches. The 2016 World Health Organization (WHO) classification integrates blast morphology and immunophenotype with cytogenetics and molecular studies to establish diagnoses with prognostic relevance. Risk stratification systems use these data to identify which patients may benefit from allogeneic hematopoietic cell transplantation or novel therapeutics. The number of recurrent genetic abnormalities has been expanded, and categories for subtypes of AML with gene mutations have been added. In 2008, the category AML with myelodysplasia-related changes was established that includes the prior category of AML with multilineage dysplasia, as well as cases with a myelodysplasia-related karyotype or history of myelodysplasia. Patients with Down syndrome are classified separately, as are patients with a history of prior cytotoxic therapy (Box 14.1). This chapter uses the 2016 WHO classification and elaborates on diagnostic difficulties that persist, as well as the differential diagnosis for many of these entities.

ACUTE MYELOID LEUKEMIA WITH t(8;21)(q22;q22.1)—FACT SHEET

Definition
- AML with bone marrow blasts showing distinctive morphologic and immunophenotypic features
- Presence of translocation diagnostic of AML regardless of blast count

Frequency
- 8% to 13% of all AMLs

Cytogenetic Event
- t(8;21)(q22;q22.1)

Molecular Event
- *RUNX1/RUNX1T1* fusion

Prognosis
- Intermediate to good with high-dose cytarabine regimens
- *KIT* mutations unfavorable in adults, less clear in children
- *FLT3* ITD mutations uncommon

■ DE NOVO ACUTE MYELOID LEUKEMIAS WITH RECURRENT GENETIC ABNORMALITIES

ACUTE MYELOID LEUKEMIA WITH t(8;21)(q22;q22.1) *RUNX1-RUNX1T1*

The t(8;21)(q22;q22.1) is present in 8% to 13% of pediatric and adult AMLs, with a lower frequency in AML of the elderly. Some patients may exhibit extramedullary tumors (myeloid or granulocytic sarcomas). The t(8;21) results in fusion of the *RUNX1* gene (or *AML1*) on chromosome 21 with the *RUNX1T1* (or *ETO*) gene on chromosome 18, and the resulting *RUNX1/RUNX1T1* chimeric protein disrupts normal function of the core-binding factor, a transcription factor complex that regulates normal hematopoiesis. Despite disruption of the core binding factor by *RUNX1/RUNX1T1,* this genetic abnormality alone is not sufficient to cause leukemic transformation.

Blast cells with abundant pink granules and slightly basophilic cytoplasm are characteristic of this disease. The blasts usually show perinuclear clearing, or hofs, and a subset of the blasts have large, irregular pink (often termed salmon-colored) cytoplasmic granules (Fig. 14.1). Thin Auer rods may also be present but may be difficult to identify with some Wright stains. Eosinophils that are morphologically normal are often increased in number in the marrow, but this feature is not specific. Mature granulocytes may show dysplastic changes with nuclear pseudo–Pelger-Huët anomaly. This finding should not be interpreted as evidence of multilineage dysplasia, which is not seen in most cases of de novo AML with t(8;21). The large number of cytoplasmic granules in the neoplastic cells of this leukemia subtype may raise concern for making a diagnosis of acute leukemia, because

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Box 14.1 2016 WHO Classification of Acute Myeloid Leukemia and Related Neoplasms

Acute myeloid leukemia with recurrent genetic abnormalities
- AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
- AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
- APL with *PML-RARA*
- AML with t(9;11)(p21.3;q23.3); *KMT2A-MLLT3*
- AML with t(6;9)(p23;q34.1); *DEK-NUP214*
- AML with inv(3)(q21.3;q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*
- AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); *RBM15-MKL1*
- *Provisional entity: AML with BCR-ABL1*
- AML with mutated *NPM1*
- AML with biallelic mutations of *CEBPA*
- *Provisional entity: AML with mutated RUNX1*

Acute myeloid leukemia with myelodysplasia-related changes
Therapy-related myeloid neoplasms
Acute myeloid leukemia, NOS
- AML with minimal differentiation
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukemia
- Acute monoblastic/monocytic leukemia
- Pure erythroid leukemia
- Acute megakaryoblastic leukemia
- Acute basophilic leukemia
- Acute panmyelosis with myelofibrosis

Myeloid sarcoma
Myeloid proliferations related to Down syndrome
- Transient abnormal myelopoiesis
- Myeloid leukemia associated with Down syndrome

ACUTE MYELOID LEUKEMIA WITH t(8;21)(q22;q22.1)—PATHOLOGIC FEATURES

Blast Cell Morphologic Features
- Abundant, large pink granules
- Basophilic cytoplasm
- Perinuclear hofs
- Thin Auer rods
- Admixed, normal eosinophils

Blast Cell Immunophenotypic Features
- Myeloid antigen positive (CD13, CD33)
- $CD34^+$
- Aberrant CD19 and PAX5 expression
- Subset of cases are $CD56^+$

Differential Diagnosis
- Growth factor effects
- Acute promyelocytic leukemia
- Myelodysplastic syndrome
- Mixed phenotype acute leukemia

the cells might not be considered true blasts by some, but the WHO classification considers all cases with this cytogenetic abnormality to represent AML regardless of blast count. Cases of this type are usually classified as AML-M2 in the French-American-British (FAB) scheme but represent a small subset of FAB M2 AMLs.

AML with t(8;21) has a characteristic immunophenotype that can be helpful in making the diagnosis. The blast cells characteristically express CD13 and CD33, similar to other types of AMLs but also usually express CD34 and the B-cell–associated antigens CD19 and PAX5. A subset of cases may also express CD56, which is associated with a worse prognosis in some studies. Rare cases will not express CD13 or CD33 but will be positive for other myeloid-associated markers, such as myeloperoxidase. Some cases may show expression of antigens associated with monocytic differentiation. The detection of the distinctive granular blast cell population with perinuclear clearing and large pink cytoplasmic granules in the setting of myeloid antigen expression and CD34 and CD19 expression is highly predictive of the t(8;21), and molecular studies are warranted in this setting if the initial karyotype is normal. AML with t(8;21) is considered to have an intermediate to good prognosis with therapeutic regimens that include high-dose cytarabine.

Low levels of the t(8;21) *RUNX1-RUNX1T1* transcript can persist in patients with clinical remission, and increases of fusion transcript evident only on serial quantitative reverse transcriptase polymerase chain reaction (RT-PCR) assays appear more predictive of recurrence. In some studies, eradication of molecular disease is necessary for long-term remission in AML with t(8;21).

The differential diagnosis of AML with t(8;21) includes growth factor changes, acute promyelocytic leukemia, myelodysplastic syndrome, and mixed-phenotype acute leukemia. Growth factor changes are described in more detail later, but the reactive promyelocytes seen after growth factor administration often show perinuclear hofs that can be confused with t(8;21) AML blasts. The detection of an aberrant immunophenotype, Auer rods, or the large pink granules of t(8;21) AML are useful in ruling out a growth factor change. Neoplastic promyelocytes of acute promyelocytic leukemia (see acute promyelocytic leukemia with *PML-RARA* below) tend to show more abundant Auer rods, finer cytoplasmic granules, and more folded nuclei than are seen with t(8;21) AML. Because some of the blast cells in AML with t(8;21) have abundant granules, blast cells may appear to be less than 20%, suggesting a myelodysplastic syndrome. However, as mentioned, cases with the characteristic features of t(8;21) AML do not usually show multilineage dysplasia and should be considered as acute leukemias rather than myelodysplasia. Finally, the aberrant expression of CD19 and PAX5 suggests an acute leukemia of ambiguous lineage. The 2016 WHO criteria for mixed phenotype acute leukemia, B-myeloid, require strong CD19 together with expression of CD79a, cytoplasmic CD22 or CD10, or weak CD19 with at least two of the additional B-cell markers noted above (strong CD79a, cytoplasmic CD22, and CD10). AML with t(8;21) typically has weak CD19 and does not commonly express CD22 or CD10.

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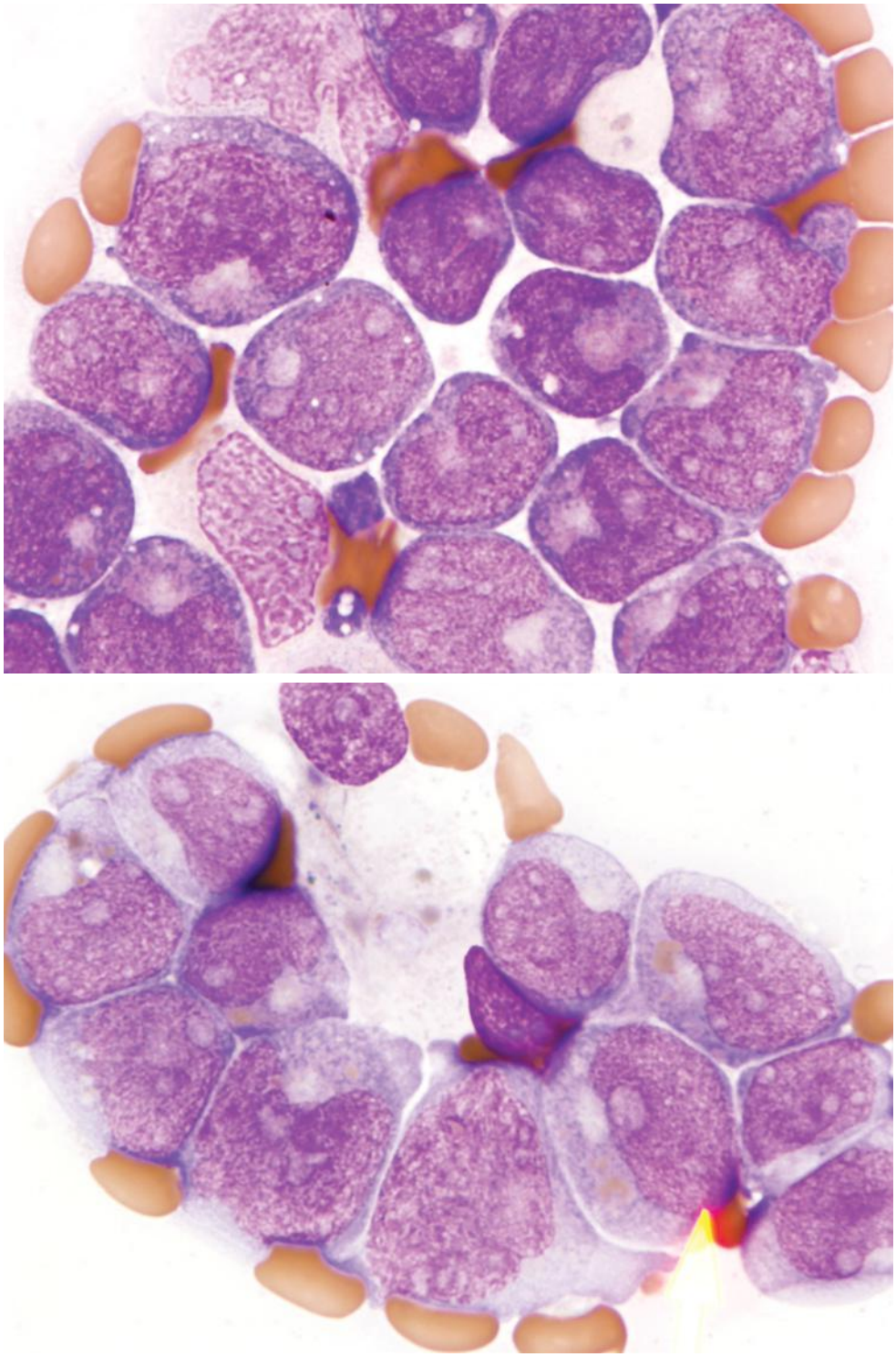


FIG. 14.1
Acute myeloid leukemia with t(8;21)(q22;q22.1). The blasts show characteristic perinuclear clearing or hofs with large pink or salmon-colored cytoplasmic granules.

Demonstration of the t(8;21) by cytogenetics, fluorescence in situ hybridization (FISH), or PCR always trumps a diagnosis of acute leukemia of ambiguous lineage. Patients with t(8;21) AML have a favorable prognosis with appropriate therapy. In contrast, patients with ambiguous-lineage leukemia often have a poor prognosis, and an ideal therapy has not been defined.

Core binding factor AML (those with *RUNX1-RUNX1T1* or *CBFB-MYH11* fusion) commonly harbors mutations of *KIT* (20% to 25%). In adults, *KIT* mutations in exons 8 and 17 appear to be associated with a worse prognosis. In children, the significance of this mutation is less certain. It is unclear whether adults with *KIT* mutated AML with t(8;21) benefit from allogeneic hematopoietic cell transplantation in first remission or tyrosine kinase inhibitor therapy. Rarely, patients with *KIT* mutated AML with t(8;21) have systemic mastocytosis. Those patients are reported to have a poor prognosis.

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Most AMLs with t(8;21) have additional cytogenetic abnormalities commonly involving loss of a sex chromosome or partial deletion of the long arm of chromosome 9 (i.e., del[9q]). The presence of additional cytogenetic abnormalities does not have prognostic significance. *FLT3* internal tandem duplication (ITD) mutations are uncommon in AML with t(8;21), as are mutations of *NPM1* or *CEBPA*. Mutations of *KRAS*, *NRAS*, *ASXL1*, and *ASXL2*, however, may occur in these patients.

ACUTE MYELOID LEUKEMIA WITH inv(16)(p13.1;q22) OR t(16;16)(p13.1;q22); *CBFB-MYH11*

Acute myeloid leukemia with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22) occurs in 5% to 10% of all AML cases and tends to occur in children or young adults, whereas approximately 1% to 3% of older adults with AML have this abnormality. Both cytogenetic abnormalities result in a fusion of the *CBFB* and *MYH11* genes on chromosome 16, which, similar to the t(8;21), disrupts the core binding factor transcription factor complex.

The bone marrow usually shows a proliferation of blasts with monocytic features and cytoplasmic granules as well as a population of abnormal eosinophils. This category generally corresponds to the AML M4Eo designation in the FAB classification. Not all cases, however, will show monocytic differentiation either by morphology or cytochemistry. The presence of an increase in normal-appearing eosinophils is not sufficient to suggest this diagnosis, because it can occur in a variety of AML subtypes. The maturing eosinophils must show abnormal basophilic granules that are large and coarse, which may give the appearance of basophils (Fig. 14.2); however, there are readily identifiable background eosinophilic granules present in the cells as well. Whereas the abnormal eosinophils are classically described as positive for chloracetate esterase by cytochemistry, as opposed to normal eosinophils that are negative for chloracetate esterase, this test is often only weakly positive and not usually necessary for the diagnosis. Rare cases will have numerous eosinophils and maturing monocytes, making the blast cell count less than 20%; however, the WHO scheme classifies this molecular genetically defined disease as acute leukemia regardless of blast cell count. Multilineage dysplasia is uncommon in this subtype. The detection of abnormal eosinophils is highly predictive of a chromosome 16 abnormality, which may be subtle on routine karyotype. Therefore additional studies, such as RT-PCR or FISH, should be performed on cases with abnormal eosinophils and an apparently normal karyotype. In a significant percentage of cases (up to 20%), the abnormal eosinophils can be scarce or absent. The eosinophils with large basophilic granules are often not evident in the peripheral blood. In cases without abnormal eosinophils, the precise diagnosis can only be made after cytogenetic or molecular genetic detection of an inv(16) or t(16;16).

AML with inv(16) or t(16;16) shows expression of the expected myeloid antigens (CD13 and CD33) and often monocyte-associated markers such as CD4, CD14, and CD64. Immunophenotyping may show a single population, or two distinct cell populations: one myeloblastic and the other monocytic. A subset of AML with inv(16) or t(16;16) cases will show aberrant expression of the T-cell–associated marker CD2, but it is not specific for this disease.

AML with inv(16) or t(16;16) is generally associated with a favorable prognosis with high-dose cytarabine. Serial quantitative RT-PCR monitoring for these genetic abnormalities is useful in identifying patients at high risk for relapse.

Because subsets of cases will not show abnormal eosinophils, especially if diagnosed in peripheral blood, or will have less than 20% blast cells, the differential diagnosis of AML

ACUTE MYELOID LEUKEMIA WITH inv(16)(p13.1;q22) OR t(16;16)(p13.1;q22)—FACT SHEET

Definition
- AML with bone marrow blasts showing distinctive morphologic and immunophenotypic features
- Presence of translocation diagnostic of AML regardless of blast count

Frequency
- 5% to 10% of all AMLs

Cytogenetic Event
- inv(16)(p13.1;q22) or t(16;16)(p13.1;q22)

Molecular Event
- *CBFB/MYH11* fusion

Prognosis
- Good with high-dose cytarabine regimens
- *KIT* mutations impart poor prognosis in adults, less clear in children
- *FLT3*-ITD mutations uncommon

ACUTE MYELOID LEUKEMIA WITH inv(16)(p13.1;q22) OR t(16;16)(p13.1;q22)–PATHOLOGIC FEATURES

Blast Cell Morphologic Features
- Often myelomonocytic appearance
- Abnormal eosinophils with large, basophilic granules

Blast Cell Immunophenotypic Features
- Myeloid or myelomonocytic antigen-positive (CD13, CD33, CD14, CD64)

Differential Diagnosis
- AML, not otherwise categorized
- Myelodysplastic syndrome

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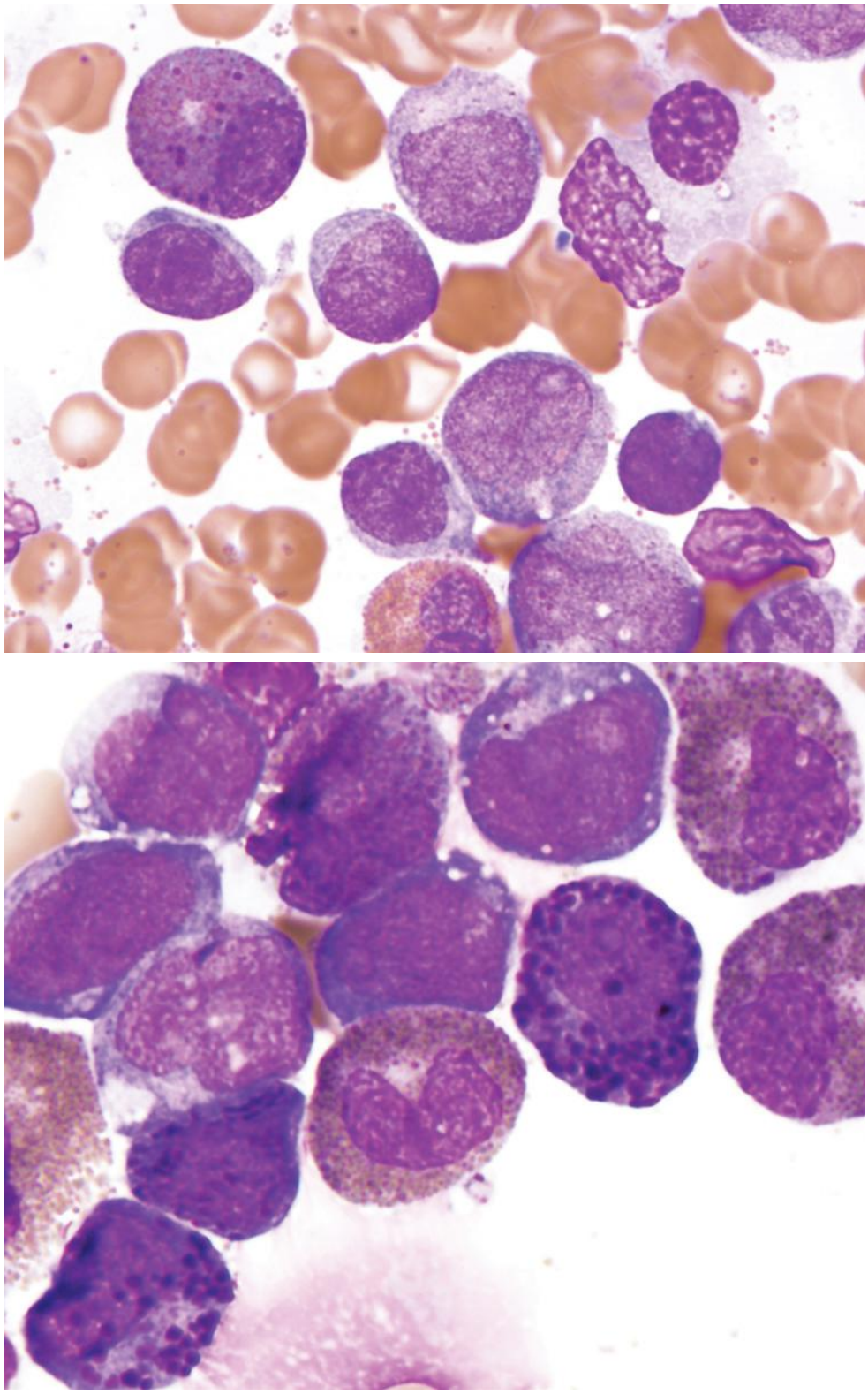


FIG. 14.2

Acute myeloid leukemia with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22). The blasts may have a myeloid or myelomonocytic appearance. They are accompanied by eosinophil precursors, some of which have large basophilic granules.

with inv(16) or t(16;16) includes AML, not otherwise specified (NOS), and myelodysplasia. Upon identification of the *CBFB-MYH11* fusion by cytogenetics, FISH, or PCR, the designation of AML with inv(16) or t(16;16) is established regardless of the blast count.

Like AML with t(8;21), this core binding factor leukemia has a favorable prognosis. *KIT* mutations are present in approximately 30% of cases and negatively affect prognosis in adults, but the prognostic significance is less certain in children. It is not yet clear whether adult cases of AML with

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an inv(16)(p13.1;q22) or t(16;16)(p13.1;q22) and mutated *KIT* benefit from allogeneic hematopoietic cell transplantation or tyrosine kinase inhibitor therapy.

ACUTE PROMYELOCYTIC LEUKEMIA WITH *PML-RARA*

Acute promyelocytic leukemia (APL) represents 9% to 12% of AMLs and is frequently associated with disseminated intravascular coagulopathy (DIC). Indeed, the DIC may be fatal if not recognized early in the management of these patients. Early recognition of this subtype of AML by the characteristic morphologic and phenotypic features is important to alert the clinician to the potential for DIC and to initiate specific therapy with all-*trans* retinoic acid (ATRA). All molecular subtypes contain mutations of the retinoic acid receptor alpha *(RARA)* gene on chromosome 17. The t(15;17)(q24.1;q21.2), the most common genetic aberration in APL, results in fusion of *RARA* with the *PML* gene on chromosome 15. This category generally corresponds to AML-M3 in the FAB classification. Of note, the chromosomal breakpoints of APL, which were considered to be t(15;17)(q22;q12-21), have been recently updated and are now recognized to be t(15;17)(q24.1;q21.2).

A variety of morphologic types of APLs are described, but they are most easily subdivided into hypergranular and hypogranular (or microgranular) forms of the disease (Fig. 14.3). The classic hypergranular APL has blast cells that somewhat resemble normal promyelocytes with abundant cytoplasmic granules. Numerous Auer rods are present in individual blasts (termed *faggot cells*). In contrast to the blasts of AML with t(8;21) or reactive promyelocyte proliferations, APL cells do not show differing stages of maturation or perinuclear clearing. Microgranular APL is usually more difficult to recognize as a type of APL and may be mistaken for myelomonocytic leukemia. The blasts characteristically have folded or bilobed nuclei ("butterfly" nuclei) with very fine to undetectable cytoplasmic granules. The hypogranular appearance is due to the submicroscopic size of granules present in the cytoplasm of blasts. Rarely, hypergranular cells may be present with Auer rods, but they often require an extensive search to be identified. Both types of APLs show strong myeloperoxidase positivity in virtually all blast cells, by both cytochemistry and flow cytometry, which is often helpful in differentiating hypogranular promyelocytes from blasts with monocytic differentiation. Some cases of APL contain a subset of blasts that are positive for nonspecific esterase by cytochemistry; this could lead to confusion with an acute myelomonocytic leukemia using FAB criteria. Multilineage dysplasia is not usually present in APL with t(15;17). Although there are no well-defined morphologic features to differentiate most of the cytogenetic variants of APL, patients with the *ZBTB16/RARA* molecular fusion are described as having distinctive features. These features include more round blast cell nuclei with more variably sized cytoplasmic granules than in other types of APLs. In addition, circulating Pelger-like neutrophils are described with clumped chromatin and hyposegmented or unsegmented nuclei. However, this molecular subtype of APL is extremely uncommon, and the predictive value of these morphologic features is unclear.

Acute promyelocytic leukemia cells express CD33 and CD13, although the latter may be weak and heterogeneous. With CD45 gating, the blast cell area is expanded because of an increase in side scatter secondary to the cytoplasmic granularity of the leukemic cells. Hypergranular APL is typically human leukocyte antigen (HLA)-DR and CD34

ACUTE PROMYELOCYTIC LEUKEMIA—FACT SHEET

Definition
- A neoplastic proliferation of bone marrow blast cells with features of promyelocytes
- Presence of translocation diagnostic of AML regardless of blast count

Frequency
- 9% to 12% of all AMLs

Most Common Cytogenetic and Molecular Events
- t(15;17)(q24.1;q21.2) *PML/RARA* fusion

Variant *RARA* Fusion Partners (Chromosome/Gene)
- 5q35.1/*NPM1*
- 11q13.4/*NUMA1*
- 11q23.2/*ZBTB16*
- 17q11.2/*STAT5B*

Prognosis
- Good with all-*trans* retinoic acid or arsenic trioxide regimens for cases with *PML/RARA*
- *FLT3* ITD mutations seen in a subset, associated with high white blood cell count (WBC) and inferior prognosis

ACUTE PROMYELOCYTIC LEUKEMIA—PATHOLOGIC FEATURES

Blast Cell Morphologic Features
- Folded, bilobed nuclei
- Abundant cytoplasmic granule
- Numerous Auer rods

Blast Cell Immunophenotypic Features
- Myeloid antigen positive (CD13, CD33)
- Weak or absent CD34 expression
- Aberrant loss of HLA-DR
- Aberrant expression of CD2 (subset)

Differential Diagnosis
- AML, not otherwise categorized, acute monocytic leukemia (can resemble microgranular variant)
- Growth factor changes
- Myelodysplastic syndrome

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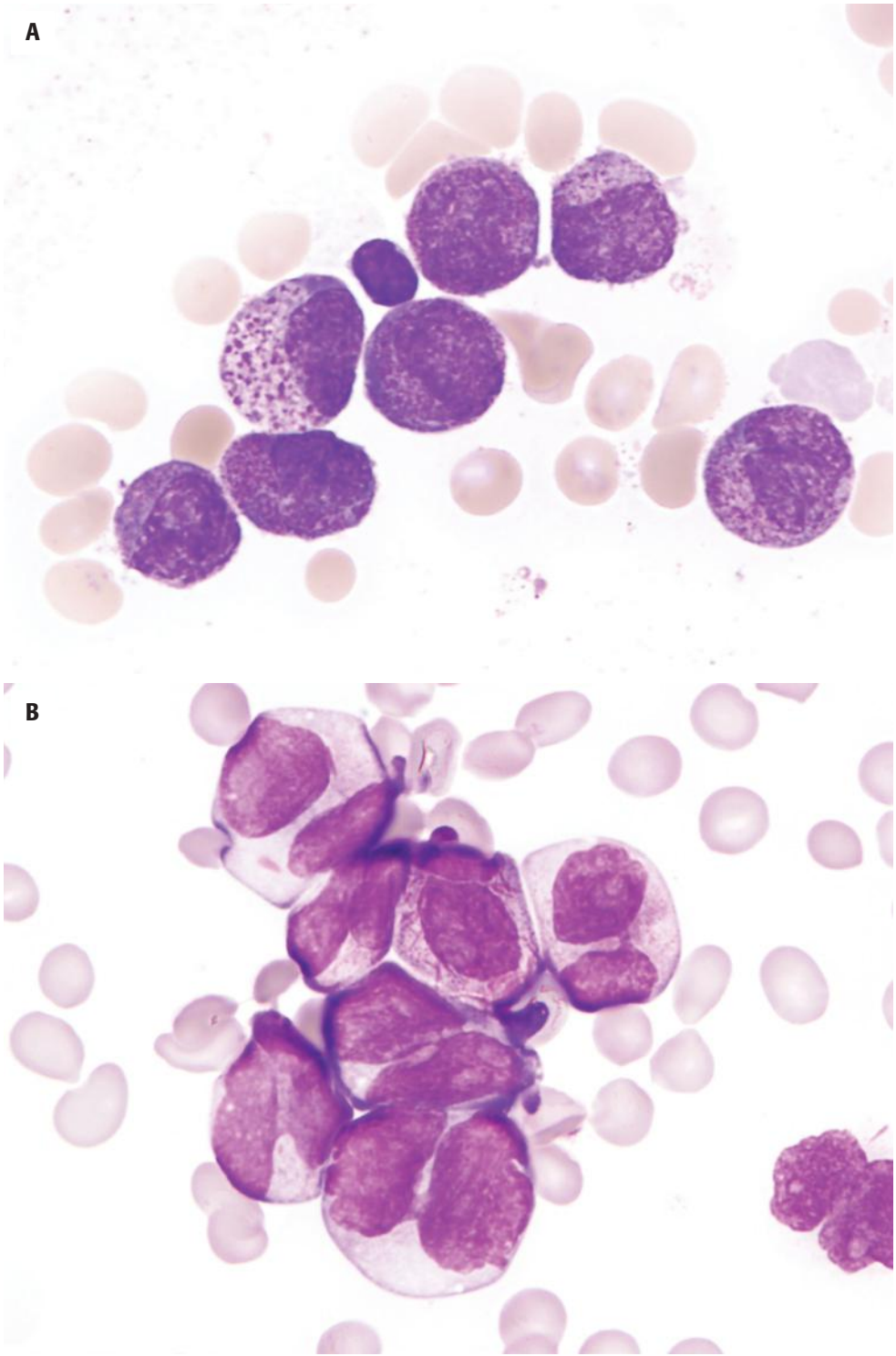


FIG. 14.3

Acute promyelocytic leukemia with *PML-RARA*. A, The granular type shows abundant cytoplasmic granules similar to normal promyelocytes. B, The microgranular variant has fine cytoplasmic granules and folded nuclei. Despite the hypogranular appearance of most cells, scattered cells with granules and numerous Auer rods can be seen.

negative. Microgranular APL may show weak or partial expression of these antigens. This immunophenotype is not unique to APL, as a subset of AML, NOS, cases without differentiation similarly lack CD34 and HLA-DR. Aberrant expression of CD2 is more commonly observed in microgranular APL. CD56 expression is described in 15% to 20% of patients and has been associated with shorter complete remission and poorer overall survival in some studies.

The *PML-RARA* fusion can arise from one of three breakpoint regions on *PML* at 15q24.1. Two (bcr1, bcr2) lead to long transcripts, and the third (bcr3) leads to the short transcript. Microgranular APL typically has the short transcript. Cytogenetics, FISH, or RT-PCR is necessary for genetic confirmation of the *PML-RARA* fusion. FISH, RT-PCR, and immunofluorescence for the micro-speckled nuclear distribution of the *PML* protein all may aid in rapid diagnosis. RT-PCR

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is the only technique that can identify the *PML-RARA* transcript isoform useful for minimal residual disease monitoring. *FLT3* ITD mutations are seen in approximately 40% of patients, more commonly in the microgranular variant, with high WBC counts.

Atypical promyelocytes can persist in the marrow for up to several weeks after induction chemotherapy, as can detection of *PML-RARA* by karyotype, FISH, or RT-PCR. These findings do not necessarily indicate resistant disease. Detection of *PML-RARA* by RT-PCR after induction does not affect subsequent clinical outcome; however, detection of *PML-RARA* after complete remission is obtained strongly predicts risk of relapse.

The majority of mutations of *FLT3* in APL are *FLT3* ITDs. *FLT3* ITD in APL is strongly associated with the microgranular subtype, high WBC counts in peripheral blood, and the bcr3 breakpoint in *PML*. In one retrospective study, patients with mutant *FLT3* had a higher rate of death during the period of induction chemotherapy but no significant difference in relapse rate or 5-year overall survival.

PML-RARA is the most common fusion product in APL. *RARA* is a nuclear hormone receptor that binds to specific DNA sequences referred to as retinoic acid–responsive elements in a heterodimeric complex with retinoid-X receptors *(RXRA)*. The *RARA-RXRA* complexes can repress transcription through a variety of mechanisms, but normal physiologic concentrations of retinoic acid in the body control this activity, allowing for normal activation of gene transcription. With the *PML-RARA* fusion, physiologic levels of retinoic acid are no longer sufficient to control the effects of *RARA*, and gene transcription is blocked. Therapeutic strategies that include ATRA provide pharmacologic concentrations of retinoic acid that allow for the return of normal transcription. Less commonly, chromosomal translocations of *RARA* on chromosome 17 can occur with other genes such as *ZBTB16* (or *PLZF*) at 11q23.2, *NUMA1* at 11q13.4, *NPM1* at 5q35.1, and *STAT5B* at 17q11.2. Patients who lack *RARA* translocations or harbor *RARA* translocations involving *ZBTB16* or *STAT5B* might not respond to ATRA and thus require a different therapeutic approach. With current therapy, using ATRA or arsenic trioxide, APL is considered to have a favorable prognosis.

The t(15;17) is usually detectable by karyotype analysis at diagnosis. Molecular detection of *PML-RARA* is usually performed by RT-PCR or FISH analysis, and these tests may be useful to rapidly confirm the initial diagnosis. However, the combined morphologic and immunophenotypic features can be extremely reliable in detecting this disease. The *PML-RARA* RT-PCR test has been shown to be useful in the early detection of residual disease in acute promyelocytic leukemia and as a predictor of relapse of that disease. Most assays for *PML-RARA* detect one translocated cell in 10,000 to 100,000 cells. The RT-PCR test appears to be less useful when more sensitive assays that detect one abnormal cell in one million are used. Results of these ultrasensitive tests will be positive in patients who are in long-term remission and do not appear to be clinically relevant. Recent studies suggest that serial quantitative assays may be useful to follow patients with low levels of disease.

The differential diagnosis of APL includes AML, NOS, myelodysplastic syndrome, and growth factor changes. As mentioned previously, the morphologic features of the microgranular variant may suggest a myelomonocytic type of AML. Because of the predominance of cells with promyelocyte features, the blast cell count may appear low and suggest a myelodysplastic syndrome, but these cases are considered as APL without regard to the blast cell count. The predominance of promyelocytes might also suggest early growth factor changes. The characteristic perinuclear hof or reactive promyelocytes, however, is not seen in acute promyelocytic leukemia, and cytogenetic abnormalities are not seen in those reactive proliferations.

ACUTE MYELOID LEUKEMIA WITH t(9;11)(p21.3;q23.3) *KMT2A-MLLT3*

Rearrangement of the *KMT2A (MLL)* gene at 11q23.3 is seen in 4% to 5% of de novo adult AMLs and in a much larger percentage of pediatric patients (up to 22%), particularly infants. Patients are at increased risk for DIC and have a propensity for extramedullary disease, such as myeloid sarcoma or tissue infiltration, commonly in the gingiva or skin. The WHO classification limits the recurrent genetic abnormalities categorization to AML with t(9;11)(p21.3;q23.3), because this subtype appears to be the most clinically homogeneous. Other rearrangements of 11q23.3 are diagnosed as AML, NOS, with note of the translocation in the diagnostic line. In the WHO classification, the t(2;11)(p21;q23.3) and t(11;16)(q23.3;p13.3) are designated myelodysplasia-associated karyotypes, and a diagnosis of AML with myelodysplasia-related changes is given (see AML with myelodysplasia-related changes and Box 14.2). *KMT2A*-rearranged AML most commonly shows monocytic differentiation, with or without nonspecific esterase cytochemical reactivity. AML with t(9;11) usually displays monocytic features, although other morphologies are observed rarely. In the bone marrow, the blast features are usually nonspecific but may have round to folded monocytoid nuclei with usually abundant, slightly basophilic, and vacuolated cytoplasm (Fig. 14.4). Promonocytes are often present, with more mature-shaped nuclei but retaining immature nuclear chromatin. More mature cells may predominate in the blood, which suggests a chronic monocytic proliferation. Multilineage dysplasia is usually not present in the blood or marrow. Because the morphologic features are not specific for this molecular subtype of AML, there is no way to reliably predict a *KMT2A* abnormality without performing the appropriate cytogenetic and molecular assays.

The blasts may variably express myeloid-associated antigens CD13 and CD33, are usually CD34 and myeloperoxidase negative, and often express monocyte-associated markers such as CD4, CD14, and CD64. They may also express CD56.

KMT2A (previously known as *MLL*) is a large, 90-kb gene containing 36 exons coding for a 431-kDa protein.

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Box 14.2 Criteria for Diagnosis of AML With Myelodysplasia-Related Changes

1. Greater than 20% myeloid blasts in the blood or bone marrow
2. No history of prior cytotoxic therapy for other disease
3. Absence of one of the seven recurrent genetic abnormality translocations [t(8;21), inv (16)t(16/16), t(15;17) or variants, t(9;11), t(6;9), inv(3)/t(3;3), t(1;22)], absence of mutation of *NPM1*[a] and absence of biallelic mutations of *CEBPA*[a], and one or more of the following features:
   1. Previous history of myelodysplastic syndrome or myelodysplastic–myeloproliferative neoplasm
   2. Multilineage dysplasia (dyspoiesis in at least 50% of the elements from two or more lineages)
   3. Presence of an MDS-associated cytogenetic abnormality, defined as the following:
      - Complex karyotype (3 or more abnormalities)
      - Unbalanced abnormalities
        - -7/del(7q)
        - del(5q)/t(5q)
        - i(17q)/t(17p)
        - -13/del(13q)
        - del(11q)
        - del(12p)/t(12p)
        - idic(X)(q13)
      - *Balanced abnormalities*
        - t(11;16)(q23.3;p13.3)
        - t(3;21)(q26.2;q22.1)
        - t(1;3)(p36.3;q21.2)
        - t(2;11)(p21;q23.3)
        - t(5;12)(q32;p13.2)
        - t(5;7)(q32;q11.2)
        - t(5;17)(q32;p13.2)
        - t(5;10)(q32;q21.2)
        - t(3;5)(q25.3;q35.1)

AML, Acute myeloid leukemia; *MDS,* myelodysplastic syndrome.
[a]Cases with mutation of *NPM1* or biallelic mutations of *CEBPA* that also have a history of MDS or have an MDS-associated cytogenetic abnormality are rare, but should be diagnosed as AML-MRC.

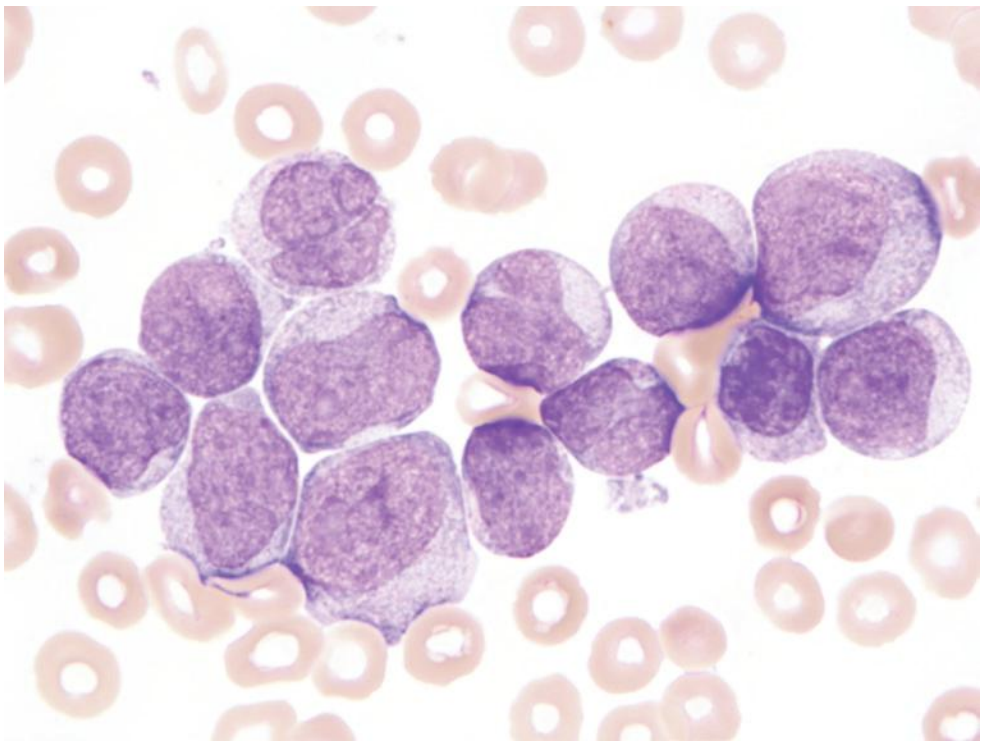


FIG. 14.4
Acute myeloid leukemia with t(9;11)(p21.3;q23.3) showing a monocytic blast cell population.

The protein contains AT hooks, which are known to regulate transcription by inducing changes in DNA conformation that permit the association of transcription factors with regulatory regions of DNA. These AT hooks recognize specific DNA structures. Other regions of *KMT2A* may repress transcription from reporter constructs, mediate homodimerization, mediate interactions with nuclear proteins, contain transcriptional-activating activity, and are involved with ATP-dependent chromatin remodeling. Partial tandem duplication mutations of *KMT2A* also occur in AML and are more common in patients with normal karyotypes or trisomy of chromosome 11.

Despite the numerous translocation partners for *KMT2A*, the most common in AML are located on chromosomes 6q27 (*MLLT4*, or *AF6*), 9p22 (*MLLT3*, or *AF9*), 19p13.3 (*MLLT1*, or *ENL*), 19p13.1 (*ELL*), 19p13.3 (*SH3GL1*, or *EEN*), 16p13.3 (*CREBBP*, or *CBP*), and 22q13.2 (*EP300*,

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ACUTE MYELOID LEUKEMIA WITH t(9;11)(p21.3;q23.3)—FACT SHEET

Definition
- AML with bone marrow blasts showing distinctive morphologic and immunophenotypic features
- Presence of translocation alone *not* diagnostic of AML
- Mandatory threshold of 20% blasts or presence of myeloid sarcoma

Frequency
- Approximately 10% of all pediatric AMLs
- 4% to 5% of de novo adult AMLs

Cytogenetic Event
- AML with t(9;11)(p21.3;q23.3)

Molecular Event
- *KMT2A-MLLT3* fusion

Prognosis
- Intermediate
- *FLT3*-ITD uncommon

ACUTE MYELOID LEUKEMIA WITH t(9;11)(p21.3;q23.3)—PATHOLOGIC FEATURES

Blast Cell Morphologic Features
- Usually monoblastic

Blast Cell Immunophenotypic Features
- Myeloid or myelomonocytic antigen-positive (CD13, CD33, CD11b, CD11c, CD4, CD14, CD64)
- CD34 and myeloperoxidase negative
- Often $CD56^+$

Differential Diagnosis
- AML, not otherwise categorized
- Chronic myelomonocytic leukemia
- Juvenile myelomonocytic leukemia

or *p300*). The t(9;11) conveys intermediate risk disease, with a complete remission rate similar to that of normal karyotype AML. In contrast, AML with other 11q23.3 translocations, with the exception of the t(9;11) and t(11;19), are considered poor risk AMLs with shorter survival. Point mutations of *FLT3* may occur in AML with t(9;11) and are currently of unknown significance. Overexpression of *MECOM (EVI1)* is associated with a very poor prognosis in this group.

AML with t(9;11) lacks specific morphologic or immunophenotypic features and can be easily mistaken for various other types of AMLs in the NOS group. These cases are only clarified with cytogenetic or molecular genetic studies. Because many monocytic leukemias show maturation of neoplastic cells in the peripheral blood, which can be interpreted as mature monocytes, these cases could be misdiagnosed as chronic myelomonocytic leukemia or juvenile myelomonocytic leukemia. Neither of these diagnoses should be made on peripheral blood samples alone. The threshold of 20% blasts must be met to confer a diagnosis of AML with t(9;11). If fewer than 20% blasts are present, a diagnosis of myelodysplastic syndrome (MDS) is more appropriate and frequent monitoring for the development of AML is required. Any history of cytotoxic or radiation therapy would designate an AML with 11q23.3 rearranged as a therapy-related myeloid neoplasm.

ACUTE MYELOID LEUKEMIA WITH t(6;9)(p23;q34.1); *DEK-NUP214*

Acute myeloid leukemia with t(6;9)(p23;q34.1) comprises less than 2% of AML in children and adults. Patients may have anemia, thrombocytopenia, and modestly elevated WBC counts. The marrow typically shows erythroid hyperplasia, dysplasia, and bone marrow basophilia (Fig. 14.5). Although erythroid dysplasia appears to be the most common, a recent report has also noted at least some granulocyte and megakaryocyte dysplasia with this abnormality. Bone marrow basophilia is unusual in other AML types other than rare t(9;22) AMLs or myeloid blast crisis of chronic myeloid leukemia. By flow cytometry, blasts typically express CD45, CD13, CD33, HLA-DR, and intracytoplasmic myeloperoxidase (MPO), with variable expression of CD34, CD15, and CD11c. Terminal deoxynucleotidyl transferase (TdT) may be positive in some cases by flow cytometry or immunohistochemistry. The t(6;9)(p23;q34.1) results in a fusion of the *DEK* gene on chromosome 6 with the *NUP214* (or *CAN*) gene on chromosome 9. *NUP214* is a nucleoporin and one of several components of nuclear pore complexes, which are structures that allow macromolecules to move in and out of the cell nucleus. It appears that nucleoporin fusion proteins act as aberrant transcription factors as well as alter nuclear transport by binding to soluble transport factors. *FLT3* ITD mutations are common in this type of AML, with a reported frequency of 70%. Although this leukemia has a generally poor prognosis in the literature, some patients appear to do well with aggressive therapy that includes hematopoietic cell transplantation.

The differential diagnosis of AML with t(6;9) includes AML with inv(16) or t(16;16). The abnormal eosinophils of the latter disease may be mistaken for basophils, but the detection of background eosinophil granules in these cells is helpful in this differential diagnosis. AML with t(6;9) is in the differential diagnosis of AML with myelodysplasia-related change because of the common presence of multilineage dysplasia. Presence of the translocation allows more precise classification. Rarely, patients have fewer than 20% blasts; the presence of the translocation in this setting is not diagnostic of AML, and close follow-up is required.

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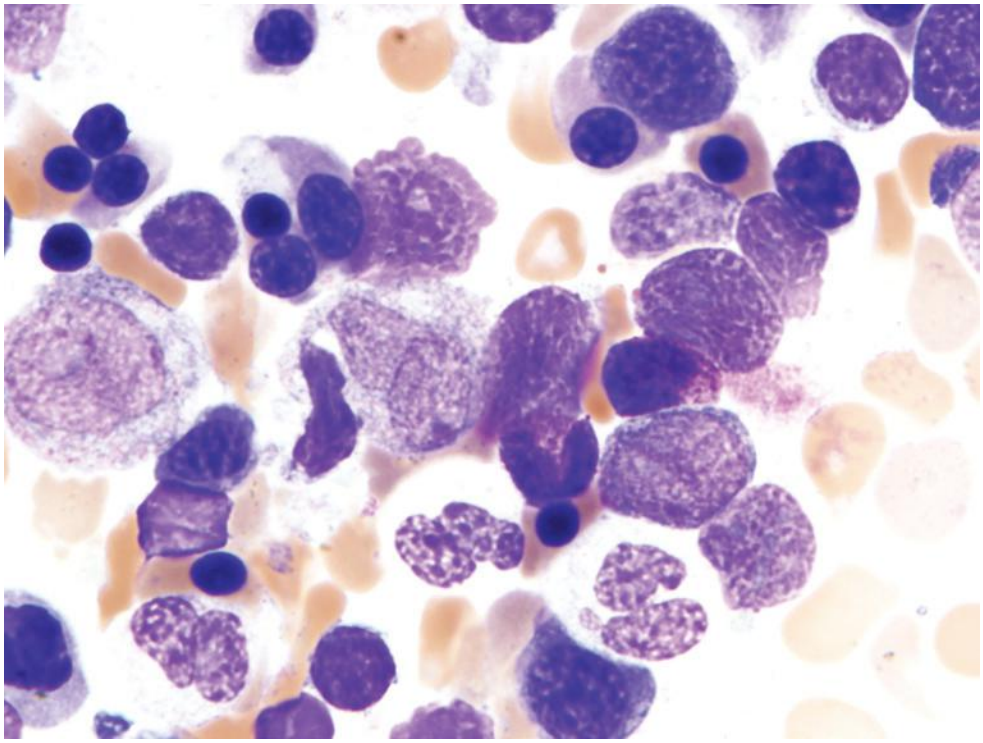


FIG. 14.5
Acute myeloid leukemia with t(6;9)(p23;q34.1). Myelomonocytic blasts are present with admixed small basophils, erythroid hyperplasia with dyserythropoiesis, and hypogranular neutrophils.

ACUTE MYELOID LEUKEMIA WITH t(6;9)(p23;q34.1)—FACT SHEET

Definition
- AML with dysplastic morphologic features frequent basophilia
- Presence of translocation alone not diagnostic of AML
- Mandatory threshold of 20% blasts or presence of myeloid sarcoma

Frequency
- Approximately 2% AMLs

Cytogenetic Event
- t(6;9)(p23;q34.1)

Molecular Event
- *DEK-NUP214* fusion

Prognosis
- Poor
- *FLT3*-ITDs commonly present

ACUTE MYELOID LEUKEMIA WITH t(6;9)(p23;q34.1)—PATHOLOGIC FEATURES

Blast Cell Morphologic Features
- Nonspecific myeloid phenotype, commonly associated with erythroid hyperplasia, multilineage dysplasia, and basophilia

Blast Cell Immunophenotypic Features
- Myeloid antigen positive (CD13, CD33)

Differential Diagnosis
- AML with myelodysplasia-related changes (AML-MRC)
- AML with t(9;22)
- AML with inv(16)
- Acute erythroid leukemia (erythroid/myeloid)
- Myelodysplastic syndrome
- Blast transformation of chronic myeloid leukemia

ACUTE MYELOID LEUKEMIA WITH inv(3)(q21.3;q26.2) OR t(3;3)(q21.3;q26.2); *GATA2, MECOM (EVI1)*

Acute myeloid leukemia with inv(3) or t(3;3) is rare, representing 1% to 2% of AML in adults and less than 1% of AML in children. The inv(3)(q21.3;q26.2) or t(3;3)(q21.3;q26.2) repositions a distal *GATA2* enhancer to activate *MECOM* expression. The translocation or inversion also simultaneously confers *GATA2* haploinsufficiency. *MECOM* (previously known as *EVI1*) is inappropriately expressed in a variety of AMLs, including AML with inv(3)(q21.3;q26.2) or t(3;3)(q21.3;q26.2), and high expression of *MECOM* is a poor prognostic indicator independent of 3q26.2 translocations. Chromosome 3q26.2 rearrangements may be cryptic on routine cytogenetic studies but detectable by FISH. This disease is associated with multilineage dysplasia and thrombocytosis (Fig. 14.6). Giant platelets may be seen in the peripheral blood that may not be counted by automated methods. The most distinctive bone marrow feature is the presence of small nonlobate or bilobate megakaryocytes. The megakaryocytes

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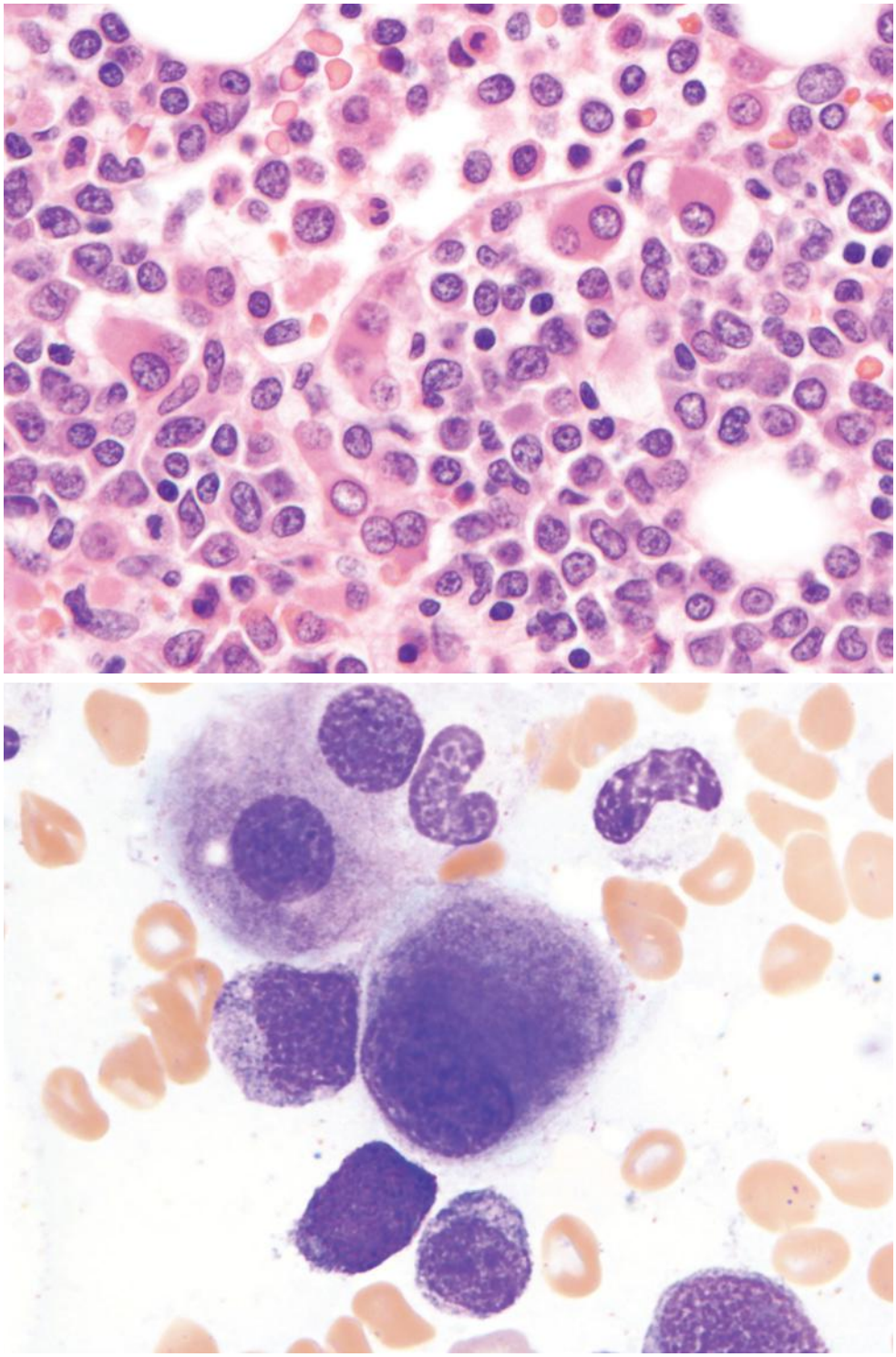


FIG. 14.6

Acute myeloid leukemia with inv(3)(p21.3;q26.2) or t(3;3)(q21.3;q26.2). Small megakaryocytes with nonlobate or bilobate nuclei are present.

are similar to those seen in the 5q-minus syndrome of myelodysplasia but are smaller and are accompanied by the presence of multilineage dysplasia and an increase in blast cells in inv(3) AML—features that are not seen with 5q-minus syndrome. This disease is usually associated with a poor prognosis. Additional poor-prognosis cytogenetic abnormalities are common, monosomy 7 (66%) in particular, as well as complex karyotypes and 5q, and these appear to worsen the already poor prognosis. *NPM1* mutations are rarely seen in this category, and *FLT3* ITDs are relatively uncommon (7%). *CEBPA* mutations are not reported. Most cases, however, have at least one other mutation, including mutations in *NRAS*, *PTPN11*, *KRAS*, *NF1*, *CBL*, *KIT*, *GATA2*, *RUNX1*, and *SF3B1*. Other translocations of 3q26 are not included in this category, and they appear to have a less severe but poor prognosis.

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ACUTE MYELOID LEUKEMIA WITH inv(3)(q21.3;q26.2) OR t(3;3)(q21.3;q26.2)—FACT SHEET

Definition
- AML with common morphologic features and prognosis
- Presence of translocation alone not diagnostic of AML
- Mandatory threshold of 20% blasts or presence of myeloid sarcoma

Frequency
- 1% to 2% of AML

Cytogenetic Event
- inv(3)(q21.3;q26.2) or t(3;3)(q21.3;q26.2), with frequent additional high-risk cytogenetic abnormalities

Molecular Event
- Involves both *GATA2* and *MECOM (EVI1)*

Prognosis
- Poor

ACUTE MYELOID LEUKEMIA WITH inv(3)(q21.3;q26.2) OR t(3;3)(q21.3;q26.2)—PATHOLOGIC FEATURES

Blast Cell Morphologic Features
- Myeloid phenotype, sometimes with megakaryocytic differentiation

Non–Blast Cell Morphology
- Multilineage dysplasia, small bilobed megakaryocytes

Blast Cell Immunophenotypic Features
- Myeloid antigen-positive (CD13, CD33, CD34, CD117, HLA-DR), variable CD61, CD41

Differential Diagnosis
- AML with myelodysplasia-related changes (AML-MRC)
- AML, NOS, acute megakaryoblastic leukemia
- Myelodysplastic syndrome, 5q-minus syndrome

ACUTE MYELOID LEUKEMIA (MEGAKARYOBLASTIC) WITH t(1;22)(p13.3;q13.1); *RBM15-MKL1*

Acute myeloid leukemia (megakaryoblastic) with t(1;22) is a rare form of AML affecting infants almost exclusively. The t(1;22)(p13.3;q13.1) results in an *RBM15/MKL1* (or *OTT/MAL*) fusion product (Fig. 14.7). Of cases, 80% are diagnosed in the first year of life. AML with t(1;22) comprises approximately 1% of childhood AML. AML with t(1;22) may mimic a solid tumor at diagnosis with hepatosplenomegaly, skeletal lesions, or both. The diagnosis can be particularly challenging in cases presenting as myeloid sarcoma with no involvement of the bone marrow. The lesions may show cohesive nests of small, round, blue cells also suggestive of a childhood solid tumor. Blasts in the blood and bone marrow show typical features of megakaryoblasts, with a modest amount of agranular cytoplasm that may show blebs or budding of platelets. The nuclear chromatin may be more condensed than myeloid blasts and infrequently nucleolated. The bone marrow aspirate may be hemodilute or aparticulate owing to marrow fibrosis. Unlike acute megakaryoblastic leukemia of Down syndrome, or acute megakaryoblastic leukemia in adults with inv(3), multilineage dysplasia is not described. Some cases may show atypical megakaryocytic maturation with micromegakaryocytes, and many may show myelofibrosis. Few cases have a reported flow cytometry immunophenotype. CD45 and CD34 may be negative, as in other AML with megakaryoblastic morphology. The myeloid antigens CD13 and CD33 are expressed inconsistently, as is HLA-DR. Immunoreactivity for megakaryocytic antigens CD41 and CD61 is commonly seen by flow cytometry, but it may be difficult to prove immunohistochemically. CD4 and CD56 may also be expressed, bringing blastic plasmacytoid dendritic cell tumor into the differential diagnosis. Additional complex karyotypic abnormalities are common in older patients (>6 months of age). The frequency of *FLT3* mutations is unclear, given the rarity of acute megakaryoblastic leukemia with t(1;22). In one study of pediatric AML (excluding patients with Down syndrome) none of the morphologic M7 cases had *FLT3* ITD mutations, although one patient had an activation loop domain point mutation. Diagnosis may be delayed in these patients because of the difficulties described previously. AML with t(1;22) was associated with poor survival in earlier studies, but more recent studies suggest they respond well to intensive AML therapy. Cases commonly present with fewer than 20% blasts in the blood or marrow, or both. Bone marrow core biopsies are often essential in the diagnosis, revealing extensive involvement when aspirates are aparticulate. Careful clinical evaluation is warranted to assess for extramedullary disease diagnostic of AML when blasts are less than 20%. In the absence of extramedullary disease, careful close clinical follow-up is indicated for evolution of AML.

ACUTE MYELOID LEUKEMIA WITH *BCR-ABL1*

The 2016 WHO classification now recognizes cases of de novo AML with *BCR-ABL1* as a provisional entity. Although most myeloblast proliferations with a *BCR-ABL1* fusion, usually caused by t(9;22)(q34.1;q11.2), represent blast transformation of chronic myeloid leukemia (CML), rare cases of de novo AML occur. This type of AML is rare, representing less than 1% of all cases, with a possible male predominance. Exclusion of an occult chronic phase of CML preceding the diagnosis of AML, or after therapy for AML, is essential for diagnosis. Cases of de novo AML with *BCR-ABL1* appear to differ from blast crisis of CML by having less frequent splenomegaly and basophilia and slightly lower

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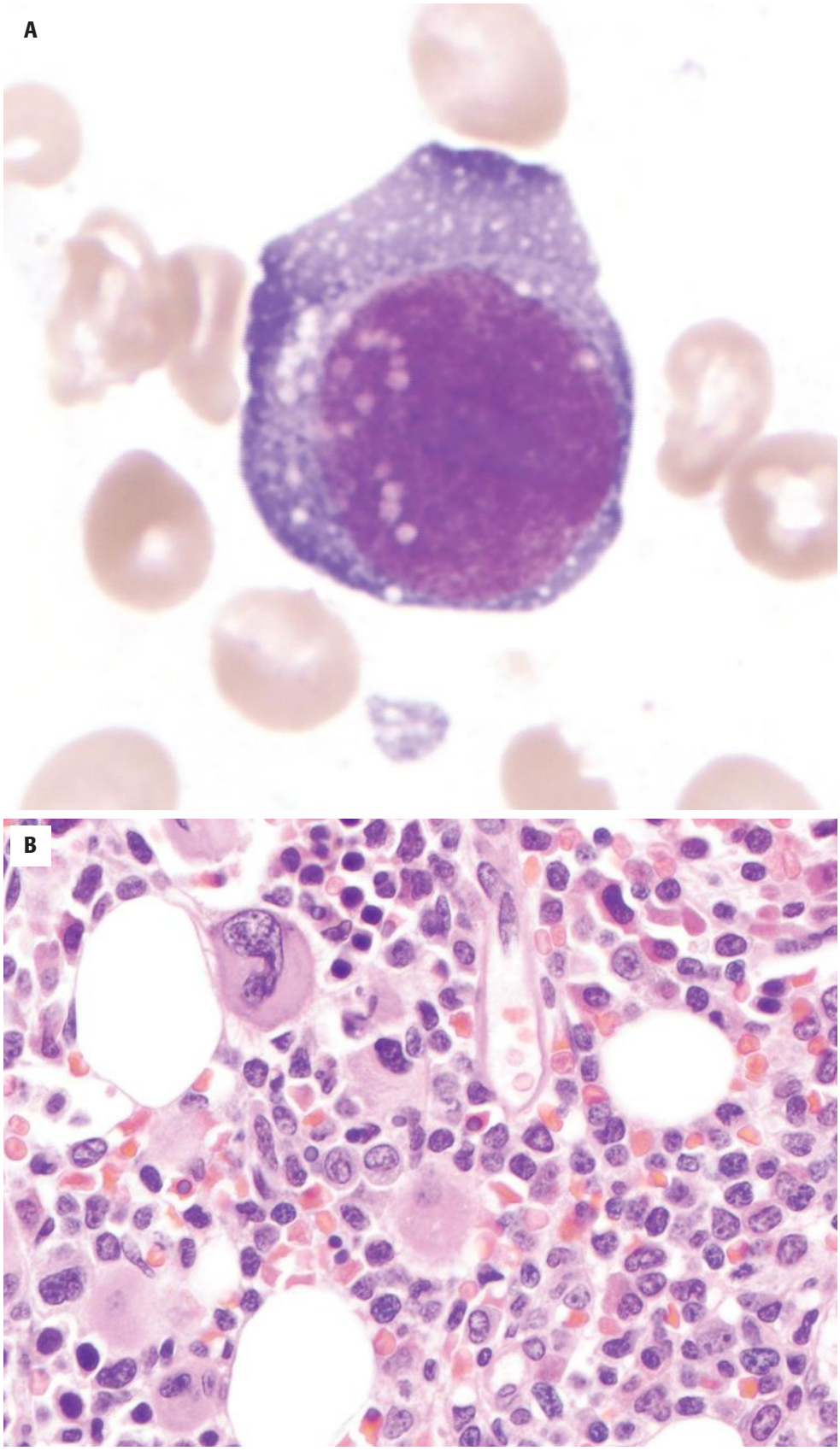


FIG. 14.7

Infant acute megakaryoblastic leukemia with t(1;22)(p13.3;q13.1). A, The blasts have basophilic, finely granular cytoplasm, similar to platelets. B, The biopsy material shows atypical megakaryocytes, blasts, and open sinuses resulting from marrow fibrosis.

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ACUTE MEGAKARYOBLASTIC LEUKEMIA WITH t(1;22)(p13.3;q13.1)—FACT SHEET

Definition
- A neoplastic proliferation of bone marrow blast cells with features of megakaryocytes, occurring almost exclusively in infants
- Presence of translocation alone not diagnostic of AML
- Mandatory threshold of 20% blasts or presence of myeloid sarcoma

Frequency, Gender, and Age
- Less than 1% of all AMLs
- Females more often than males
- Infants, usually younger than 6 months

Cytogenetic Event
- t(1;22)(p13.3;q13.1)

Molecular Event
- *RBM15-MKL1* fusion

Prognosis
- Intermediate

ACUTE MEGAKARYOBLASTIC LEUKEMIA WITH t(1;22)(p13.3;q13.1)—PATHOLOGIC FEATURES

Blast Cell and Bone Marrow Morphologic Features
- Nonspecific cytoplasmic blebs
- Variable marrow fibrosis

Blast Cell Immunophenotypic Features
- Variably myeloid antigen positive (CD13, CD33)
- Aberrant CD7 commonly positive
- Megakaryocyte antigen positive (CD41, CD42, CD61)

Differential Diagnosis
- AML, not otherwise categorized
- Myeloid proliferations of Down syndrome
- Acute panmyelosis with myelofibrosis

ACUTE MYELOID LEUKEMIA WITH *BCR-ABL1*—FACT SHEET

Definition
- A neoplastic proliferation of bone marrow blast cells with variable morphologic features
- Presence of gene fusion alone not diagnostic of AML
- Mandatory threshold of 20% blasts or presence of myeloid sarcoma

Frequency, Gender, and Age
- Less than 1% of all AMLs
- Possible slight male predominance
- Usually occurs in adults

Cytogenetic Event
- t(9;22)(q34.1;q11.2)

Molecular Event
- *BCR-ABL1* fusion

Prognosis
- Poor with traditional AML therapy

ACUTE MYELOID LEUKEMIA WITH *BCR-ABL1*—PATHOLOGIC FEATURES

Blast Cell and Bone Marrow Morphologic Features
- Non-specific marrow features
- Basophils may be increased

Blast Cell Immunophenotypic Features
- Variably myeloid antigen-positive (CD13, CD33)
- Aberrant expression of lymphoid antigens common

Differential Diagnosis
- AML with t(6;9)
- Blast transformation of chronic myeloid leukemia

marrow cellularity. The morphologic features and immunophenotype of the blast cells in AML with *BCR-ABL1* are non-specific with expression of CD13, CD33, and CD34. Aberrant expression of CD19, CD7, and TdT is reportedly common. In some cases, the translocation may be cryptic, but the *BCR-ABL1* fusion, usually the p210 transcript, is present in all cases. Most cases have additional cytogenetic abnormalities, including a complex karyotype, monosomy 7, or trisomy 8. AML-associated mutations, particularly *NPM1* and *FLT3* ITD, are reported in AML with *BCR-ABL1*, but not in blast transformation of CML, but these mutations are relatively uncommon. Loss of *IKZF1* and *CDKN2A* in AML with *BCR-ABL1* and cryptic deletions within the *IGH* and *TRG* genes have recently been described as potentially useful in differentiating de novo AML from myeloid blast transformation of CML, but these tests are not currently available in most diagnostic laboratories. Although rare, AML with *BCR-ABL1* appears to demonstrate a poor response to traditional AML therapy or to tyrosine kinase inhibitor therapy alone. However, survival may be improved with tyrosine kinase inhibitor therapy followed by allogeneic hematopoietic cell transplantation.

ACUTE MYELOID LEUKEMIA WITH GENE MUTATIONS

A wide number of genetic abnormalities occur in de novo acute myeloid leukemia, but the significance of many is not

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yet well understood. Some are thought to ensure proliferation and survival of the abnormal clone (type I), others are thought to impair differentiation (type II). Type II abnormalities include those that characterize distinct subtypes of AML with recurrent genetic abnormalities (e.g., *PML-RARA, RUNX1-RUNX1T1, CBFB-MYH11, KMT2A* fusions). Type I mutations include receptor tyrosine kinase or GTPase alterations, such as mutations of *FLT3, KIT, RAS, PTPN11,* and *JAK2*. Although current knowledge of gene mutations suggests more complexity than only two groups, classic type II abnormalities are generally considered to be disease-defining whereas others may only impact prognosis.

In recent years, it has become clear that mutations of the *FLT3* gene are the most common genetic abnormality in AML. *FLT3* encodes a receptor tyrosine kinase that is constitutively activated by either ITD of the juxtamembrane region or by point mutations in the second tyrosine kinase domain (D835) in AML. The FLT3 protein is expressed in early hematopoietic progenitor cells and is involved in early stem cell survival and myeloid differentiation. *FLT3* mutations occur in 20% to 28% of adult AMLs and 11.5% of all de novo pediatric AMLs. The mutations occur with multiple entities of the WHO classification. Among AML with recurrent translocations, they are frequent in APL with *PML-RARA* and AML with t(6;9). *FLT3* ITD mutations are associated with shorter duration of complete remission and overall survival. The ratio of mutant *FLT3* to wild-type allele can identify patients with poor outcomes. FLT3 inhibitor drugs are under study for these patients. A recently completed phase 3 trial (CALGB 10603) has shown improved event-free and overall survival for patients with *FLT3* ITD mutated AML treated with induction and consolidation chemotherapy plus the kinase inhibitor midostaurin. In April of 2017, midostaurin was approved for the treatment of FLT3- mutated AML. Because of the relatively high frequency and clinical significance of these mutations in AML, *FLT3* mutation testing is performed commonly and guides targeted therapy.

ACUTE MYELOID LEUKEMIA WITH MUTATED *NPM1*

Mutation of the nucleophosmin gene *(NPM1)* is seen in approximately 35% of primary nonelderly adult AML. Mutations occur in exon 12 and affect the C-terminal portion of the protein, thus altering its subcellular localization. The mutations occur most frequently in cases of AML with normal karyotype (85%). They are rare in AML with the recurrent genetic abnormalities listed previously. Less than 10% of AML with mutated *NPM1* have karyotypic abnormalities, most of which are non-specific. Some cases also show multilineage dysplasia. Most do not have myelodysplasia-related cytogenetic abnormalities with the exception of del 9q, which, when present, does not impact prognosis. The 2016 WHO classification allows for multilineage dysplasia in de novo cases without myelodysplasia-related cytogenetic abnormalities in this category. Most cases have monocytic blast cell features with nuclear invaginations common. The myeloblasts are often negative for HLA-DR and CD34. Overall, AML with mutated *NPM1* in the absence of an *FLT3* mutation appears to have a favorable prognosis similar to core binding factor AML. The mutation leads to aberrant cytoplasmic localization of the nucleophosmin protein. Initial studies suggested that immunohistochemistry was an appropriate screening tool for this diagnosis; however, subsequent studies have shown discordance between immunostaining results and *NPM1* mutation status. Molecular assessment is the preferred method for assessing this mutation.

Secondary mutations in *FLT3*, *DNMT3A*, *TET2*, *IDH1*, and *IDH2* are relatively common in AML with mutated *NPM1* and may impact prognosis. Minimal residual disease testing for the mutant *NPM1* gene is also useful in predicting prognosis.

ACUTE MYELOID LEUKEMIA WITH BIALLELIC MUTATIONS OF *CEBPA*

Mutations of the *CEBPA* gene occur in 7% to 11% of acute myeloid leukemia and 15% to 18% of normal karyotype AML. More than 100 different non-silent mutations are identified. *CEBPA* encodes the basic zipper transcription factor CCAAT/enhancer binding protein α (C/EBPα), which is critical for normal differentiation of mature granulocytes. Mutations may occur in both the C- and N-terminal of the gene. C-terminal mutations of *CEBPA* disrupt the basic zipper region that contains both DNA-binding and dimerization domains. N-terminal mutations disrupt the reading frame and result in premature termination and truncation of the normal 42-kDa protein and enhanced translation of the 30-kDa protein initiated downstream. N-terminal mutations of *CEBPA* result in inhibition of myeloid and erythroid cells in humans. Mutations in the *CEBPA* gene are reported in 10% of patients with AML. Although the *CEBPA* mutants are known to display distinct biological function during leukemogenesis, the molecular basis for this subtype of AML remains elusive. Mutation of *CEBPA* appears to disrupt the regulation of microRNA control of granulocytic differentiation in AML, and these microRNAs may represent a novel therapeutic target in AML with *CEBPA* mutations. It is now understood that the favorably prognostic importance of *CEBPA* mutations only correlates with biallelic mutations, and this category was modified in the 2016 WHO classification to restrict this group to only cases with biallelic mutations. As with AML with mutated *NPM1*, multilineage dysplasia is acceptable in cases of AML with biallelic mutations of *CEBPA*.

Acute myeloid leukemias with biallelic mutations of *CEPBA* are associated with intermediate risk cytogenetics and are more commonly associated with FAB M1 or M2 morphology and less commonly with monocytic features. Some authors suggest that patient with *CEBPA* mutation should be considered a favorable risk category, not requiring transplant in first remission. Unlike *NPM1* mutated AML, *FLT3* ITDs are uncommon in AML with biallelic mutations of *CEBPA*.

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ACUTE MYELOID LEUKEMIA WITH MUTATED *RUNX1*

AML with mutated *RUNX1* is a provisional entity in the 2016 WHO classification. It is restricted to de novo AML that does not meet criteria for any other WHO AML type (especially therapy-related AML and AML with myelodysplasia-related changes). The incidence of this mutation is 4% to 16%, but it is also associated with prior MDS, radiation exposure, or chemotherapy, findings that would disqualify a case from the category of AML with mutated *RUNX1*. It is more common in older adults without a definite sex predilection. Morphologically, the blasts may have a variable morphologic appearance, although a majority of so-called minimally differentiated AML cases have this mutation. There are no specific immunophenotypic features of this disorder. Most *RUNX1* mutations are monoallelic and involve the runt homology domain represented by exons 3 to 5 or the transactivation domain composed of exons 6 to 8. They are most commonly frameshift or missense mutations. Karyotypic abnormalities, including monosomy 7 or del(7q), trisomy 8, and trisomy 13, are commonly associated with the mutations but are often associated with myelodysplasia-related changes or prior therapy, which would result in reclassification of the case. Cases with these features should be classified as AML with myelodysplasia-related changes or therapy-related myeloid neoplasm. The recurring cytogenetic abnormalities of specific WHO categories are not commonly associated with mutation of *RUNX1*, and the presence of such an abnormality would also take diagnostic precedence over this provisional entity. Cooperating mutations are common with *RUNX1* and commonly involve *ASXL1*, *KMT2A*, *FLT3*, *IDH1*, and *IDH2*.

Mutations of *RUNX1* in AML are associated with worse overall survival, and the presence of combined mutations in *RUNX1* and *ASXL1* is associated with a particularly adverse prognosis. Survival may be improved with allogeneic hematopoietic cell transplantation. Similar to *CEBPA* mutations, a subset of patients with AML with mutated *RUNX1* has germline mutations of the gene, and when either mutation is detected, germline studies should be performed or careful family histories obtained. Affected family members may have autosomal-dominant thrombocytopenia and platelet-dense granule storage pool deficiency as well as an increased risk for development of AML or MDS.

ACUTE MYELOID LEUKEMIA WITH MYELODYSPLASIA-RELATED CHANGES

The 2008 WHO classification expanded the category of AML with multilineage dysplasia to AML with myelodysplasia-related changes (AML-MRC), now encompassing cases with multilineage dysplasia as defined in the previous classification, a myelodysplasia associated karyotype, or a history of myelodysplastic syndrome or myelodysplastic–myeloproliferative neoplasm. This category, however, is slightly modified in the 2016 revision of the WHO classification. Dysplastic changes may be evident in the non-blast marrow and peripheral blood elements. To meet the morphologic criteria for AML-MRC (multilineage dysplasia), there must be 20% or more bone marrow or peripheral blood blast cells and 50% or more dysplastic cells in at least two cell lines (erythroid, granulocytic, or megakaryocytic). The 50% cutoff for dysplastic changes is arbitrary and excludes some cases with definite associated dysplastic changes.

Dysplastic changes may be seen in both peripheral blood and bone marrow samples (Fig. 14.8). Red cell changes manifest as anisopoikilocytosis of peripheral blood red cells, including hypochromic teardrop-shaped cells and macrocytes, dimorphic red cell populations of the blood, nuclear-cytoplasmic asynchrony of red cell precursors, megaloblastic changes, and irregularities of red cell precursor nuclei. Megaloblastic changes differ from a left shift of erythroid cells by the presence of more immature nuclear chromatin, often associated with more mature, red-staining erythroid cell cytoplasm (dyssynchrony of nuclear and cytoplasmic maturation). Nuclear irregularities, including multinucleation, nuclear blebs, and irregular nuclear contours, are commonly seen in dysplastic erythroid precursors. Granulocyte dysplasia is most easily recognized in the more mature granulocyte forms of the blood and marrow. These changes include uneven cytoplasmic granulation or completely agranular mature neutrophils. Nuclear changes include clumping of nuclear chromatin that is usually associated with abnormalities of nuclear lobation, particularly monolobate or bilobate nuclei (pseudo–Pelger-Huët cells). Some nuclear abnormalities

ACUTE MYELOID LEUKEMIA WITH MYELODYSPLASIA-RELATED CHANGES—FACT SHEET

Definition

- AML with 20% blasts and at least one of the following:
  - Dysplastic changes in two or more bone marrow cell lines
  - Myelodysplasia syndrome–related karyotypic abnormality
  - History of myelodysplastic syndrome or myelodysplastic–myeloproliferative neoplasm, excluding cases with a recurrent genetic abnormality, history of cytotoxic therapy, or Down syndrome
  - De novo cases with a normal karyotype and either a mutation in *NPM1* or biallelic mutations of *CEBPA* are excluded

Frequency and Age Distribution

- Up to 50% of adult AML, approximately 20% of pediatric AML
- Most common in elderly

Most Common Cytogenetic and Molecular Events

- Complex karyotypes, balanced and unbalanced abnormalities (see Box 14.2)

Prognosis

- Poor

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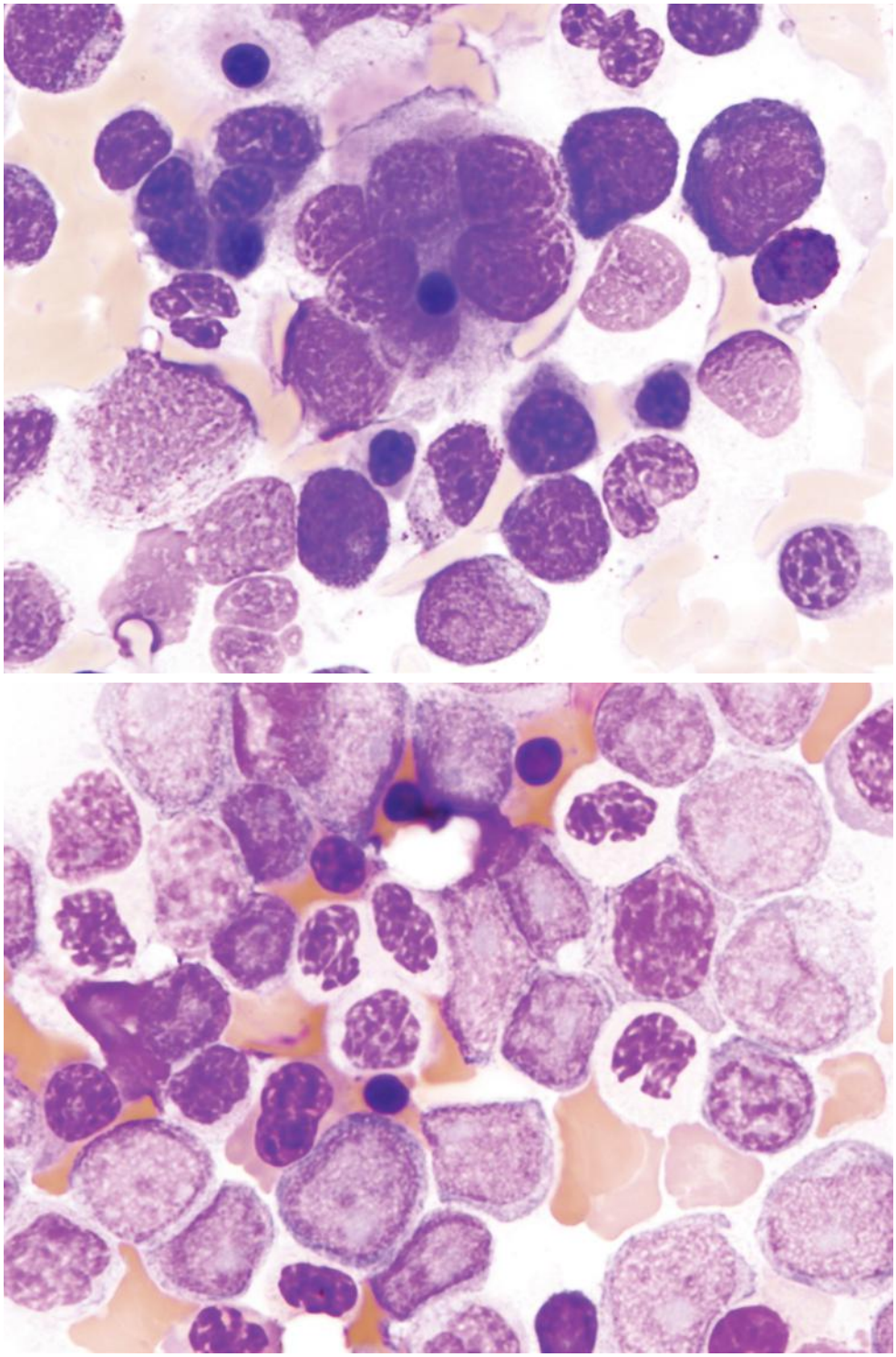


FIG. 14.8

Acute myeloid leukemia with myelodysplasia-related changes. The blasts are associated with trilineage dysplasia that includes erythroid precursors with irregular nuclear contours, hypolobated neutrophils with hypogranular cytoplasm, and a megakaryocyte with irregular nuclear lobation.

may be seen with drug therapy and do not constitute true dysplasia. These abnormalities are usually not accompanied by hypogranulation; therefore, identification of both features in the granulocyte line for dysplasia appears more specific. Peripheral blood platelets may show variation in platelet size with hypogranular platelets. Megakaryocytes may show great variation in size with detached, hyperlobate nuclei or hypolobate or monolobate forms with hyperchromatic nuclei.

Some cases have insufficient numbers of non-blast elements to make a morphologic assessment of multilineage dysplasia. A diagnosis of AML-MRC may be rendered based on a set of myelodysplasia-associated karyotypic abnormalities, including a complex karyotype (three or more unrelated abnormalities), certain unbalanced abnormalities, and a set of nine balanced translocations (see Box 14.2). Of note, del(9q), which was previously included as a myelodysplasia-associated

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ACUTE MYELOID LEUKEMIA WITH MYELODYSPLASIA-RELATED CHANGES—PATHOLOGIC FEATURES

Blast Cell Morphology
- Heterogeneous blast features

Non–Blast Cell Morphology (Usual Types)
- Erythroid cells
  - Nuclear-cytoplasmic dyssynchrony
  - Irregular nuclear contours
- Granulocytes
- Hypolobated nuclei with clumped nuclear chromatin
- Hypogranular cytoplasm
- Megakaryocytes and platelets
  - Hypogranular platelets
  - Megakaryocytes with hyperchromatic, hypolobated nuclei
  - Megakaryocytes with detached nuclear lobes

Immunophenotypic Features
- Nonspecific myeloid or myelomonocytic lineage

Differential Diagnosis
- AML with t(6;9)
- AML with inv(3), t(3;3)
- AML with mutated *NPM1*
- AML with biallelic mutations of *CEBPA*
- Myeloid proliferations of Down syndrome
- Therapy-related myeloid neoplasms
- Myelodysplastic syndrome

cytogenetic abnormality, but which appears not to alter the prognosis when present with *NPM1* or biallelic *CEBPA* mutations, is no longer included as diagnostic of this category in the 2016 WHO classification. In addition, a history of a prior myelodysplastic syndrome or myelodysplastic–myeloproliferative neoplasm is sufficient for the diagnosis of AML-MRC.

Although not considered a distinct entity, cases of AML-MRC with t(3;5)(q25.3;q35.1) *NPM1-MLF1* translocation tend to occur in young adult males and may respond well to hematopoietic cell transplantation (Fig. 14.9).

AML-MRC does not show specific blast cell morphologic features. Therefore, no specific cytochemical or immunophenotypic features of the blast cells exist for this disease category. The AML-associated karyotypes, particularly the chromosomal monosomies, generally have a poor prognosis. Multilineage dysplasia without an MDS-associated cytogenetic abnormality is also associated with a poor prognosis. Mutations of *U2AF1*, *ASXL1*, and *TP53* are relatively common in AML-MRC, particularly when compared with AML, not otherwise specified (NOS). *TP53* mutations are almost always associated with a complex karyotype but may suggest an even worse prognosis than in other cases in this generally poor prognostic group. The differential diagnosis of AML with multilineage dysplasia includes the more specific diagnoses of AML with t(6;9) and AML with inv(3)/t(3;3). Correlation with cytogenetics is always necessary because these specific karyotype abnormalities trump the category of AML-MRC. As mentioned, some cases of AML with mutated *NPM1* or AML with biallelic

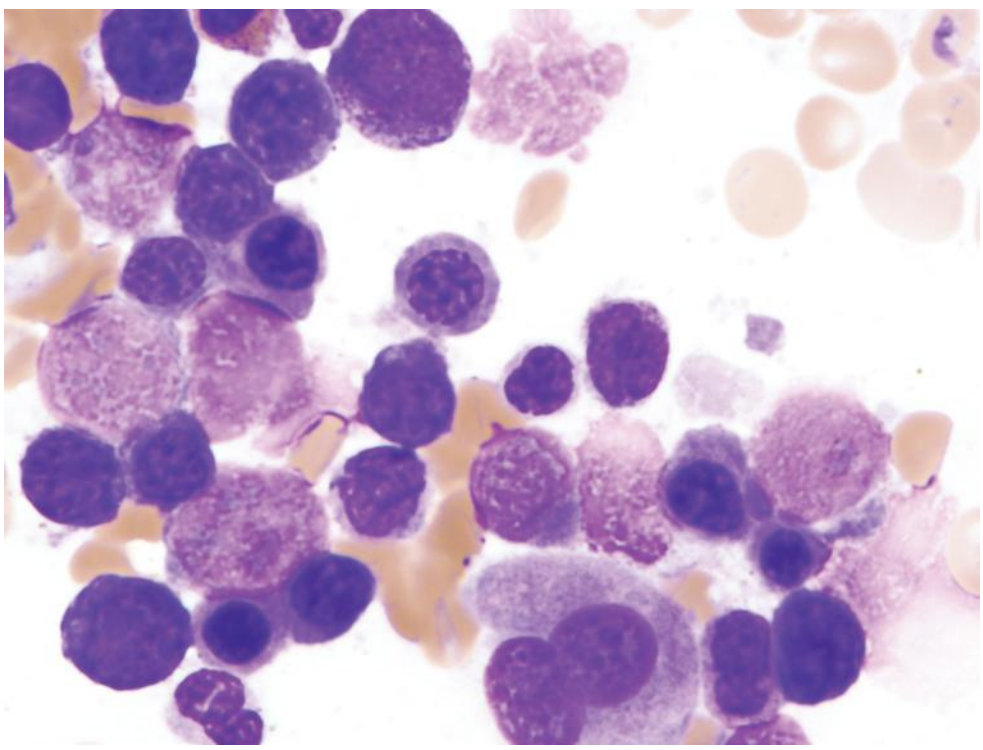


FIG. 14.9
Acute myeloid leukemia with t(3;5)(q25;q35.1). Blasts with associated multilineage dysplasia (erythroid and megakaryocytic in this field) are present.

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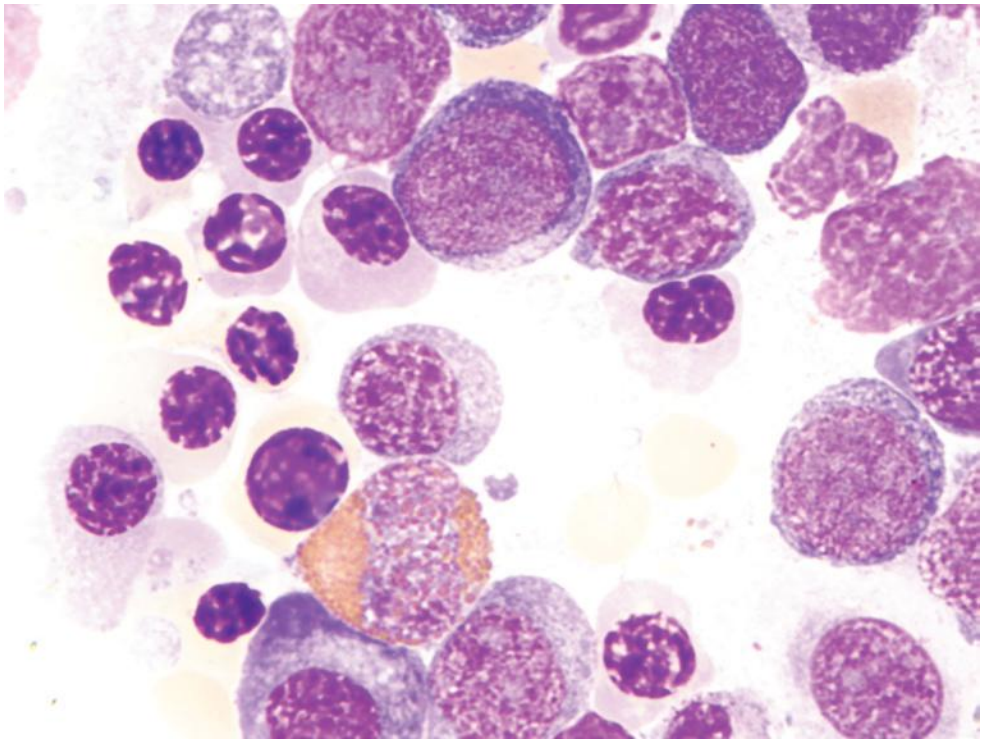


FIG. 14.10

Acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) with erythroid hyperplasia. Dysplastic erythroid cells represent more than 50% of the marrow cells. Because blast cells comprise more than 20% of the total cells (erythroid and nonerythroid nucleated cells) and multilineage dysplasia is present, this case is best classified as AML-MRC instead of acute erythroid leukemia.

mutations of *CEBPA* demonstrate multilineage dysplasia but do not have a history of MDS or a mixed myelodysplastic–myeloproliferative syndrome and do not have an MDS-associated cytogenetic abnormality. These cases should no longer be diagnosed as AML-MRC since their prognosis is based on the gene mutations. In addition, the diagnosis of therapy-related myeloid neoplasm or myeloid proliferation of Down syndrome is preferred in these clinical contexts, which commonly have multilineage dysplasia. Cases of AML-MRC may have erythroid hyperplasia with erythroid cells comprising greater than 50% of the marrow elements. If greater than 20% myeloblasts are present (out of all nucleated cells), a diagnosis of AML-MRC is appropriate. The previous category of AML, NOS, acute erythroid leukemia (erythroid/myeloid type) is now eliminated from the 2016 WHO classification (Fig. 14.10). Some patients with multilineage dysplasia and MDS-associated karyotypic abnormalities have erythroid or megakaryocytic blast phenotypes. It is not clear whether there is a meaningful prognostic distinction of these entities from other cases of AML-MRC with myeloid blasts. Recognition of the blast phenotype is certainly important for monitoring response to therapy. The marked dysplastic changes in some cases of myelodysplasia and AML with multilineage dysplasia can make enumerating blast cells difficult. Careful, high-power examination of the blast cell nuclei is often necessary to separate dysplastic, hypolobated granulocytes from blasts in these cases, because both may contain cytoplasmic granules. The more mature dysplastic granulocyte population has clumped nuclear chromatin compared with the fine nuclear chromatin of a blast cell.

THERAPY-RELATED MYELOID NEOPLASMS

The WHO category of therapy-related myeloid neoplasms recognizes that myelodysplasia, AML, and myelodysplastic–myeloproliferative neoplasms all may occur after cytotoxic therapy and have a similarly poor prognosis. Therefore, these neoplasms are considered together as a distinct clinical syndrome. The distinction into alkylating agent–related and topoisomerase II inhibitor–related types is no longer necessary. It is currently recognized that large-field radiation therapy, as well as cytotoxic therapy for non-neoplastic disease, can be an inciting factor. In some patients, myeloid disease as a second malignancy may follow a primary diagnosis of cancer treated with surgery alone. These cases are not truly therapy related but may be related to the primary neoplasm in other ways such as host or environmental factors. Transformation from myeloproliferative neoplasms (such as polycythemia vera or primary myelofibrosis) are excluded from this category because it is difficult to separate disease progression and evolution from therapy-related disease.

Therapy-related myeloid neoplasms may occur with a short latency (e.g., 2 years), often with abnormalities of *KMT2A* at 11q23.3 or *RUNX1* at 21q22.1 (Fig. 14.11) and associated with topoisomerase II inhibitors. Longer latency therapy-related disease (e.g., 5 or more years) often has myelodysplastic features including a prolonged MDS phase, multilineage dysplasia, and MDS-associated karyotypic abnormalities such as loss of genetic material of chromosomes 5 or 7, or both (Fig. 14.12). These features are more likely to be related to

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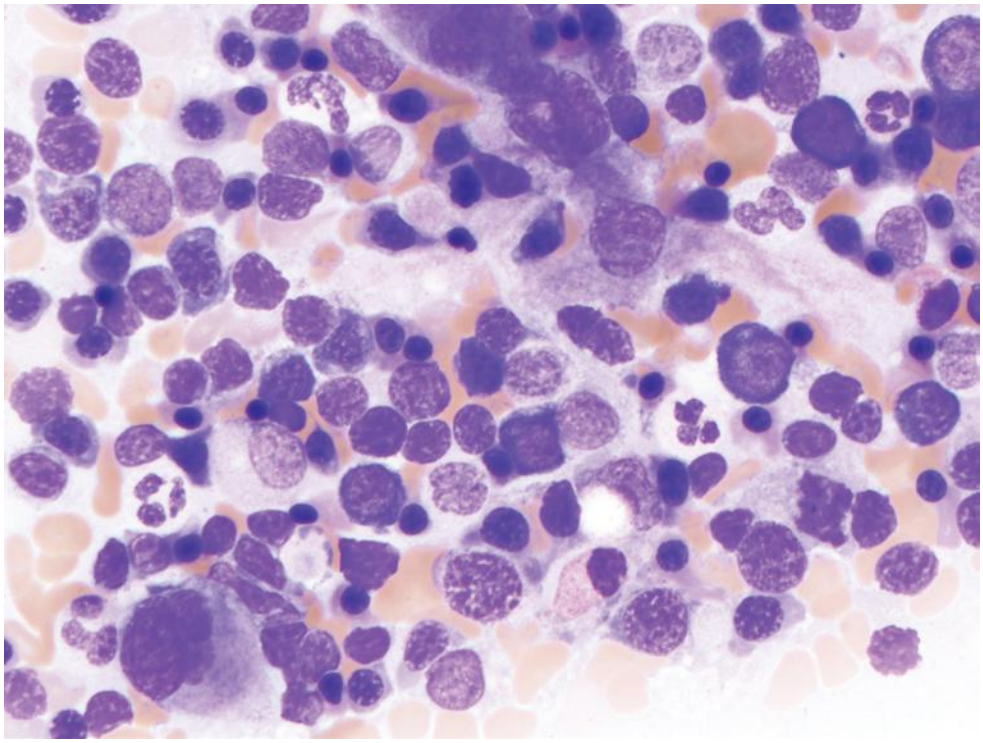


FIG. 14.11
Therapy-related acute myeloid leukemia following alkylating agent therapy showing multilineage dysplasia with erythroid hyperplasia.

THERAPY-RELATED MYELOID NEOPLASMS—FACT SHEET

Definition
- Myeloid neoplasms that occur after chemotherapy or radiation therapy for other malignancies or chronic diseases

Long Latency: Clinical Features
- Occurs 5 to 7 years after therapy, classically after alkylating agent exposure

Short Latency: Clinical Features
- Occurs 2 to 3 years after therapy, often topoisomerase II inhibitor therapy

Prognosis
- Poor

THERAPY-RELATED MYELOID NEOPLASMS—PATHOLOGIC FEATURES

Long Latency

Bone marrow morphologic features
- Similar to AML-MRC

Cytogenetic features
- Complex karyotypes
- Abnormalities of chromosomes 5 and 7

Short Latency

Morphologic features
- Usually monoblastic or myelomonocytic without associated dysplasia

Cytogenetic features
- Abnormalities at 11q23.3 or 21q22.1

alkylating agents or ionizing radiation. Mutations in *TP53*, *TET2*, *PTPN11*, *IDH1*, *IDH2*, *NRAS*, and *FLT3* have been reported in therapy-related myeloid neoplasms.

A history of prior therapy trumps a diagnosis of AML-MRC. The pathologic features of therapy-related myeloid neoplasms are not specific and are those of an MDS with multilineage dysplasia, MDS-myeloproliferative neoplasm (MPN) overlap such as chronic myelomonocytic leukemia (CMML), or AML. It appears that the exact blast percentage, marrow cellularity, or pathologic subclassification is less important than the history of prior therapy. However, from a practical aspect, it is appropriate to report such cases as therapy-related(t)-AML/t-MDS or t-AML/t-MDS/MPN with a description of the findings and blast percentage.

A number of recurrent genetic abnormalities can occur in therapy-related myeloid neoplasms, including the more favorable subtypes of inv(16) and t(15;17). Some studies demonstrate cytogenetic prognostic significance similar to that of de novo AML. Treatment of therapy-related disease is challenging as a result of drug resistance and limited tolerance to chemotherapy because of previous exposure.

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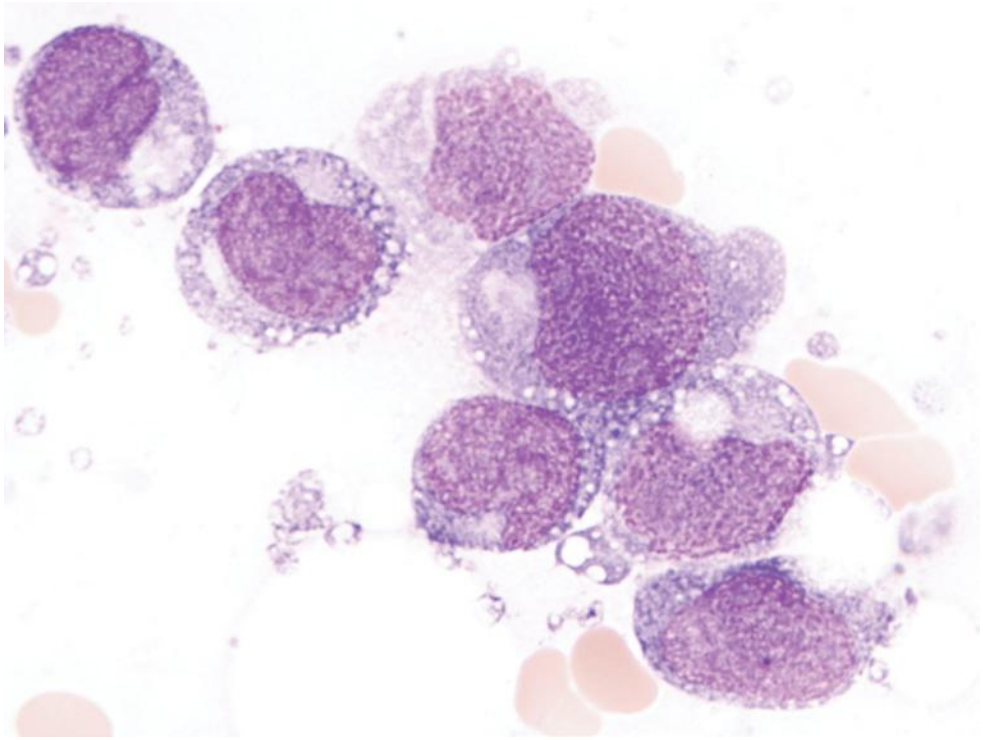


FIG. 14.12
Therapy-related acute myeloid leukemia following topoisomerase II inhibitor therapy. The blast cells have a monoblastic appearance.

ACUTE MYELOID LEUKEMIA, NOT OTHERWISE SPECIFIED, AND THE FRENCH-AMERICAN-BRITISH COOPERATIVE GROUP CLASSIFICATION OF ACUTE MYELOID LEUKEMIA

The WHO classification of AML includes an NOS disease group with nine different subgroups, but due to a lack of clinical difference among the subgroups it is not essential to subclassify all cases. Many of these subgroups have similarities to the original FAB disease groups but differ in several ways. The WHO AML, NOS, category does not include cases with the recurring cytogenetic abnormalities mentioned previously, cases meeting criteria for AML-MRC, or those occurring in patients with a history of cytotoxic therapy or Down syndrome. AML, NOS, defines acute leukemia as bone marrow proliferations with 20% or more blasts as opposed to a 30% or higher cutoff for the FAB classification.

The category of AML, NOS, AML with minimal differentiation is similar to the FAB category of minimally differentiated acute myeloid leukemia (M0). This leukemia is acute with uniform blast cells without cytoplasmic granules, with no Auer rods, and negative blast-cell cytochemical reactions for myeloperoxidase, Sudan black B, and nonspecific esterase (Fig. 14.13). Many laboratories today no longer perform cytochemical stains, making it difficult to distinguish this subtype from other AMLs. The lineage of the blast cells is only recognizable by immunophenotyping methods, which usually show the blast cells to express two of the more common AML markers, including myeloperoxidase, CD13, CD33, or CD117. This is considered evidence of myeloid lineage commitment. Antigens associated with monocytic or myeloid maturation, or erythroid or megakaryocytic lineage are not expressed. Myeloperoxidase may be positive by flow cytometry, but the WHO does not describe a threshold for "positive." The FAB classification required 3% myeloperoxidase (MPO) reactivity by cytochemistry, and the European Group for the Immunological classification of Leukemias (EGIL) classification required 10% positivity by flow cytometry to designate a case as MPO positive. It can be difficult to differentiate minimally differentiated AML from acute undifferentiated leukemia in the absence of immunophenotypic evidence for MPO positivity. Acute leukemias with only one myeloid lineage marker, and no definitive evidence of erythroid, megakaryocytic, lymphoid, or blastic plasmacytoid dendritic cell lineage are best characterized as acute undifferentiated leukemia. AML with minimal-differentiation blast cells may be TdT positive and frequently express CD7. There may be rearrangements of T- and B-cell receptor genes, and these gene rearrangements are not to be interpreted as evidence of lymphoid lineage. Specific evidence of lymphoid lineage must be excluded by immunophenotyping. The WHO definition of lymphoid lineage is detailed in Chapter 16. The most specific marker of T-cell lineage is CD3. B-cell lineage is determined by a combination of antigens, including CD19, cytoplasmic CD22, CD10, and CD79a. Blastic plasmacytoid dendritic cell (BPDC) tumor is detailed below in a separate section. Expressions of CD4, CD56, and CD123 are hallmarks of this entity, and it must be excluded by further immunostains before a diagnosis of AML with minimal differentiation is established. *RUNX1* (or *AML1*) gene mutations are more common in this FAB type than others, and de novo cases with mutated *RUNX1* would now be considered as part of the 2016 WHO provisional

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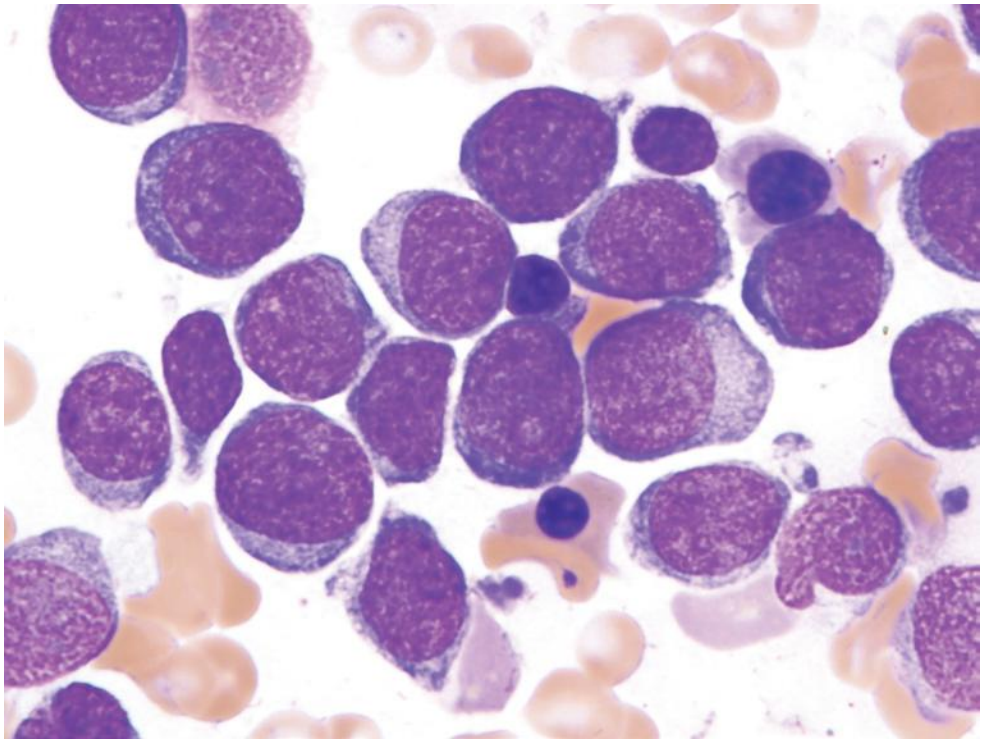


FIG. 14.13
Minimally differentiated acute myeloid leukemia. The blasts mark with CD13 and CD33 by flow cytometry but are myeloperoxidase and nonspecific esterase negative by cytochemical study.

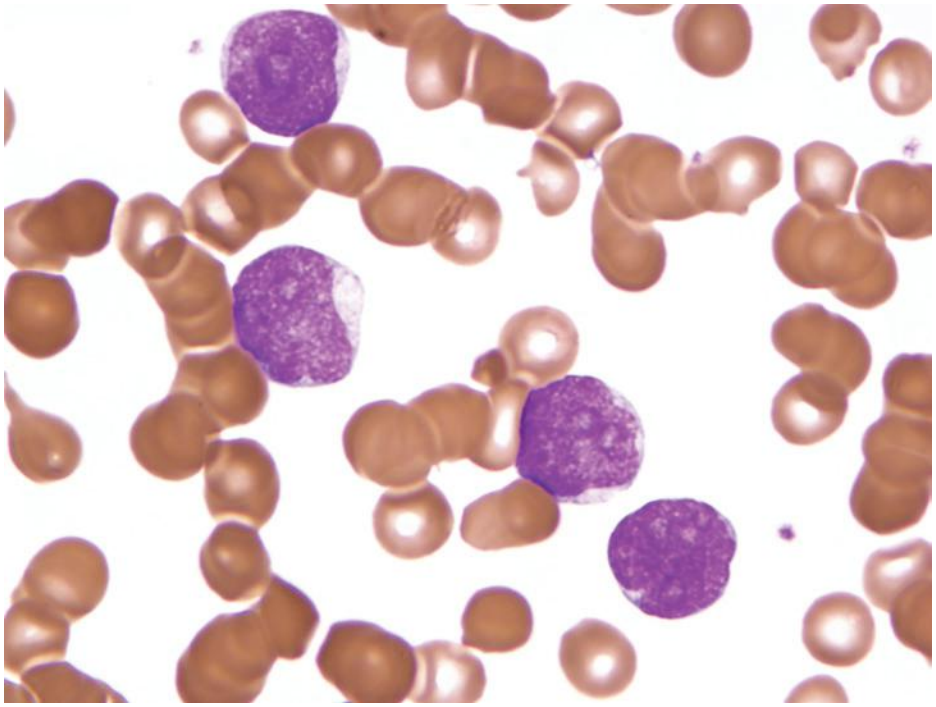


FIG. 14.14
Acute myeloid leukemia without maturation. The blasts are similar in appearance to those in Fig. 14.13 but were myeloperoxidase positive and nonspecific esterase negative.

entity of AML with mutated *RUNX1* rather than this subcategory of AML, NOS. The largest series of FAB M0 cases with karyotype suggests that occasional cases (0.5%) will be diagnosed with a specific recurrent genetic abnormality (inv [3]/t[3;3] and t[9;11]), and approximately 22% will be reclassified as AML-MRC based on the frequently complex karyotype. *FLT3* ITD mutations were reported in 22%.

AMLs that are myeloperoxidase positive and nonspecific esterase negative by cytochemistry and that show less than 10% bone marrow cells with maturation to or beyond the promyelocyte level of differentiation are designated AML, NOS, without maturation in the WHO classification or FAB M1 (Fig. 14.14). In the absence of cytochemical stains, this diagnosis is based on the flow cytometric detection of

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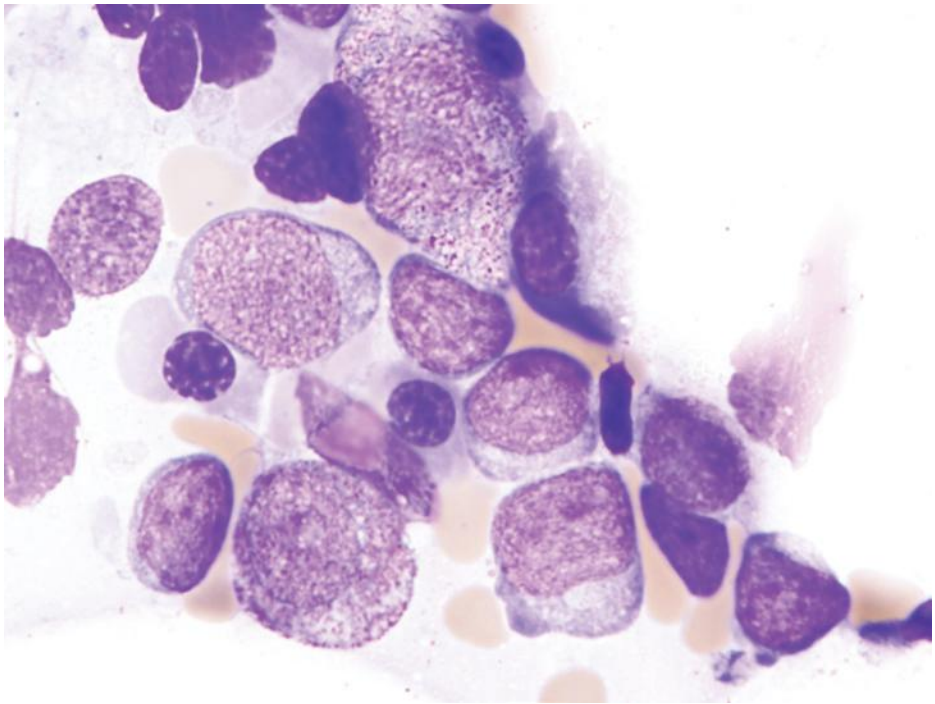


FIG. 14.15

Acute myeloid leukemia with maturation. More than 10% of marrow cells contain sufficient granules to be considered at the promyelocyte stage of maturation.

MPO-positive blasts lacking evidence of monocytic maturation. Without cytochemical stains, no criteria exist to distinguish flow cytometry MPO-positive blasts of AML with minimal differentiation that efface the marrow from AML without maturation. AML, NOS, with maturation (FAB M2) is defined as cytochemically similar to AML M1 but with 10% or more bone marrow cells showing maturation to or beyond the promyelocyte level (Fig. 14.15).

Acute promyelocytic leukemia corresponds to FAB M3 and has no corresponding subcategory in the WHO AML, NOS, group. All such cases would fall into the category of AML with recurrent cytogenetic abnormalities (see previous discussion). Rarely, a case of AML, NOS, will exhibit granular blasts resembling promyelocytes but lack cytogenetic or molecular evidence of a *PML-RARA* rearrangement. Such cases are best designated AML with maturation.

AML, NOS, acute myelomonocytic leukemia (FAB M4) shows substantial myeloid (granulocytic) and monocytic differentiation. There are 20% or more blasts that may be the sum of myeloblasts, monoblasts, and promonocytes. Monoblasts are large with round nuclei, fine chromatin, variably prominent nucleoli, and moderate amounts of pale gray-blue cytoplasm with sparse azurophilic granules. The cytoplasm may also show vacuoles and blebs (pseudopods). Promonocytes have nuclear chromatin similar to monoblasts, with slightly indented nuclei with nuclear creases or folds and moderate amounts of gray-blue cytoplasm with more noticeable granules and vacuoles. Auer rods can be seen (Fig. 14.16). A subset of the blast cells are myeloperoxidase positive, but the strong positivity of virtually all cells seen in the microgranular variant of acute promyelocytic leukemia, which may mimic acute myelomonocytic leukemia, is not usually present. The blast cells of acute myelomonocytic leukemia are usually nonspecific esterase positive, reflecting monocytic differentiation. By definition, 20% to 79% of bone marrow neoplastic cells show monocytic differentiation by morphology or cytochemistry. Indeed, double staining for chloracetate esterase and nonspecific esterase may show dual-positive cells in some cases. A subtype of M4 AML (acute myelomonocytic leukemia with eosinophilia or M4Eo) is included in the FAB classification and corresponds to AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22) of the WHO AML with recurrent cytogenetic abnormalities (see Fig. 14.2).

When there are more than 20% bone marrow or blood blasts (again a combination of myeloblasts, monoblasts, and promonocytes) and 80% of neoplastic cells are nonspecific esterase positive or are morphologically monoblasts with immunophenotypic evidence of monocytic differentiation, a diagnosis of AML, NOS, acute monocytic and monoblastic leukemia (FAB M5) is made (Fig. 14.17). These cases may be myeloperoxidase negative but differ from minimally differentiated AML by their positivity for nonspecific esterase. The blasts of acute monocytic leukemia have abundant, often agranular cytoplasm that may contain vacuoles. The blast nuclei may be round and uniform or folded. Cases with more immature nuclear features in the bone marrow (typically when the monocytic cells are 80% or more true monoblasts) are termed *monoblastic* (FAB M5a), whereas those with evidence of monocytic maturation in the bone marrow are termed *monocytic* (FAB M5b). Monocyte maturation may be prominent in the peripheral blood in either acute monocytic or myelomonocytic leukemia, and the subclassification is best made on bone marrow specimens. However, the blood may help to confirm the monocytic component of the leukemia.

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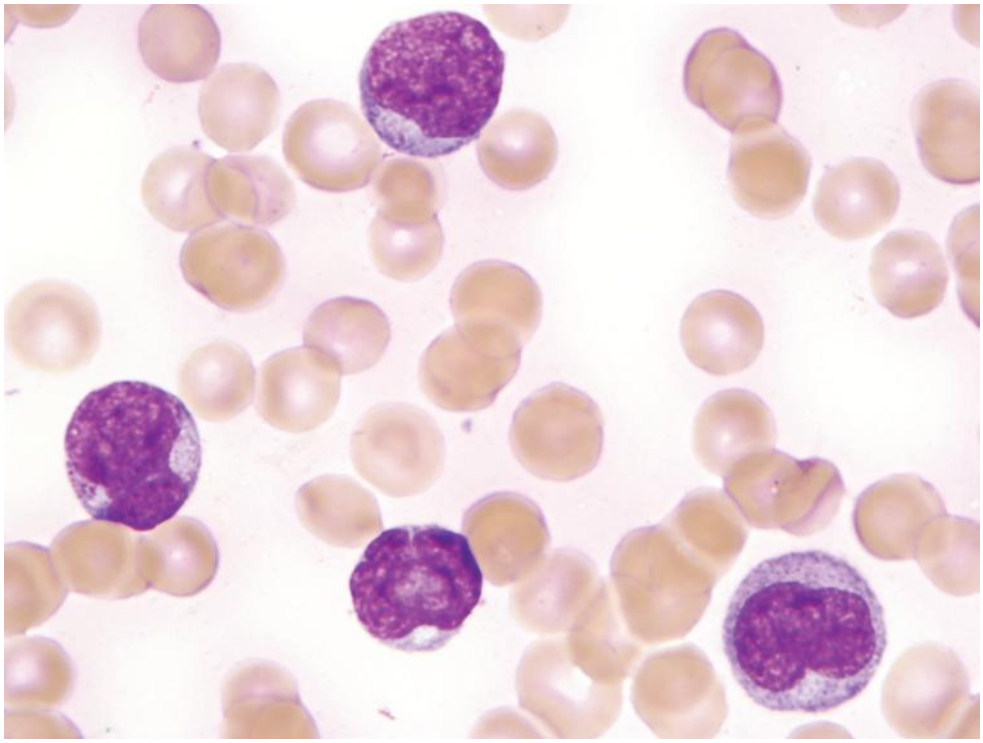


FIG. 14.16

Acute myelomonocytic leukemia. Some blasts have granules and others have folded nuclei suggestive of monocytic differentiation. These cells are partially myeloperoxidase positive and show more than 20% nonspecific esterase positivity by cytochemistry.

A subset of FAB M4 and M5 AMLs will have 11q23.3 abnormalities. Only the t(9;11) is recognized as a recurrent genetic abnormality. The variant 11q23.3 translocation should be specified in the diagnosis, because some are now recognized to have some specific prognostic importance as well (see previous discussion).

Acute erythroid leukemia previously consisted of at least two different categories in the WHO classification that roughly correspond to the original Di Guglielmo syndrome and disease. Acute erythroleukemia, erythroid/myeloid type of the 2008 WHO classification is no longer included in the 2016 WHO classification. It was a myeloid blast cell proliferation that occurred in association with erythroid hyperplasia. By definition, erythroid precursors represented 50% or more of the bone marrow cells, and blast cells were 20% or more of the nonerythroid elements for this diagnosis. Therefore, cases with high numbers of bone marrow erythroid precursors and relatively low blast cell counts were diagnosed as acute leukemia. Such cases are now diagnosed as myelodysplastic syndromes based on the absolute blast percentage. Most cases of AML with erythroid hyperplasia fulfill criteria for AML-MRC by virtue of greater than 20% myeloblasts (out of all nucleated cells) and multilineage dysplasia, or this blast percentage and an MDS-associated karyotype; these cases should not be called AML, NOS. As described previously, AML with t(6;9) commonly has erythroid hyperplasia. Pure erythroid leukemia is now the only type of acute erythroid leukemia in the WHO AML, NOS, category. It is defined as a bone marrow proliferation of more than 80% neoplastic, immature erythroid cells with at least 30% proerythroblasts and without a significant myeloblast component (Fig. 14.18). These cases are also frequently associated with multilineage dysplasia and myelodysplasia-related karyotypes. It is not clear whether these cases have a clinical behavior distinct from other cases of AML-MRC or whether they should be classified together.

Acute megakaryoblastic leukemia corresponds to FAB M7. The WHO defines acute megakaryoblastic leukemia as an acute leukemia in which 50% or more of the blasts are of megakaryocytic lineage. Excluding cases with t(1;22), inv(3)/t(3;3), and Down syndrome, this morphology remains more common in children. Most cases of adult acute megakaryoblastic leukemia are associated with multilineage dysplasia and could be considered as AML-MRC. The morphologic, immunophenotypic, and ultrastructural features are similar to those described previously for other types of megakaryoblastic leukemias, with detection of CD41 and CD61 expression being the most common means of defining megakaryocytic lineage. These cases are commonly associated with marked marrow fibrosis and an inaspirable marrow ("dry tap"), making diagnosis difficult in many cases.

The WHO AML, NOS, group includes two additional subcategories that do not correspond to previous FAB categories. The first is acute basophilic leukemia, which is defined as an AML with primary differentiation to basophils. It is unclear whether this subcategory represents a distinct entity, because some specific AML types are associated with basophilia and should not be placed in this category, such as t(9;22) AML, blast crisis of chronic myeloid leukemia, t(6;9) AML, t(3;6) AML, and AMLs with 12p abnormalities. In addition, cases of AML with the abnormal eosinophils of inv(16) and cases of mast cell leukemia may be mistaken for basophilic leukemias. Therefore the lack of specificity of this diagnosis should be recognized, and attempts should be made

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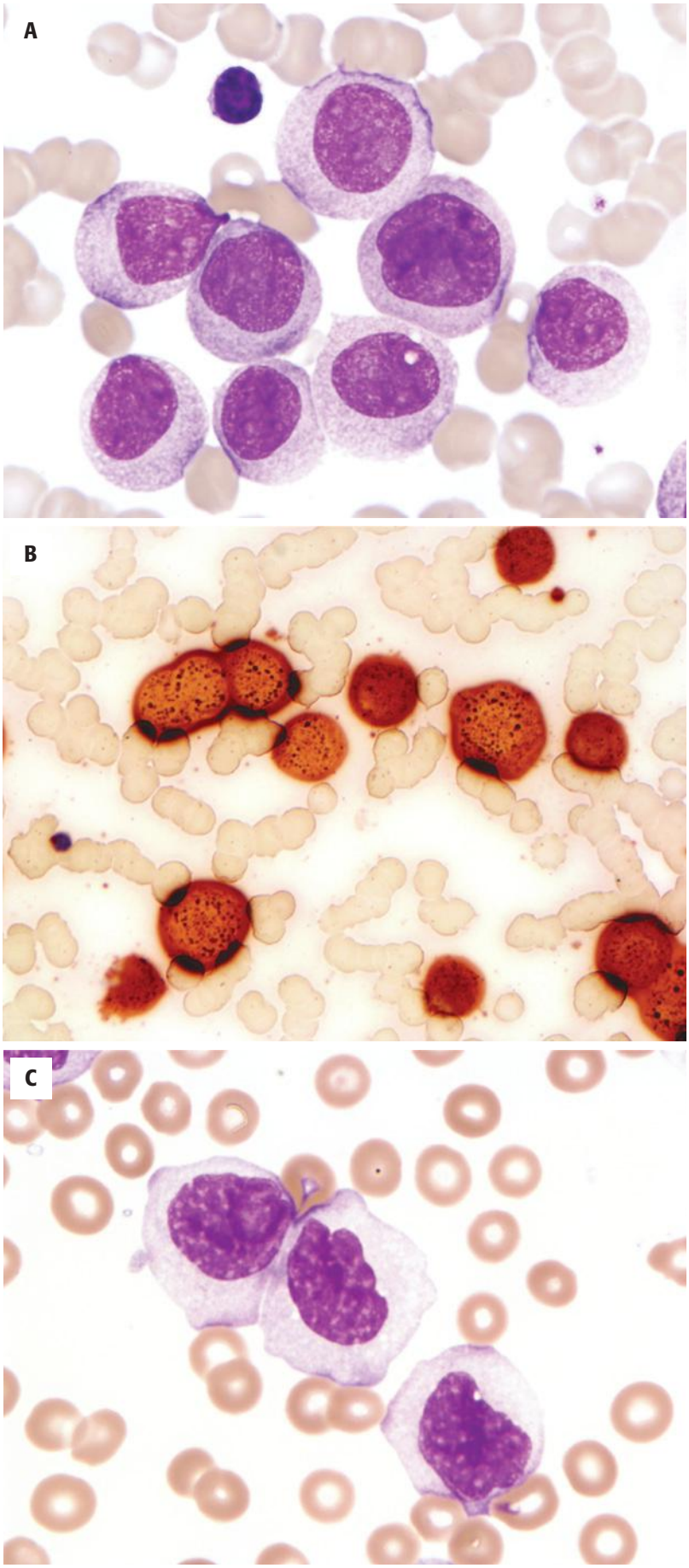


FIG. 14.17

Acute monocytic leukemia. A, Monoblastic cases are more immature without nuclear folds. B, More than 80% of the blasts are nonspecific esterase positive. C, Monocytic cases show more monocytic maturation (promonocytes) with more irregular nuclear contours. These cells are also more than 80% nonspecific esterase positive.

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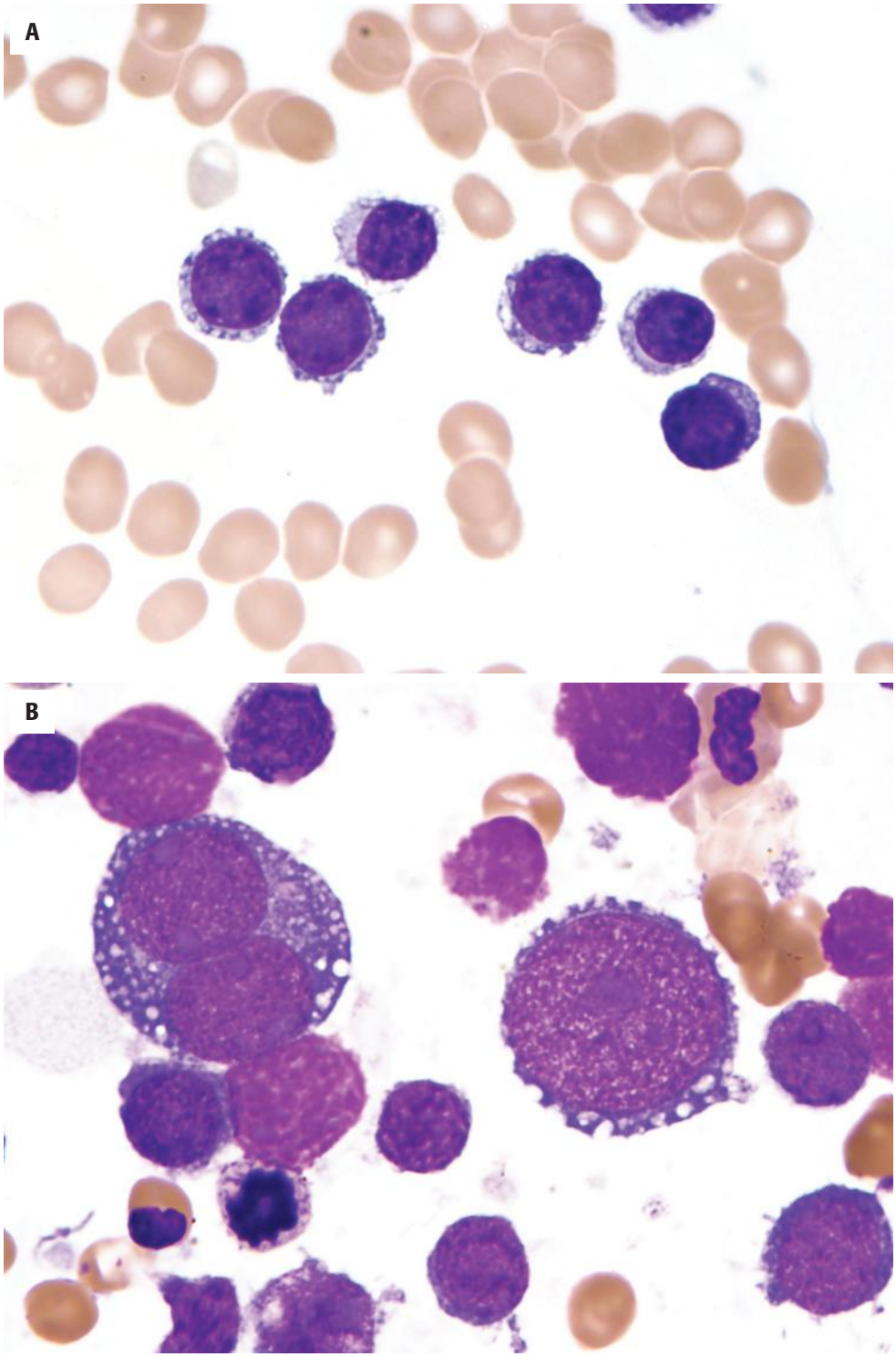


FIG. 14.18

Pure erythroid leukemia. A, Rare cases show a monotonous population of immature erythroid cells with cytoplasmic vacuoles. B, Most cases, however, show more variation in erythroid cells and are associated with multilineage dysplasia.

to diagnose cases with increased basophils or basophil-like cells into more specific disease categories.

Acute panmyelosis with myelofibrosis is defined by the WHO as an acute panmyeloid proliferation with accompanying bone marrow fibrosis (Fig. 14.19). Some authors suggest that this disease may be a subtype of acute megakaryoblastic leukemia, because they commonly share marrow fibrosis, multilineage dysplasia, and complex cytogenetic abnormalities. Most cases of acute panmyelosis with myelofibrosis have 20% to 25% blasts; however, unlike the other entities on the differential diagnosis (e.g., AML-MRC, acute megakaryoblastic leukemia, spent phase of a myeloproliferative disorder), there is a prominent proliferation of myeloblasts, proerythroblasts, and megakaryoblasts (panmyelosis).

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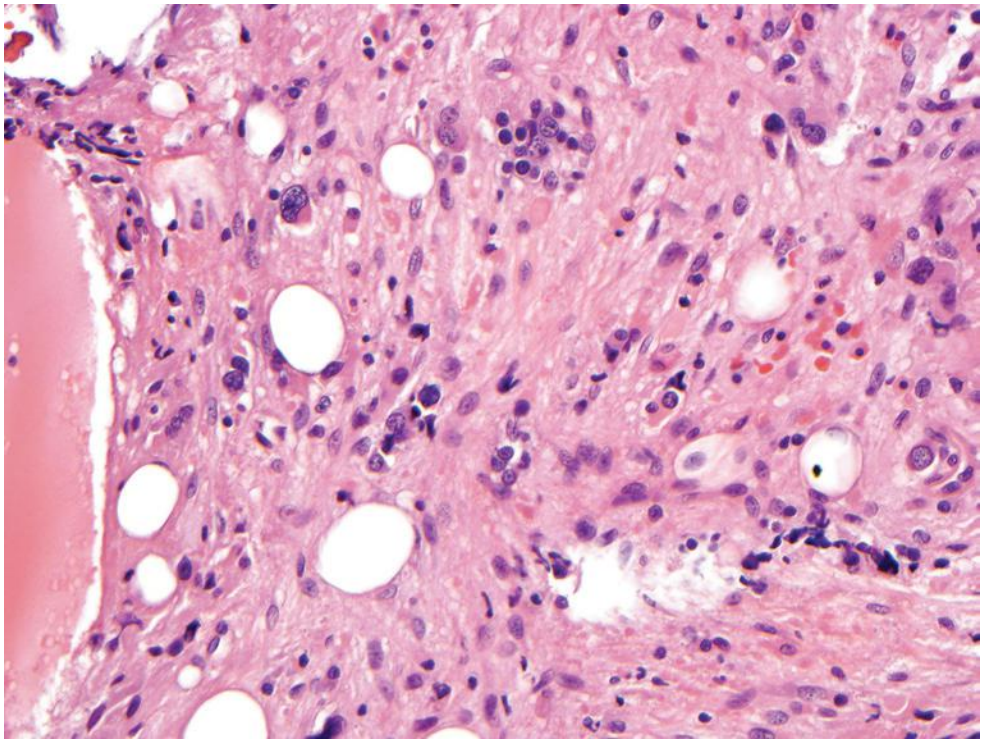


FIG. 14.19

Acute panmyelosis with myelofibrosis. The marrow is inaspirable with marked marrow fibrosis, dysplastic megakaryocytes, and scattered immature-appearing cells. This appearance is similar to many cases of adult megakaryoblastic leukemia but with less than 20% megakaryoblasts.

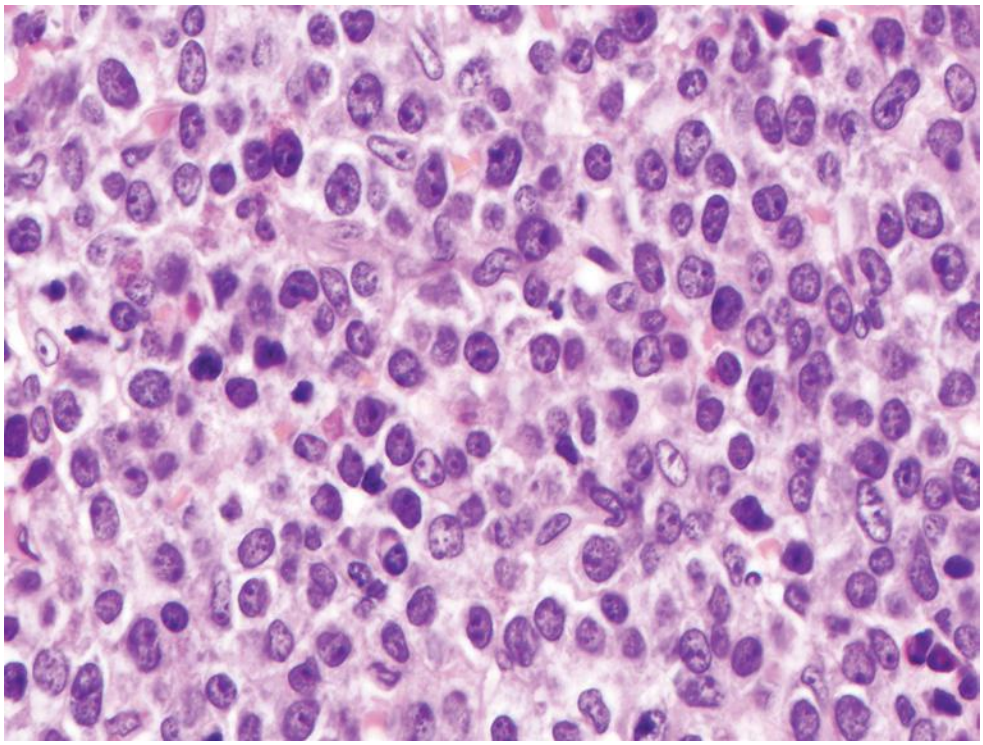


FIG. 14.20

Extramedullary myeloid cell tumor (myeloid sarcoma) involving a lymph node. The tissue is infiltrated by a monotonous population of mononuclear cells with a subset showing granular cytoplasm.

MYELOID SARCOMA

Myeloid sarcoma (also known as *granulocytic sarcoma, chloroma,* and *extramedullary myeloid tumor*) is defined as an extramedullary mass-forming lesion of immature myeloid cells (Fig. 14.20) and is diagnostic of AML, even without 20% blood or marrow blasts. Myeloid sarcoma can occur before, simultaneously with, independent of, or as a relapse of an acute or chronic myeloproliferative disorder. Although myeloid sarcoma has its own category, this type of myeloid proliferation is commonly associated with one of the AMLs with recurrent cytogenetic abnormalities, particularly in children. In adults, it can accompany AML-MRC, MDS,

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accelerated or blast phase of a chronic myeloproliferative neoplasm, or myelodysplastic or myeloproliferative neoplasm. Myeloid sarcoma is classically described as being associated with eosinophilic myelocytes, a clue to a myeloid proliferation. Cases with this morphology are often recognized as extramedullary presentations of AML with t(8;21). Other cases have a monotonous blastic or large cell appearance. Such cases may mimic lymphoblastic, Burkitt, or diffuse large cell lymphomas. Correlation with peripheral blood or bone marrow samples to evaluate for acute leukemia is usually helpful, but some cases represent isolated presentation or relapses of myeloid tumors. Myeloid sarcomas are usually diagnosed using paraffin section immunohistochemistry; they fail to react with specific lymphoid markers, such as CD2, CD3, CD20, or CD79a, and are usually immunoreactive for myeloperoxidase and CD33 or express monocyte markers such as CD14, CD163, or CD68. CD68 expression is nonspecific and should not be used alone for diagnosis of monocytic lineage. AML with t(8;21) commonly develops as myeloid sarcoma in the head and neck region of children. It should be remembered that this entity shows aberrant expression of B-cell markers such as PAX-5 and CD79a in some cases. In addition, AML with t(1;22) may develop as myeloid sarcoma and be negative for B- and T-cell markers, MPO, CD33, and CD68. CD43 will often be expressed, and megakaryocytic antigens such as CD61, CD41, or factor VIII–related antigen are diagnostic of the megakaryocytic lineage. Blastic plasmacytoid dendritic cell tumor commonly develops as a skin lesion and should be remembered in the differential diagnosis. These lesions share CD123 with many other types of AMLs but express TCL1, commonly lack CD13 and CD33, and do not express MPO. Repeated biopsy for cytogenetics and molecular studies should be strongly considered if the marrow is not informative in cases of myeloid sarcoma, because a more specific diagnosis is necessary for optimal risk stratification.

MYELOID PROLIFERATIONS OF DOWN SYNDROME

Approximately 10% of neonates with Down syndrome, as well as rare fetuses, develop a hematopoietic disorder that is morphologically indistinguishable from acute megakaryoblastic leukemia or a traditional myelodysplastic syndrome termed *transient abnormal myelopoiesis* (TAM). These infants have a marked leukocytosis with numerous peripheral blood and bone marrow megakaryoblasts (Fig. 14.21). Some patients will also show peripheral blood basophilia and erythroid dysplasia. As in acute megakaryoblastic leukemia, the blasts are negative for myeloperoxidase and express myeloid and megakaryocyte-associated antigens. These transient proliferations may show karyotypic abnormalities in addition to trisomy 21, but they do not have the t(1;22) of infant acute megakaryoblastic leukemia, and they differ from true acute megakaryoblastic leukemia by clinical resolution in 1 to 3 months. Therefore, caution should be used in diagnosing a proliferation of this type as acute leukemia in a neonate with Down syndrome. Although cases may spontaneously remit, they can be associated with severe morbidity and mortality when associated with hepatic fibrosis and occlusion of small vessels of vital organs by the megakaryoblasts. Of note, TAM may be the first clinical indication of Down syndrome in

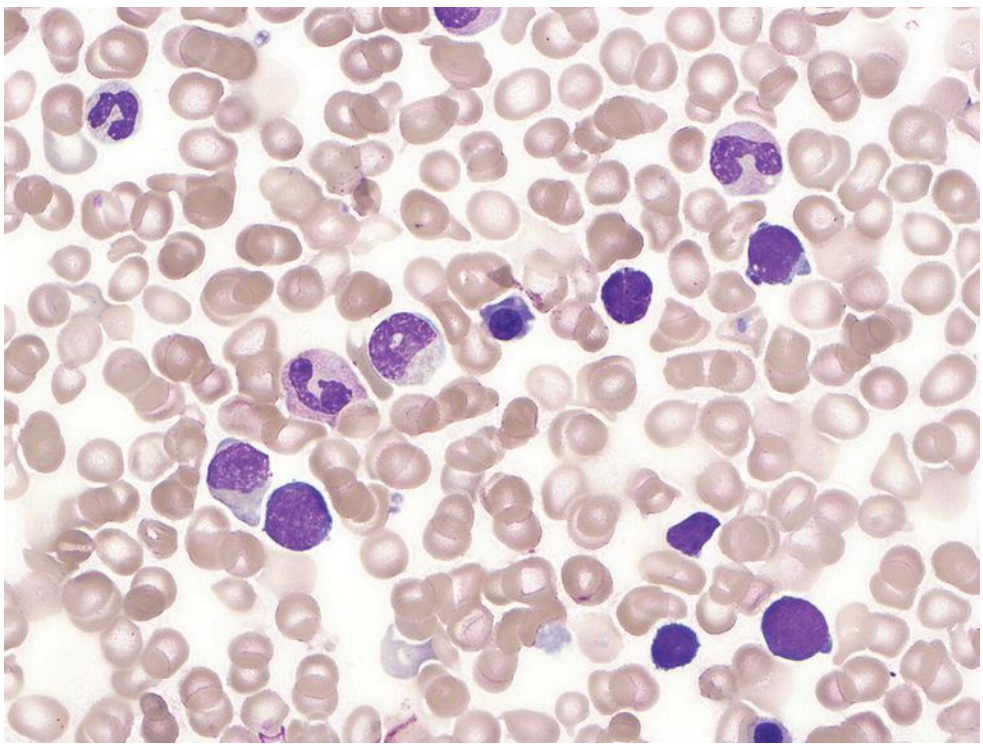


FIG. 14.21

Transient abnormal myelopoiesis of Down syndrome. The peripheral blood of this infant shows an elevated white blood cell count with circulating blast cells. Some blasts show cytoplasmic blebs, suggestive of megakaryoblasts. This lineage was confirmed by detection of CD61 and CD41 on the blast cell population.

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MYELOID PROLIFERATIONS OF DOWN SYNDROME—FACT SHEET

Definition
- Myeloid proliferations in infants and children with Down syndrome

Transient Abnormal Myelopoiesis

Frequency and age distribution
- Fetus and neonates
- Approximately 10% of patients with Down syndrome

Clinical Features
- Leukocytosis with resolution in 1 to 3 months

Cytogenetic and Molecular Features
- Trisomy 21 (constitutional), random nonclonal abnormalities, *GATA1* mutations, *JAK3* mutations

Prognosis
- Good, if not complicated by hepatic fibrosis

Myeloid Leukemia of Down Syndrome

Frequency and age distribution
- 2 years of age or older
- Approximately 2% of children with Down syndrome

Clinical Features
- Leukocytosis without resolution until treated, typically from TAM, which does not resolve

Cytogenetic and Molecular Features
- Trisomy 21 (constitutional), additional unbalanced clonal abnormalities (trisomy 1, trisomy 8, monosomy 7); *GATA1* mutations, *JAK2, JAK3* and other mutations

Prognosis
- Generally good in children less than 4 years of age

TRANSIENT MYELOPROLIFERATIVE DISORDER AND ACUTE MEGAKARYOBLASTIC LEUKEMIA OF DOWN SYNDROME—PATHOLOGIC FEATURES

Blast Cell Features
- Often megakaryoblastic with cytoplasmic blebbing

Non–Blast Cell Morphology
- Associated multilineage dysplasia

Blast Cell Immunophenotype
- Myeloid antigen positive (CD13, CD33)
- Megakaryocyte antigen positive (CD41, CD42, CD61)

Differential Diagnosis
- AML with t(1;22)
- AML-MRC
- AML, NOS, acute megakaryoblastic leukemia
- Myelodysplastic syndrome

patients with mosaicism for the trisomy 21 who do not display the typical physical features of Down syndrome. Therefore, awareness of this possibility in the neonatal setting and correlation with cytogenetic findings may prompt further investigation of the germline karyotype in nonmyeloid tissues.

Acute megakaryoblastic leukemia of Down syndrome (Fig. 14.22) is clinically distinct from TAM of Down syndrome, and it can occur 1 to 2 years after the resolution of a TAM or follow neonatal presentations of TAM that never completely abate. The vast majority of Down syndrome–associated AML are acute megakaryoblastic leukemias. These leukemias usually occur around 2 years of age and have a generally favorable prognosis until the age of 4 years, after which the prognosis worsens with advancing age.

Both acute megakaryoblastic leukemia and TAM of Down syndrome are characterized by acquired mutations of the *GATA1* gene. A single gene array study identified three transcriptional differences between the two entities: *CDKN2C* and *PRAME* transcripts were increased in acute megakaryoblastic leukemia, whereas expression of the *MYCN* gene was increased in TAM. Confirmation of these transcriptional differences may provide useful future molecular markers to distinguish these related but prognostically distinctive diseases. Acute leukemia is reportedly associated with acquisition of additional mutations, including *CTCF*, *EZH2*, *KANSL1*, *JAK2*, *JAK3*, *MPL*, *SH2B3*, and *RAS* pathway genes

MYELOID NEOPLASMS WITH GERMLINE PREDISPOSITION

Most cases of MDS or acute leukemia are sporadic diseases, but it is now clear that a subgroup of cases are associated with germline mutations and are familial. For this reason, the 2016 revision of the WHO classification has added a section on myeloid neoplasms with germline predisposition. This section includes cases of myelodysplastic syndrome, myelodysplastic-myeloproliferative neoplasms, and acute leukemias, usually AML, that occur on the background of a predisposing germline mutation. These patients may have a history of multiple prior neoplasms themselves or in family members that are sometimes associated with thrombocytopenia and may be accompanied by non-neoplastic clinical features associated with specific syndromes.

The presence of the specific underlying genetic defect or predisposition syndrome should be noted as part of the diagnosis since the abnormality may be present in other family members without disease. Such identification should raise awareness of the need to screen family members for these aberrations and hopefully allow for early detection of disease in affected relatives. The major categories of such familial cases are summarized in Box 14.3.

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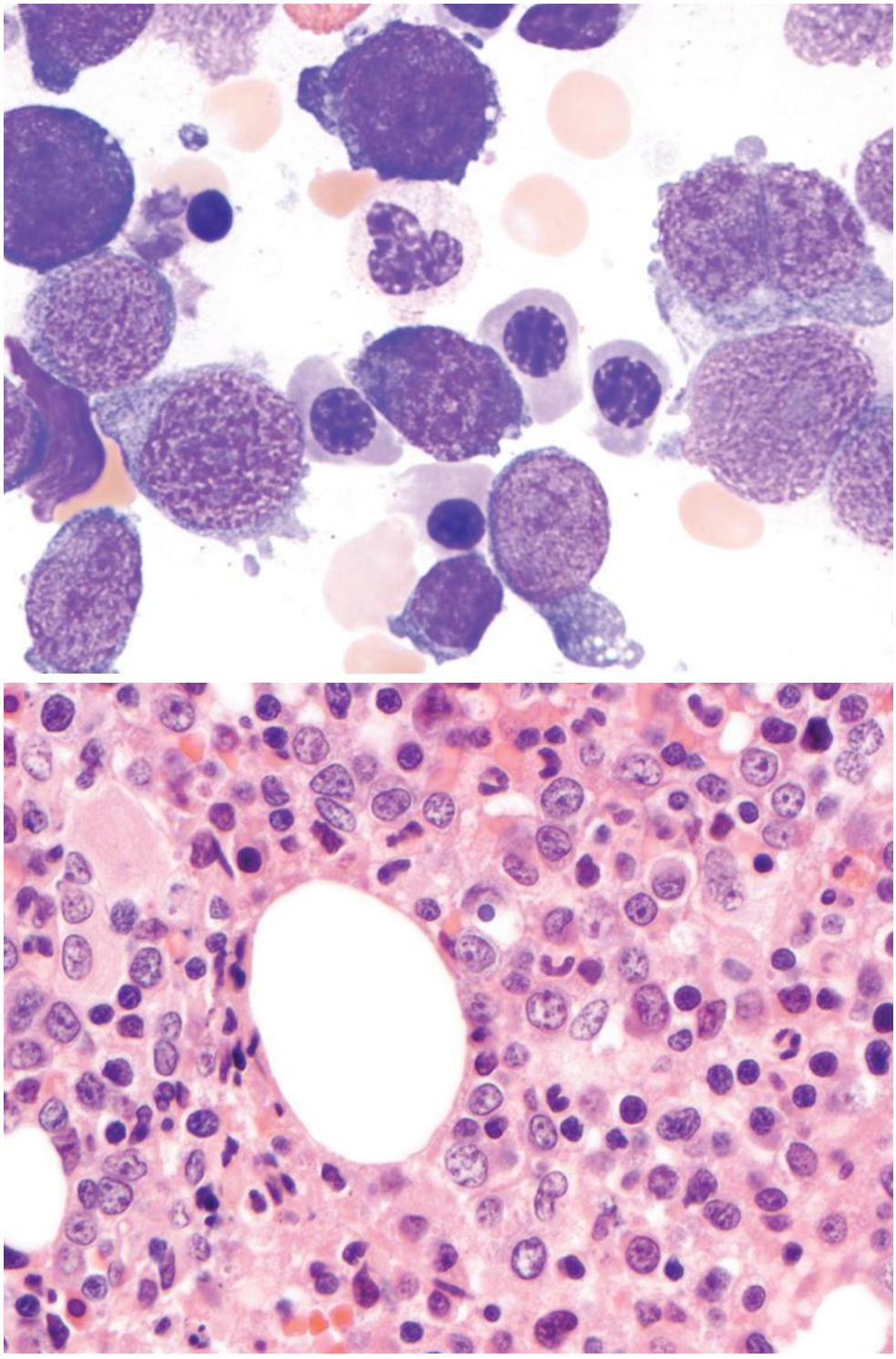


FIG. 14.22

Acute megakaryoblastic leukemia of Down syndrome. The bone marrow is infiltrated by cells similar to the blasts seen in Fig. 14.21, but this occurred in an older child.

BLASTIC PLASMACYTOID DENDRITIC CELL TUMOR (BPDC)

Blastic plasmacytoid dendritic cell tumor is the entity previously known as *agranular CD4$^+$ CD56$^+$ hematodermic neoplasm,* and *blastic natural killer (NK) cell leukemia–lymphoma*. Since those descriptions, the morphologic and immunophenotypic resemblance of this neoplasm to plasmacytoid dendritic cells has been recognized, and the lineage has been established. Whereas BPDC is not an AML, it is included in this chapter because it can resemble AML or myeloid sarcoma. Plasmacytoid dendritic cells are the major type I interferon-producing cells in the body in response to the binding of

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Box 14.3 2016 WHO Classification of Myeloid Neoplasms With Germline Predisposition

Myeloid Neoplasms With Germline Predisposition Without a Preexisting Disorder or Organ Dysfunction

Acute myeloid leukemia with germline *CEBPA* mutation
Myeloid neoplasms with germline *DDX41* mutation[a]

Myeloid Neoplasms With Germline Predisposition and Preexisting Platelet Disorders

Myeloid neoplasms with germline *RUNX1* mutation[a]
Myeloid neoplasms with germline *ANKRD26* mutation[a]
Myeloid neoplasms with germline *ETV6* mutation[a]

Myeloid Neoplasms With Germline Predisposition and Other Organ Dysfunction

Myeloid neoplasms with germline *GATA2* mutation
Myeloid neoplasms associated with BM bone marrow failure syndromes
Myeloid neoplasms associated with telomere biology disorders
Juvenile myelomonocytic leukemia associated with neurofibromatosis, Noonan syndrome or Noonan syndrome–like disorders
Myeloid neoplasms associated with Down syndrome[a]

[a]Lymphoid neoplasms also reported.

BLASTIC PLASMACYTOID DENDRITIC CELL TUMOR—FACT SHEET

Frequency and Age Distribution

- Uncommon, less than 1% of acute leukemias
- Older adults, less commonly infants and young children

Clinical Features

- Usually develops as skin lesion
- Common association with other myeloid proliferations (MDS, myeloproliferative, myeloproliferative-myelodysplastic, AML)

Prognosis

- Poor

BLASTIC PLASMACYTOID DENDRITIC CELL TUMOR—PATHOLOGIC FEATURES

Morphology

- Skin: dermal medium-sized blast infiltrates with periadnexal accumulation and sparing of the epidermis
- Bone marrow: on smears, blasts showing a variable amount of gray-blue cytoplasm, frequently with pseudopodia and small polarized vacuoles; irregular nuclei and one to several small nucleoli; aggregates or sheets of monomorphic-appearing blasts on core biopsy

Immunophenotype

- $CD4^+$, $CD43^+$, $CD56^+$, $CD123^+$, $TCL1^+$, $CD303^+$, $CD68^+$, HLA-DR^+ with variable CD7, CD2, and rarely dim CD33

Cytogenetic Features

- Complex karyotype, (5q-, 12p-, 13q, 6q, 15q, and 9 abnormalities)

nucleic acid type ligands on the cell surface. The precise pathway of derivation from a hematopoietic stem cell is not known.

Most commonly reported in adults, BPDC cases are seen in infants and children. BPDC is an aggressive neoplasm that typically develops as a skin lesion, with subsequent rapid marrow dissemination. Spreading to lymph nodes and spleen is commonly reported. Up to 20% of patients will have an associated myeloid neoplasm (e.g., myelodysplasia, myeloproliferative–myelodysplastic neoplasm, or AML) recognized either before or after the diagnosis of BPDC.

Skin lesions show dermal infiltrates of the blasts with periadnexal accentuation and sparing of the epidermis. In the peripheral blood, blasts are intermediate in size with minimal cytoplasm. On bone marrow aspirate smears, they display a variable amount of gray-blue cytoplasm. Distinctive elongate pseudopodia, often with accumulation of small vacuoles, are a helpful clue to the diagnosis (Fig. 14.23). The marrow may be effaced or minimally and interstitially involved. Nodules of plasmacytoid dendritic cells in the marrow or lymph nodes can be seen in other myeloid conditions; these are distinguished from involvement by BPDC by their focal nature and more mature appearance with abundant cytoplasm, unlike the blastic morphology of BPDC.

BPDC commonly expresses CD4, CD56, CD123, CD43, CD45RA, CD303, and TCL1. Variable expression of CD7, CD33, CD38, CD71, TdT, and a dot-like cytoplasmic reactivity for CD68 are also reported. Rare cases may lack either strong CD4 or CD56 reactivity. CD34, CD117, CD14, CD15, CD57, CD14, CD163, lysozyme, and MPO are not expressed. Granzyme B, but not TIA1 or perforin, is detected by flow cytometry. Immunohistochemistry for granzyme B in paraffin section is usually negative. The immunophenotype $CD4+$, $CD56+$, $CD123+$, lacking CD34 or CD117 overlaps significantly with AML with monocytic differentiation. BPDC lacks cytochemical esterase reactivity and will not express lysozyme. The presence of small vacuoles, with expression of CD71, may suggest acute erythroleukemia. Erythroblast vacuoles are usually PAS positive. Expression of TCL1 is largely restricted to B-cell neoplasms and T-cell prolymphocytic leukemia. CD123 is widely expressed in other types of AMLs, as well as in cases of B- and T-acute lymphoblastic leukemia. Newer markers, such as CD303 (BDCA-2) and CD2AP, may be useful in difficult cases. Some cases may harbor a normal karyotype, but most have a complex karyotype, including 5q, 12p, 13q, 6q, 15q, and 9 abnormalities. Mutations in *TET2* are also common.

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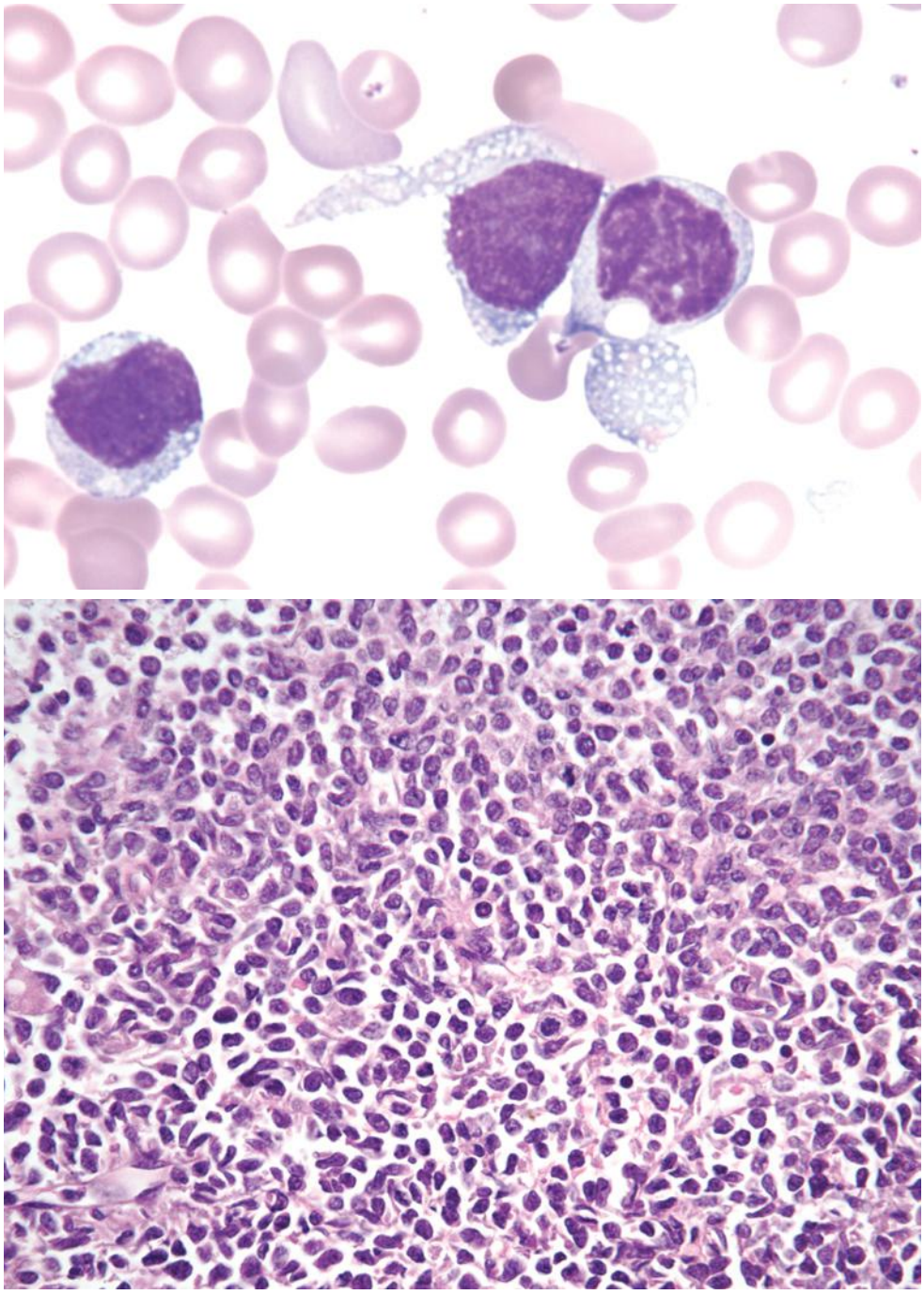


FIG. 14.23

Blastic plasmacytoid dendritic cell tumor. Neoplastic cells of blastic plasmacytoid dendritic cells have oval, variably folded nuclei. The cytoplasm is pale gray-blue with elongate pseudopodia and small vacuoles in many forms. Sheets of immature cells with folded nuclei efface the marrow on the core biopsy.

GROWTH FACTOR CHANGES THAT CAN MIMIC ACUTE MYELOID LEUKEMIA

Growth factors are administered for a variety of reasons, including enhancing bone marrow recovery after chemotherapy and priming the marrow or peripheral blood prior to stem cell collection. These agents can cause changes that might suggest a new diagnosis of AML or relapsed AML. The most commonly used agents in this setting are human recombinant granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), which cause both peripheral blood and bone marrow changes (Box 14.4). Both agents cause a peripheral blood leukocytosis with a granulocyte left shift. The common presence of toxic granulation and Döhle bodies give the appearance of a reactive proliferation. Enlarged neutrophils or neutrophils with vacuolated cytoplasm can also occur.

The bone marrow shows a granulocytic hyperplasia (Fig. 14.24). Depending on the timing of bone marrow examination, the marrow may show the complete spectrum of granulocytic maturation, give the appearance of maturation arrest, or show a predominance of segmented neutrophils. The maturation arrest-type changes usually occur just after administration

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Box 14.4 Peripheral Blood and Bone Marrow Changes Associated With Growth Factors (G-CSF and GM-CSF)

- Peripheral blood changes
- Circulating nucleated red blood cells
- Neutrophilia
- Vacuolated and giant neutrophils
- Granulocyte left shift
- Toxic granulation and Döhle bodies
- Hypogranular neutrophils
- Increase in large granular lymphocytes
- Eosinophilia
- Transient blast cells
- Early bone marrow changes
- Granulocytic hyperplasia with increased numbers of promyelocytes and myelocytes
- Toxic granulation of granulocytes
- Enlarged promyelocytes and myelocytes
- Increased mitotic activity of granulocyte precursors
- Transient blast cell increase
- Biopsy hypocellularity with left-shifted granulocytic precursors
- Late bone marrow changes
- Binucleated promyelocytes
- Marrow neutrophilia
- Marrow eosinophilia
- Toxic granulation
- Variable biopsy cellularity

G-CSF, Granulocyte-colony stimulating factor; *GM-CSF,* granulocyte-macrophage colony-stimulating factor.

of the growth factor and offer the most diagnostic problems, including features worrisome for AML or myelodysplasia. A predominance of promyelocytes and myelocytes is usually present. In some cases, bone marrow and peripheral blood blast cells may exceed 5%, but this increase is usually accompanied by an even greater increase in promyelocytes. Blast proliferations that are not accompanied by an increase in promyelocytes should be considered highly suggestive of leukemia and not simply attributed to growth factor changes.

In a patient with a history of acute myeloid leukemia, it might not be possible to entirely exclude the possibility of leukemia in the setting of an increase in blast cells and cytogenetic studies; evaluation for a prior aberrant leukemia immunophenotype may be useful in that setting. The promyelocytes that occur with G-CSF and GM-CSF therapy usually have prominent perinuclear hofs, which should be a clue to the possibility of growth factor administration. These cells differ from those of acute promyelocytic leukemia, which usually do not show perinuclear cytoplasmic clearing and demonstrate Auer rods that are not present in reactive promyelocytes. A repeated bone marrow examination 1 to 2 weeks after cessation of the growth factor will usually demonstrate more complete granulocyte maturation, and such a study is advisable in cases that are worrisome for residual leukemia. Less common changes that have been reported after G-CSF and GM-CSF therapy include marrow necrosis and marrow histiocyte proliferations.

POST-THERAPY CHANGES IN ACUTE MYELOID LEUKEMIA

CHANGES ASSOCIATED WITH MYELOABLATIVE THERAPY

Several studies have evaluated the marrow changes after high-dose chemotherapy, or chemotherapy with radiation, including regimens used for hematopoietic stem cell transplantation, approaches used for many types of AMLs. The changes are similar to the toxic changes of other drug injuries of the marrow. Complete marrow aplasia is the immediate expected change in the first week after myeloablative therapy (Box 14.5, Fig. 14.25). The marrow cellularity is essentially zero with an absence of normal marrow fat. There is edema with dilated marrow sinuses and scattered stromal cells, histiocytes, plasma cells, and lymphocytes. Normal maturing granulocytes, nucleated red blood cells, and megakaryocytes are usually not identifiable. Histiocytes containing cellular debris are often present, and acellular areas of fibrinoid necrosis often predominate. The development of mild reticulin fibrosis and the reappearance of fat cells follow these initial changes. The early fat in a regenerating marrow is loculated. Although the marrow remains markedly hypocellular, the loculated fat is accompanied by focal areas of early hematopoiesis in the second week after treatment; this may be represented by islands of erythroid cells alone or in combination with areas of left-shifted granulocytes. Both elements are usually present after 2 weeks. Megakaryocytes, often occurring in clusters with hypolobated nuclei, occur later in this process but are usually easily identified by the third week. In some patients, particularly children, early regeneration may be accompanied by an increase in precursor B cells or hematogones.

The expected later changes after high-dose therapy usually include a loss of the mild reticulin fibrosis of early regeneration and a return to normal or even slightly increased marrow cellularity. All three normal marrow cell lines are present, although a left shift of granulocytes and erythroid cells and atypical megakaryocyte clustering may persist for some time.

MARROW AFTER RECOVERY FROM ACUTE MYELOID LEUKEMIA THERAPY

Guidelines for the definition of morphologic remission in patients treated for acute myeloid leukemia require a morphologic leukemia-free state (defined as <5% bone marrow blasts, absence of Auer rods, or extramedullary disease, and the absence of a unique leukemia immunophenotype by flow cytometry), as well as an absolute neutrophil count of greater than 1000/μL and platelet count of 100,000/μL or greater. Before demonstrating these changes, however, several morphologic features of the peripheral blood and bone marrow have prognostic significance. Failure to demonstrate a reduction in blast cells and cellularity at day 6 of induction

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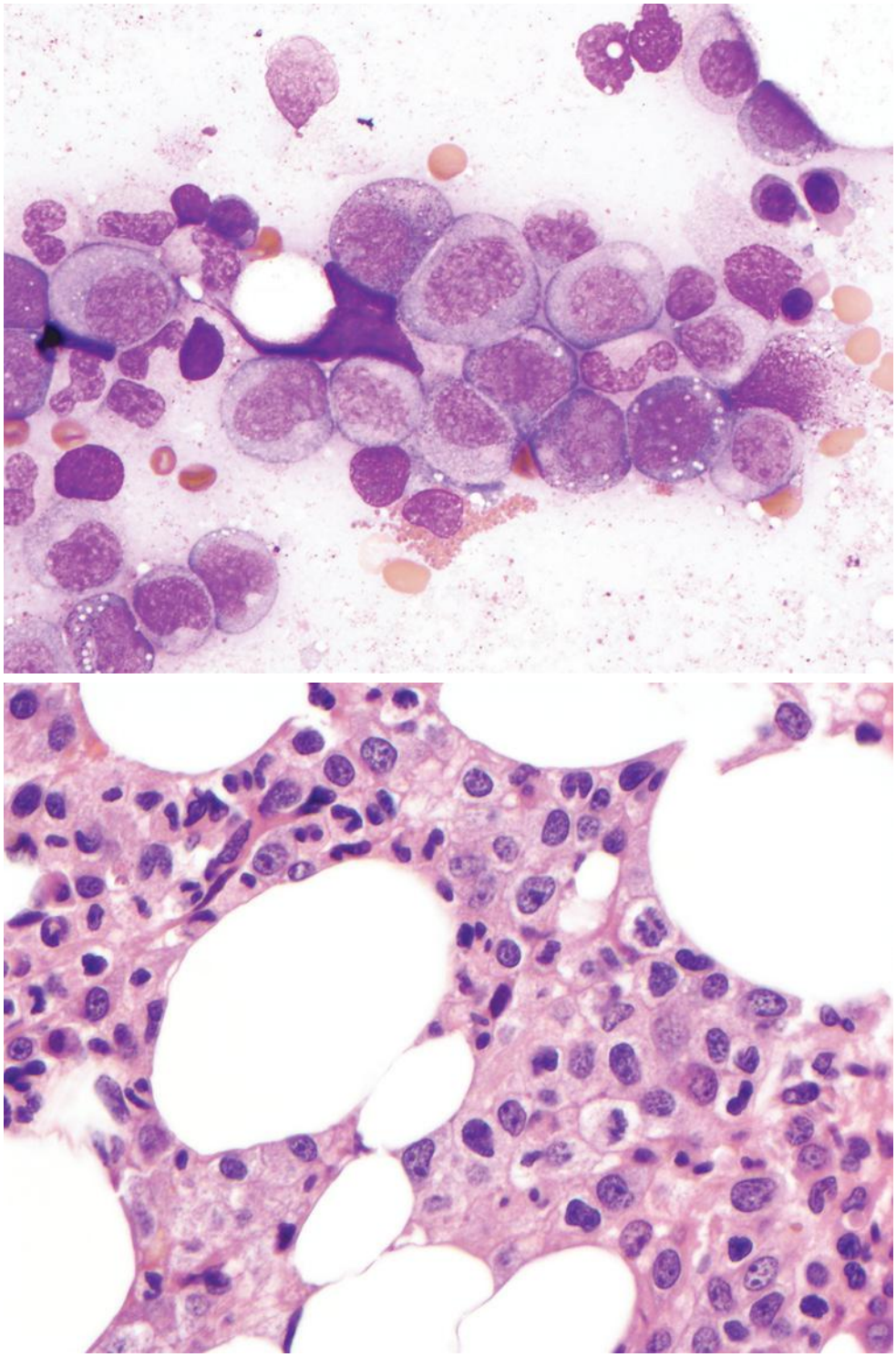


FIG. 14.24

Growth factor changes. This patient received growth factor after therapy for lymphoma. The marrow shows an increase in promyelocytes with perinuclear hofs and a paucity of erythroid precursors.

chemotherapy will usually result in a change or augmentation of induction chemotherapy. Not surprisingly, the presence of residual leukemic cells at the end of induction chemotherapy is a poor prognostic indicator. Even after meeting the criteria for remission, patients with bone marrow hypercellularity, anemia, bone marrow blast cell counts of 1% or more, or peripheral blood blast cell counts of over 3% have a shortened duration of remission and shortened survival. Therefore, more detailed evaluation of bone marrow and peripheral blood samples is needed than is suggested from most remission criteria.

The blast cells in patients with a history of acute myeloid leukemia should be compared to the patient's original disease material. The presence of an increased number of cells with features similar to the original material should be regarded with suspicion (Fig. 14.26). Auer rods, rod-shaped cytoplasmic aggregates of granules, are not a feature of regenerating or non-neoplastic myeloblasts and should be considered as

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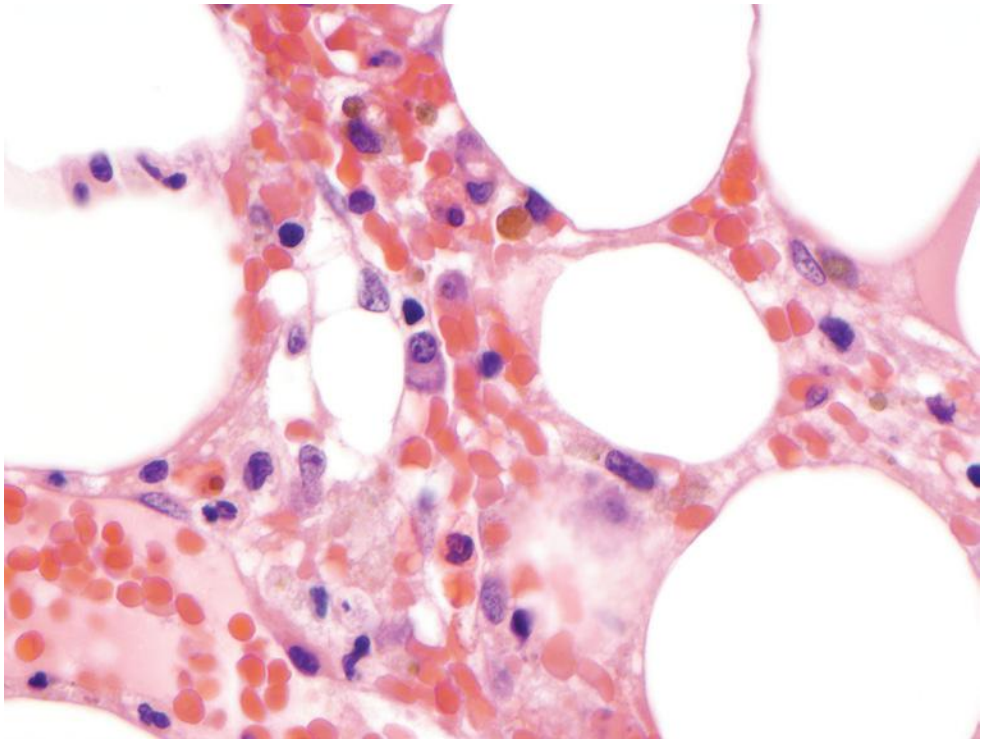


FIG. 14.25

Changes immediately after myeloablative therapy for acute myeloid leukemia. The marrow shows hemorrhage and fibrin deposition without scattered lymphocytes, plasma cells, and histiocytes. Maturing myeloid, erythroid, and megakaryocytic cells are essentially absent.

Box 14.5 Bone Marrow Changes Immediately Following Myeloablative Therapy

- Initial changes (≈1 week)
- Marrow aplasia
- Absence of fat cells
- Edema and fibrinoid necrosis with or without tumor necrosis
- Dilated sinuses
- Rare stromal cells, histiocytes, lymphocytes and plasma cells
- Intermediate changes (≈2 weeks)
- Reappearance of fat, often showing lobulated cytoplasm
- Mild reticulin fibrosis
- Left-shifted erythroid and granulocyte islands
- Increase in precursor B cells
- Late changes (≈3 to 4 weeks)
- Resolution of reticulin fibrosis
- Clusters of small megakaryocytes
- Normal or slightly increased marrow cellularity

evidence of residual disease. Auer rods may be encountered rarely in maturing granulocytes but are still considered an abnormal finding. Regenerating blast cells are usually admixed with promyelocytes and maturing granulocytes, and the presence of sheets of blasts on a smear is generally a sign of recurrent disease. In contrast, specimens with blast cells in numbers equal to or lower than promyelocytes usually represent regeneration. Clustering of blast cells is often difficult to interpret on hematoxylin and eosin (H&E)-stained biopsy material, and aggregates of regeneration may be difficult to differentiate from leukemic blast cell aggregates. Regeneration usually occurs adjacent to bony trabeculae, and the presence of immature cell aggregates away from the bone is generally considered abnormal. This abnormal localization of immature cell precursors has been used as a sign of a myelodysplastic syndrome, but caution should be used in applying these criteria in patients after hematopoietic stem cell transplantation. After transplantation, the normal bone marrow architecture of regeneration may change, and regenerating immature-appearing cells on H&E-stained sections may be present away from the bone. Paraffin section immunohistochemistry may also be of value in selected cases, particularly in the presence of left-shifted cell aggregates on H&E-stained sections. By immunophenotyping, the immature cell aggregates of regeneration can be shown to represent a spectrum of left-shifted cells that are not exclusively blast cells, whereas recurrent leukemia blast cell aggregates are a more uniform population of neoplastic cells. Therefore, the identification of clusters of cells expressing the immature cell antigen CD34 in biopsy material is a feature favoring residual or recurrent disease. Correlation with cytogenetic, molecular genetics, or FISH studies may also be useful to search for a characteristic genetic abnormality associated with a given patient's original disease.

Acute myeloid leukemia with myelodysplasia-related changes may show the presence of multilineage dysplasia before an increase in blast cells at relapse. Again, the features of the original multilineage dysplasia should be reviewed with the possible relapse sample. Caution should be used not to overcall multilineage dysplasia during or immediately following therapy. Dyserythropoietic changes are common during chemotherapy and often include a left shift of erythroid precursors and multinucleation of erythroid cells. In addition, regenerating megakaryocytes often cluster and are small during or immediately after chemotherapy. Granulocyte changes after therapy are usually restricted to a left shift without the

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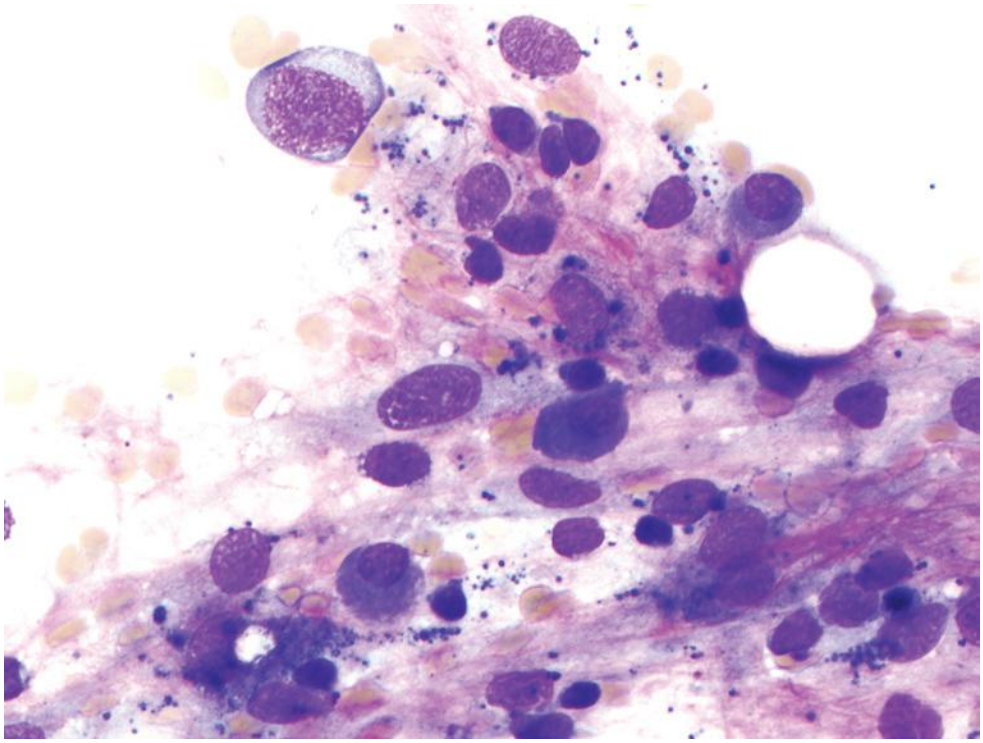


FIG. 14.26

Post-therapy bone marrow aspirate showing evidence of ablation, but a few blast cells are present. These cases should be correlated with prior blast cell morphology, immunophenotyping, and cytogenetic studies to resolve the differential diagnosis of regeneration versus residual disease. A biopsy should be repeated in 1 to 2 weeks if these additional studies are not available or are ambiguous.

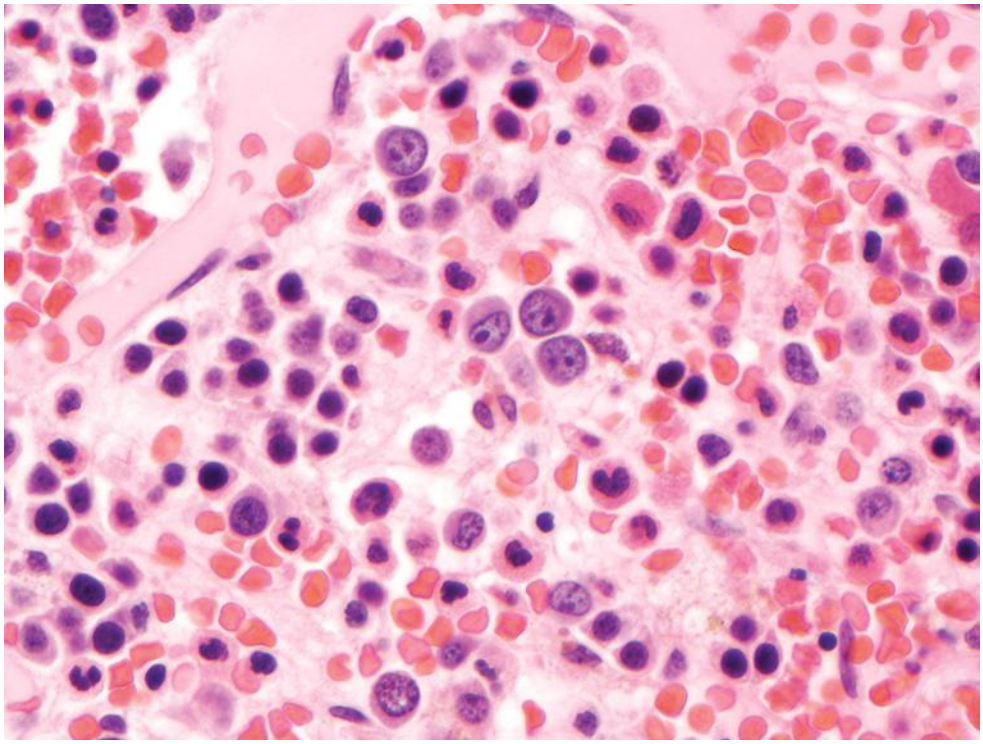


FIG. 14.27

Acute promyelocytic leukemia post all-*trans* retinoic acid therapy. Residual promyelocytes, including degenerating promyelocytes, may be present after therapy. These patients should be followed closely. This finding in an early bone marrow specimen is not sufficient for an interpretation of relapse or recurrent disease.

hypogranulation commonly seen in association with myelodysplasia. Therefore, dysplastic changes of maturing granulocytes are probably more reliable than erythroid abnormalities alone for identifying recurrent AML with myelodysplasia-related changes during or immediately after chemotherapy.

Currently, most patients with acute promyelocytic leukemia are treated with both standard chemotherapy and ATRA or a combination of ATRA and arsenic trioxide, and they demonstrate bone marrow changes that are usually similar to other AML samples. However, some patients treated with ATRA or combination chemotherapy without ATRA might not show an initial marrow aplasia. The bone marrow in these patients may remain hypercellular with markedly elevated numbers of promyelocytes (Fig. 14.27). These cells will

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usually slowly undergo maturation secondary to the therapy with loss of the t(15;17) cytogenetic abnormality associated with acute promyelocytic leukemia. In this subgroup of patients, it should be understood that the presence of sheets of promyelocytes may not indicate treatment failure, and the patients should be followed closely with additional marrow examinations to confirm that the maturational changes are occurring.

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The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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Precursor Lymphoid Neoplasms

John Kim Choi, MD, PhD

INTRODUCTION

Acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma (LBL) constitute a biologic continuum of neoplastic lymphoid disorders characterized by the proliferation of immature (blast) cells of B-cell or T-cell lineage. For clinical purposes, an arbitrary cutoff has been used for many years to separate lymphoblastic leukemias and lymphomas. Cases with tissue involvement and less than 25% replacement of the marrow cellularity by lymphoid blasts have been designated as *lymphoblastic lymphomas*. Cases with 25% or greater marrow involvement have been designated *acute lymphoblastic leukemias*. For practical purposes, this separation is a measure of disease burden or disease stage, leading to different duration of the same chemotherapy. Pure T-lymphoblastic lymphoma versus leukemia have biologic differences based on gene expression profile studies, but it is not known what percent, if any, of bone marrow involvement distinguishes one from the other. Most cases of LBL are T-cell, whereas B-cell equivalents are rare, most likely reflecting that most B-cell lymphoblastic neoplasms originate in the bone marrow, whereas most T-cell equivalents originate most frequently in the thymus. In the past, the original French-American-British (FAB) classification included the leukemic phase of Burkitt lymphoma in the category of ALL. These cases were classified as L3 ALL. In the current classification system, Burkitt lymphoma/leukemia is a neoplasm with a mature B-cell immunophenotype and is no longer considered a lymphoblastic neoplasm. It will be addressed in this chapter only as it applies to the differential diagnosis of ALL.

Clinical Features

Acute lympoblastic leukemia and LBL can occur at any age, but most cases arise in children, constituting the most common type of childhood malignancy. The disease has a bimodal age distribution, a higher incidence peak in early childhood with median ages of 4 years for B-cell ALL (B-ALL) and 10 years for T-cell ALL (T-ALL) as well as a second lower incidence peak in older adults. In the Western countries, approximately 80% to 85% of ALL are B-ALL, whereas 15% to 20% are T-ALL. In the Middle East, North Africa, and Sicily, the proportion of T-ALL is higher (up to one third of all ALLs) for unknown reasons. T-ALL also occurs with increased frequency in patients with ataxia telangiectasia. ALL can occur as de novo disease or, rarely, as a secondary neoplasm following chemotherapy for a different malignancy.

ALL may present clinically as an acute illness or with symptoms that develop slowly and persist for months. The most common findings include fever, fatigue, bone or joint pain, bleeding, anorexia, abdominal pain, and hepatosplenomegaly. Approximately 50% to 60% of the patients with T-ALL develop an anterior mediastinal mass that can lead to superior vena cava syndrome.

ALL is a highly aggressive neoplasm that requires intensive chemotherapy. The modern approach to the treatment of ALL involves tailoring the intensity of the chemotherapy to risk groups defined by the presenting clinical features, lineage, cytogenetics, and molecular findings, as well as by the early response to therapy.

Pathologic Features

Gross Findings

When involving lymph nodes or other extramedullary tissues, LBL has the characteristic pale pink "fish-flesh" appearance typically associated with lymphomas.

Microscopic Findings

Lymphoblastic leukemia involving lymph nodes has a diffuse growth pattern with subtotal or complete effacement of the normal lymph node architecture. When partially involving the lymph node, T-cell LBL usually has a predominantly interfollicular distribution. There may be a focal associated "starry-sky" appearance imparted by the presence of tingible-body macrophages. In paraffin-embedded tissue sections of lymph nodes and bone marrow biopsy samples, the lymphoma cells are typically intermediate in size, with oval, often indented nuclei, finely dispersed nuclear chromatin, and small

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ACUTE LYMPHOBLASTIC LEUKEMIA—FACT SHEET

Definition
- Malignant lymphoid neoplasm composed of blasts of B-cell (B-ALL) or T-cell (T-ALL) lineage

Incidence and Location
- Incidence in United States (1997 to 2001): 1.4 per 100,000 population (all ages); 6.9 per 100,000 in children 1 to 4 years of age
- 3200 new cases reported in the United States in the year 2000
- Frequently involves bone marrow and peripheral blood, as well as liver, spleen, lymph nodes, and mediastinum (the last in T-ALL)

Morbidity and Mortality
- 80% to 90% cure rate in children treated with modern intensive chemotherapy, depending on prognostic features
- 30% to 40% 5-year survival in adults treated with dose-intensive regimens
- Less than 10% 3-year survival in patients older than 60 years

Gender, Race, and Age Distribution
- Slight male predominance (male-to-female, 1.2:1 to 1.8:1)
- Median age at diagnosis: 11 years
- Bimodal age distribution with an incidence peak in children up to 18 years of age and a lower incidence peak in adults older than 65 years

Clinical Features
- Most common presenting signs: fever, fatigue, bone or joint pain, bleeding, anorexia, lymphadenopathy, hepatosplenomegaly
- T-ALL: commonly anterior mediastinal mass with or without superior vena cava syndrome

Prognosis and Therapy
- Poor prognostic factors in adults: age >35 years, leukocyte count >30,000, >4 weeks of therapy required to achieve complete remission, Philadelphia chromosome present in the leukemic blasts
- Poor prognostic factors in children: age ≥10 years, leukocyte counts >50,000, unfavorable cytogenetic changes (Philadelphia chromosome, infant B-ALL with *MLL* gene rearrangements, hypodiploidy, iAMP21), Ph-like associated molecular mutations, and detectable residual leukemia (≥0.01%) after induction therapy. The last is the most predictive.
- All patients require intensive risk-adapted multi-agent chemotherapy and, in some cases, followed by allogeneic bone marrow translation
- Adjuvant targeted therapy directed against mutated kinase improves outcome for subtypes of B-ALL, such as Philadelphia chromosome and Ph-like B-ALLs
- Prolonged continuation therapy (up to 3 years) is critical in preventing disease relapse

inconspicuous nucleoli (Fig. 15.1). Fewer cases are composed of large blasts with prominent centrally located nucleoli. In Wright-Giemsa–stained cytologic preparations, including smears of blood, bone marrow, and fine-needle aspiration samples, the ALL blasts are usually of intermediate size (typically up to 1.5 to 2 times the size of a small lymphocyte). Several cytologic subtypes of ALL, designated L1, L2, and L3, have been defined by the FAB classification in the 1970s and early 1980s. The L3 subtype is known to correspond to the leukemic phase of Burkitt lymphoma and therefore will be addressed in this chapter mainly as it pertains to the differential diagnosis of ALL. The L1 and L2 subtypes do not accurately correlate with disease subtypes or prognosis and therefore are mostly of descriptive importance at the current time. The most common cytologic subtype is L1 (Fig. 15.2), characterized by relatively uniform, intermediate-sized blasts, with scant lightly basophilic cytoplasm, homogeneous, somewhat condensed chromatin, and inconspicuous or absent nucleoli. The L2 subtype (Fig. 15.3) consists of more heterogeneous blasts that include larger forms, moderate amounts of lightly basophilic cytoplasm, finely dispersed nuclear chromatin, and prominent nucleoli. In any of these variants the blast cytoplasm may contain clear vacuoles (Fig. 15.4) or granules (Fig. 15.5), or rarely large unusual inclusions. ALL with granules, typically azurophilic or pale pink on the Wright-Giemsa stains, have been called granular ALL. The granules are of no prognostic consequence and can occur in rare cases of B-ALL and T-ALL. In addition, the granules may be positive for Sudan black B or nonspecific esterase on cytochemical staining, characteristics usually associated with acute myeloid leukemia. Rare cases of ALL, both T- and B-cell, may have a predominance of hand-mirror–shaped blasts that have an eccentric nucleus and a pale basophilic cytoplasmic tail. This finding is likewise of no prognostic significance. Last, the L3 ALL (Fig. 15.6) is characterized by moderate to large blasts with regular nuclear outline, fine to slightly clumped nuclear chromatin, prominent and frequently multiple nucleoli, and moderately abundant deeply basophilic vacuolated cytoplasm. The vacuoles characteristically contain lipid droplets that can be demonstrated by oil-red O staining of touch imprints. The abnormal lipid vacuoles can also be identified by the abnormal expression of adipophilin by immunohistochemistry, which appears to be relatively specific for BL.

Ancillary Studies

Immunophenotypic Analysis

Immunophenotypic analysis of ALL should provide at a minimum a lineage (T- or B- cell) that is important for guiding further workup and treatment. The modalities involved typically include flow cytometry (requiring fresh cell suspensions) and immunohistochemistry (when only fixed tissue is available).

When evaluated by flow cytometry, the immunophenotype of B-ALL typically includes the following surface antigens:

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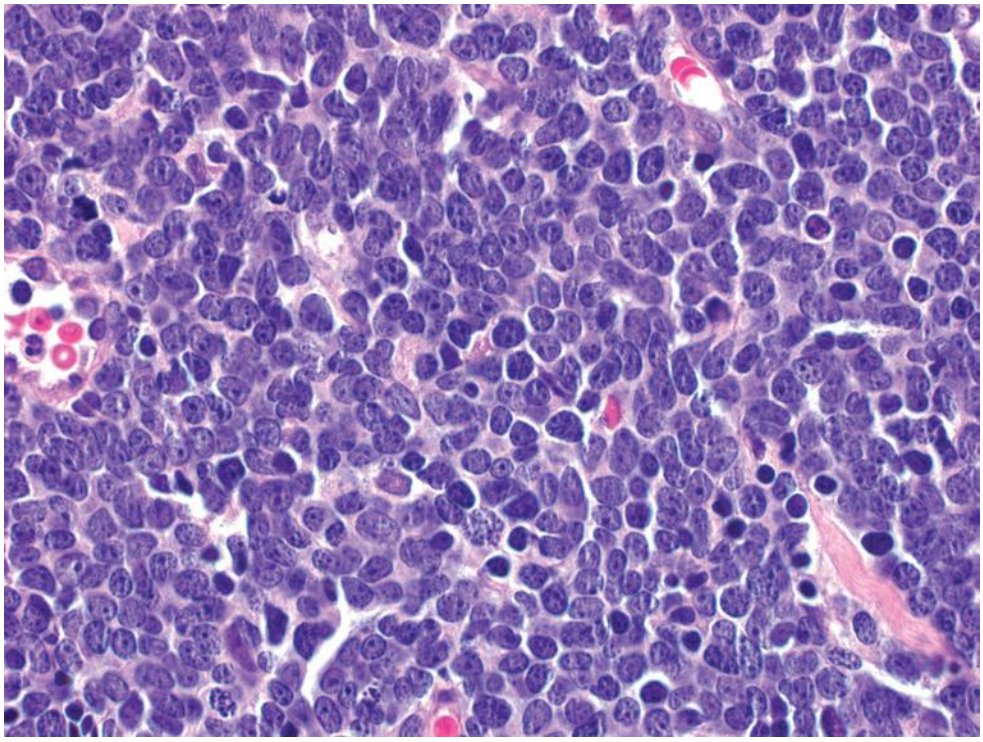


FIG. 15.1

Lymphoblastic lymphoma, morphologic features in paraffin-embedded tissue sections. The tumor is characterized by a diffuse growth pattern and is composed of relatively uniform blasts that are 1.5 to 2 times the size of small mature lymphocytes and have finely dispersed chromatin with small or absent nucleoli. Original magnification, ×60; oil immersion.

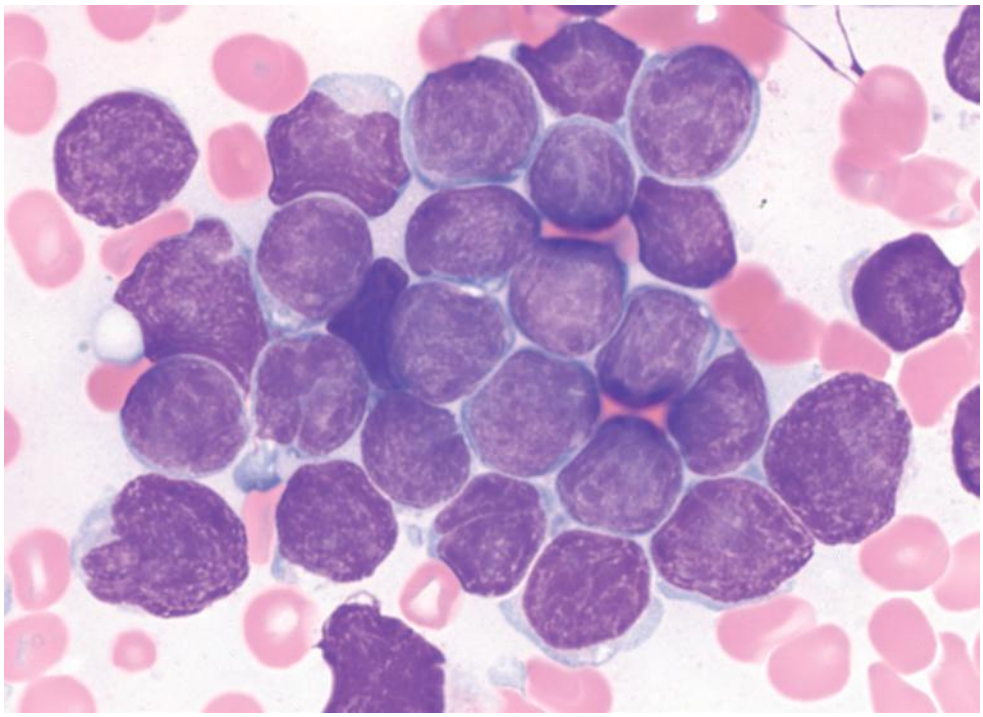


FIG. 15.2

Acute lymphoblastic leukemia (ALL) with L1 morphology. The most common cytologic appearance of lymphoblasts. The blasts have small to medium-sized round nuclei, scant cytoplasm, slightly condensed nuclear chromatin, and indistinct nucleoli. Some blasts can have cleaved and folded nuclear outlines. Wright-Giemsa stain; original magnification, ×100; oil immersion.

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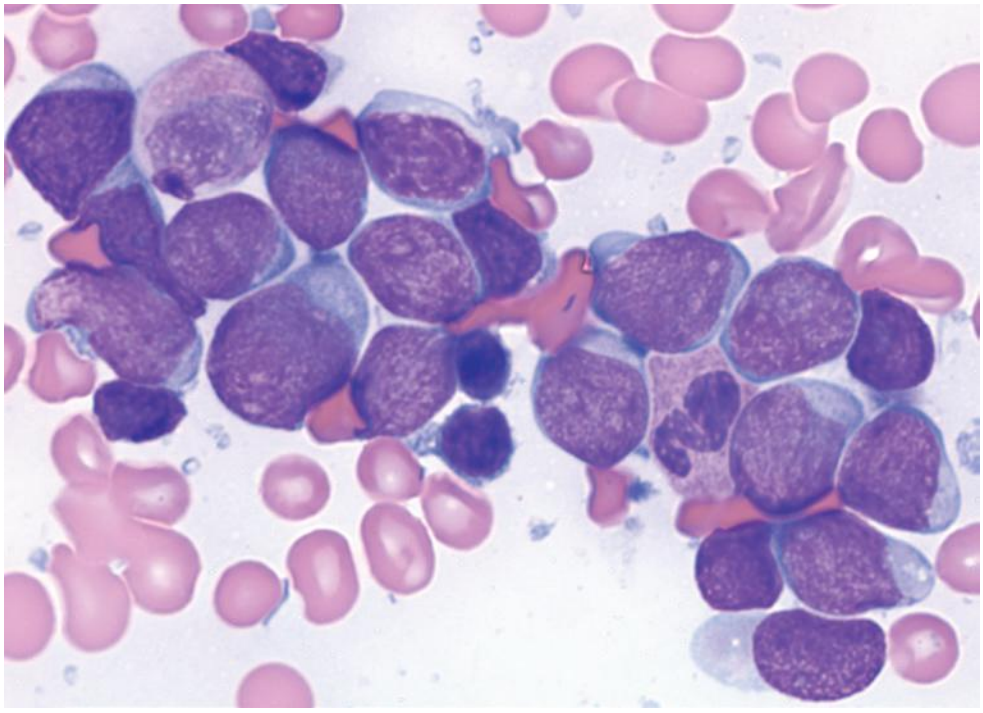


FIG. 15.3

Acute lymphoblastic leukemia (ALL) with L2 morphology, seen in some cases, raises the differential diagnosis of acute myeloid leukemia. The blasts have variable sized and shaped larger nuclei, moderate amounts of cytoplasm, finely dispersed nuclear chromatin, and one to several prominent nucleoli. Wright-Giemsa stain; original magnification, ×100; oil immersion.

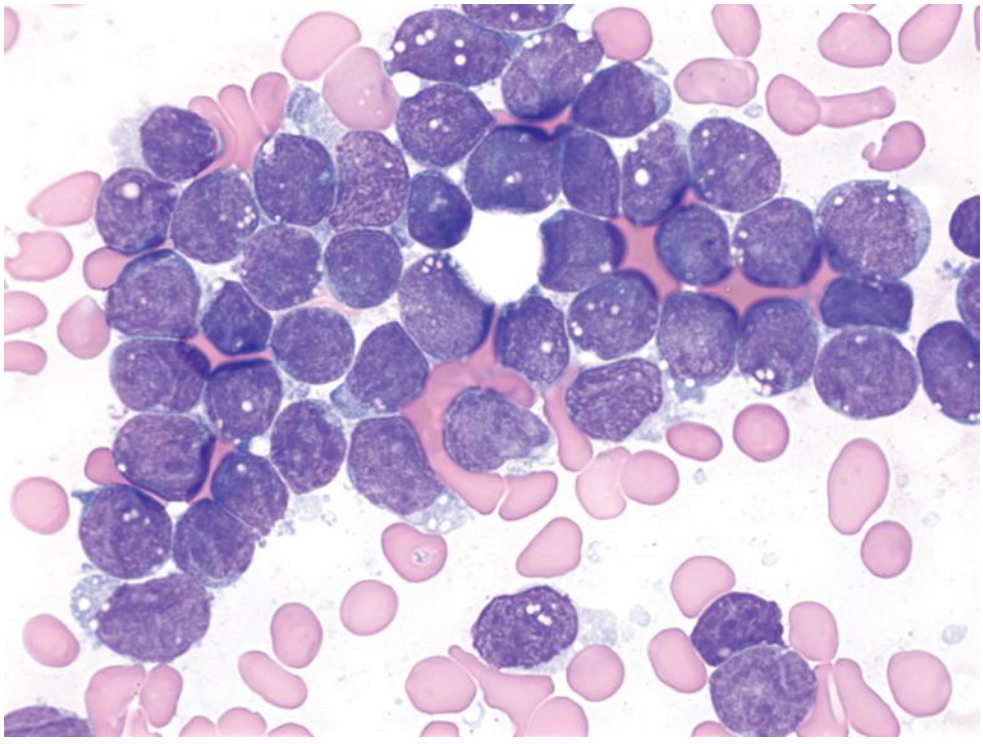


FIG. 15.4

Acute lymphoblastic leukemia (ALL) blasts with cytoplasmic vacuolization. This case of L1 ALL shows prominent clear vacuoles present in the cytoplasm of the blasts and occasionally overlying the blast nuclei. Vacuolization is not unusual in ALL and should be differentiated from that seen in Burkitt leukemia–lymphoma. Wright-Giemsa stain; original magnification ×60; oil immersion.

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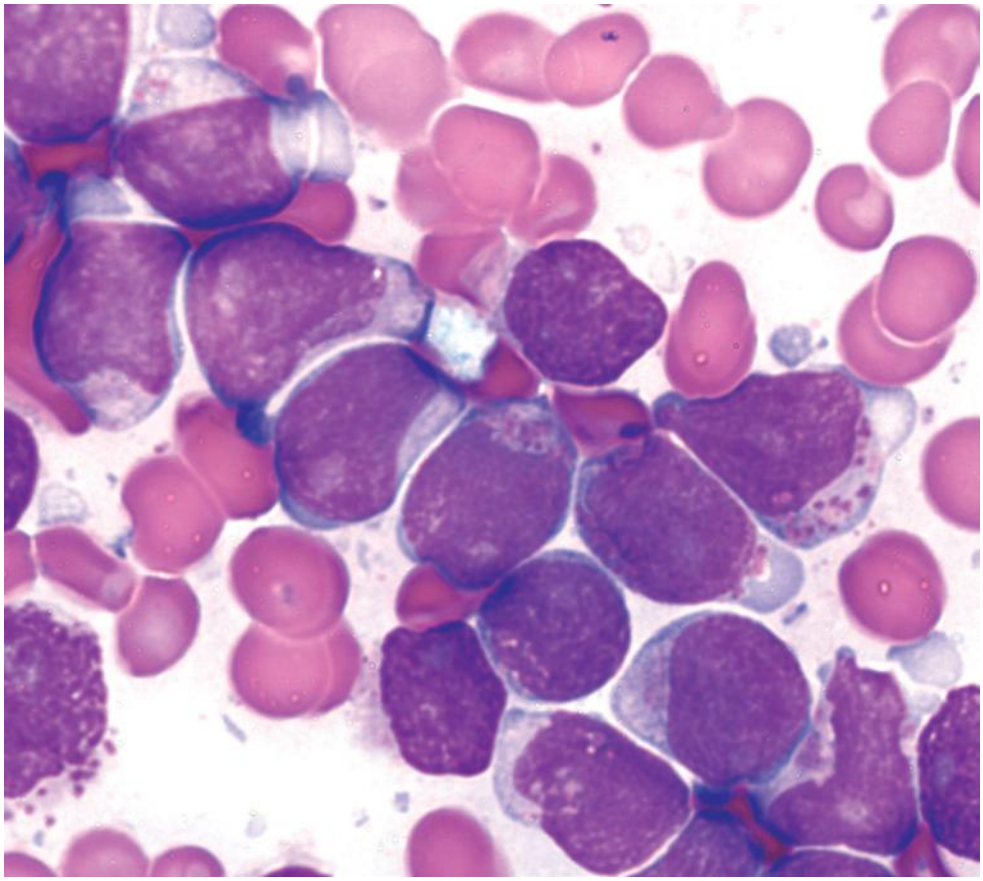


FIG. 15.5

Acute lymphoblastic leukemia (ALL) blasts with prominent cytoplasmic granules (i.e., granular ALL). In the cases of granular ALL, the blasts contain pale pink to magenta cytoplasmic granules. This morphologic finding has no prognostic implications. Wright-Giemsa stain; original magnification, ×100; oil immersion.

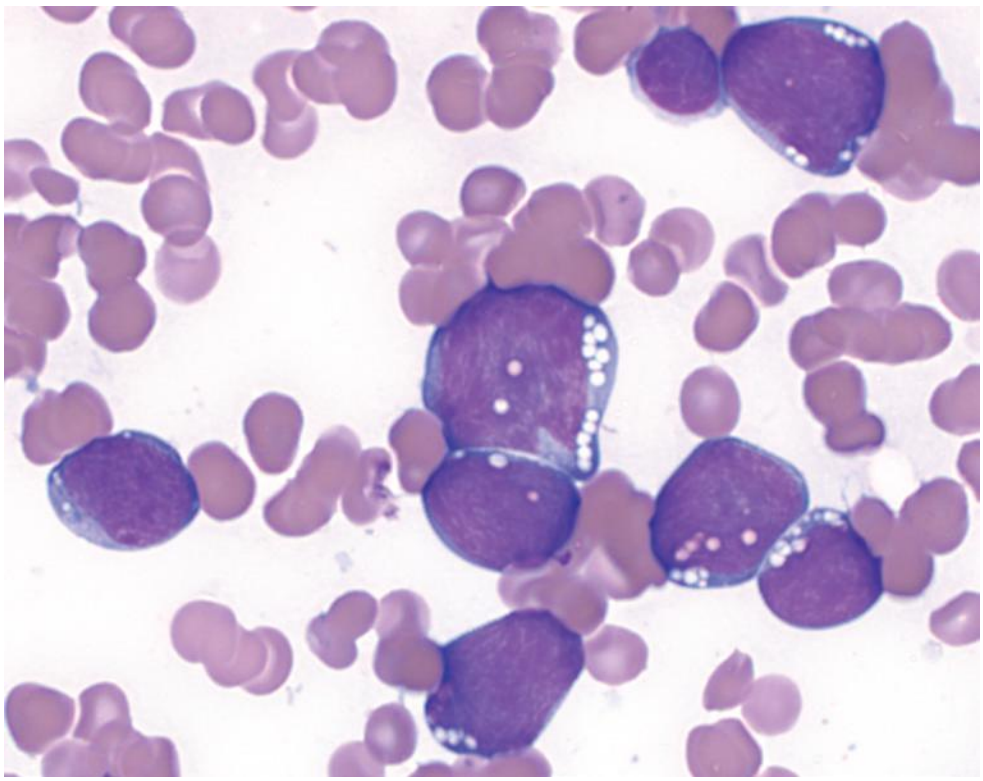


FIG. 15.6

Burkitt lymphoma/leukemia (previously called FAB ALL-L3), cytologic features. The blasts have intermediate to large sized nuclei, finely clumped nuclear chromatin, several small nucleoli, and moderate amounts of deeply basophilic cytoplasm with prominent vacuolization. Wright-Giemsa stain; original magnification, ×60; oil immersion.

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ACUTE LYMPHOBLASTIC LEUKEMIA—PATHOLOGIC FEATURES

Gross Findings

- When involving lymph nodes: homogeneous, pale pink, "fish-flesh" appearance of cut sections

Microscopic Findings

- In tissue sections: a diffuse growth pattern (sheets of cells); there may be focal "starry-sky" appearance
- On Wright-Giemsa-stained smears:

L1 blasts:

  - Cells are small to intermediate in size (approximately twice the size of normal lymphocytes) and uniform in appearance
  - Nuclei are round or ovoid, sometimes cleaved, with homogeneous, often condensed chromatin
  - Nucleoli are small and inconspicuous or not visible
  - Cytoplasm is scant and lightly basophilic

L2 blasts with similar morphology to myeloblasts:

  - Cells are heterogeneous in size, including intermediate and large forms
  - Nuclei are round, with finely dispersed chromatin
  - Nucleoli are large and prominent

- The FAB classification had criteria for L1 versus L2 ALL based on percentage of blasts with each of the mentioned features. These criteria are not commonly used, but the L1 and L2 blast terminology remains popular
- 4.5% to 7% of cases may have slightly pink or orange cytoplasmic granules (visible on Wright staining); some cases contain cytoplasmic vacuoles

Ultrastructural Findings

- Cytoplasmic organelles are sparse and include small mitochondria, a Golgi region, scattered polyribosomes, and rarely strands of rough endoplasmic reticulum
- In cases with cytoplasmic granules, these are 0.2- to 1.5-μm electron-dense structures clustered near the Golgi region or 1.5- to 2.5-μm membrane-bound cytoplasmic inclusions; some may show ultrastructural peroxidase positivity

Immunophenotypic Features

- B-ALL: vast majority positive for CD19, CD22, CD24, CD79a, CD34, TdT, HLA-DR; frequently positive for CD10, CD20 (weak), and myeloid-associated antigens such as CD13 and CD33; almost always lack surface immunoglobulin expression
- T-ALL: vast majority positive for CD2, cytoplasmic CD3, CD5, CD7; frequently positive for CD1a, surface CD3 (dim), CD4, CD8, CD10, CD21, TdT, CD34, HLA-DR
- Most ALL are dim to moderate $CD45^{+}$; rare cases are $CD45^{-}$ or bright $CD45^{+}$

Pathologic Differential Diagnosis

- Benign expansions of normal bone marrow B-cell precursors (hematogones)
- Normal cortical thymocytes (for mediastinal mass)
- Burkitt lymphoma
- Blastic variant of mantle cell lymphoma
- Peripheral T-cell non-Hodgkin lymphoma
- Acute myeloid leukemia or granulocytic sarcoma

CD10, CD19, CD20 (may be very weak or absent), CD22, CD24, CD34, CD45 (weaker than normal lymphocytes), and HLA-DR (Fig. 15.7). Terminal deoxynucleotidyl transferase (TdT) is a nuclear enzyme that is an excellent marker of lymphoid blasts that is expressed in more than 98% of B-ALL (some cases of acute myeloid leukemias express aberrant TdT). Cytoplasmic antigens include CD22, CD79a, and, in a subset of ALL, μ immunoglobulin heavy chains (Igμ). Some of the cases that have cytoplasmic Igμ expression may also express weak surface Igμ (so-called transitional pre-B ALL). However, B-ALLs typically lack surface immunoglobulin light chain expression, and the rare exceptional cases should be diagnosed with great caution. Some subtypes of ALL, in particular those with hyperdiploid karyotypes, may lack CD45 expression, an important consideration when using CD45 alone to rule out a hematopoietic neoplasm in the evaluation of a small blue cell tumor in tissue sections.

Certain genetic subtypes may be associated with specific immunophenotypic patterns. Some common associations include B-ALL with *AF4/MLL* ($CD19^{+}$, $CD10^{-}$, $CD15^{+}$, $CD34^{+}$, TdT^{+}), *TEL/AML1* (*ETV6/RUNX1*; $CD19^{+}$, $CD10^{+bright}$, $CD34^{+}$ [variable intensity], $CD45^{+very\ dim}$, $HLA\text{-}DR^{+bright}$, TdT^{+}, cytoplasmic $Ig\mu^{-}$, frequent expression of myeloid markers such as CD13 and CD33), *BCR/ABL1* ($CD19^{+}$, $CD10^{+}$, $CD34^{+}$, $CD45^{dim\pm}$, TdT^{+}, $CD13^{+}$, $CD33^{+}$), and *E2A/PBX1* (TCF3-PBX1; $CD19^{+}$, $CD10^{-/dim+}$, $CD34^{-}$, $CD45^{dim\pm}$, cytoplasmic $Ig\mu^{+}$). These associations are not sufficiently specific, and final genetic subtyping requires confirmation by karyotype or molecular genetic testing.

T-ALLs are more heterogeneous in their pattern of T-antigen expression. All T-ALLs express cytoplasmic CD3, and many cases may show weak surface CD3 expression. Other T-cell antigens that are consistently expressed include CD2, CD5, and CD7. Commonly, T-ALL blasts have a cortical thymic immunophenotype that also includes expression of CD1a, CD4, CD8, and CD21 (Fig. 15.8). Other antigens that may be expressed in these cases are CD10 and CD56. A constellation of a specific immunophenotype—(1) positive for cCD3 and 1 stem cell/myeloid marker (CD117, CD34, HLA-DR, CD13, CD33, CD11b, or CD65), (2) negative to dim positive for CD5 (<75% positive), and (3) negative for CD1a, CD8, and MPO—defines a distinct subentity, early T-precursor lymphoblastic leukemia that is a new entity in the upcoming WHO 2016 classification. Occasional cases of T-ALLs may be negative for CD34, TdT and careful exclusion of peripheral T-cell lymphoma may be required. In addition to immunophenotype, cell cycle analysis for the DNA content (DNA index) of the leukemic blasts is used for risk stratification. In B-lymphoblastic leukemia, DNA index greater than 1.15 correlates with high hyperdiploidy and a good prognosis; in contrast, a DNA index less than 1.00 indicates hypodiploidy and a poor prognosis.

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Finally, in many cases of ALL (B-cell and T-cell) there is lineage-inappropriate expression of one or several myeloid-associated antigens. These most commonly include CD11b, CD13, CD33, and CD66c. The myeloid antigen expression has no prognostic significance and does not warrant a diagnosis of mixed-phenotype acute leukemia, unless specific criteria required for that entity are met. These criteria will be reviewed in Chapter 16 (mixed phenotype acute leukemias). Importantly, immunophenotypic aberrancies of ALL have provided a basis for monitoring minimal residual disease using flow cytometry, as part of some treatment protocols.

Immunohistochemical (IHC) analysis is usually more limited in the number of antigens that can be evaluated but is necessary in a dry aspirate tap requiring bone marrow biopsy or in resource-limited regions without access to flow cytometers. B-cell LBL and ALL are typically positive for CD19, CD79a, PAX5, CD10, and TdT (Fig. 15.9). These should be included in an IHC workup since other markers CD45, CD20, and CD34 can be negative in some B-lymphoblastic lymphoma/leukemia. T-lymphoblastic lymphoma/leukemia are always positive for CD3; other T-cell lineage–associated markers CD2, CD5, CD7 are almost equally sensitive but can be seen in B and myeloid lineages. In addition to the immature markers TdT and CD34, CD1a can also be seen in T-lymphoblastic lymphoma/leukemia.

Molecular and Cytogenetic Features

B-cell acute lymphoblastic leukemia includes several clinically important subgroups defined by recurrent cytogenetic and molecular abnormalities (Table 15.1). These groups have distinct gene expression profiles and are associated with a different response to various chemotherapy agents and with different therapy outcomes. In most therapeutic protocols, the molecular and cytogenetic information is used to assign patients to distinct risk groups that receive chemotherapy regimens of different intensities. In most centers high-risk groups, *MLL* translocation–positive infant ALL and hypodiploidy ALL, will also require bone marrow transplantation. Some of the translocations associated with a major prognostic effect, such as the *TEL-AML1* fusion, may not be detected by conventional cytogenetics, and additional techniques such as fluorescence in situ hybridization (FISH) or reverse transcription-polymerase chain reaction (RT-PCR) may be

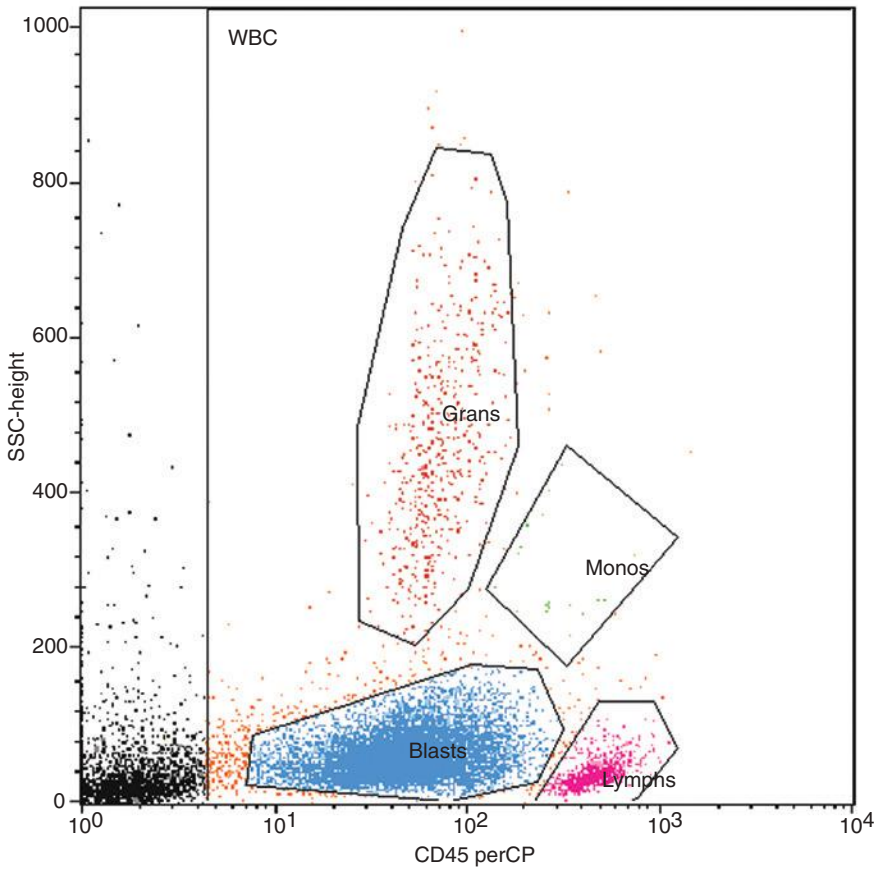
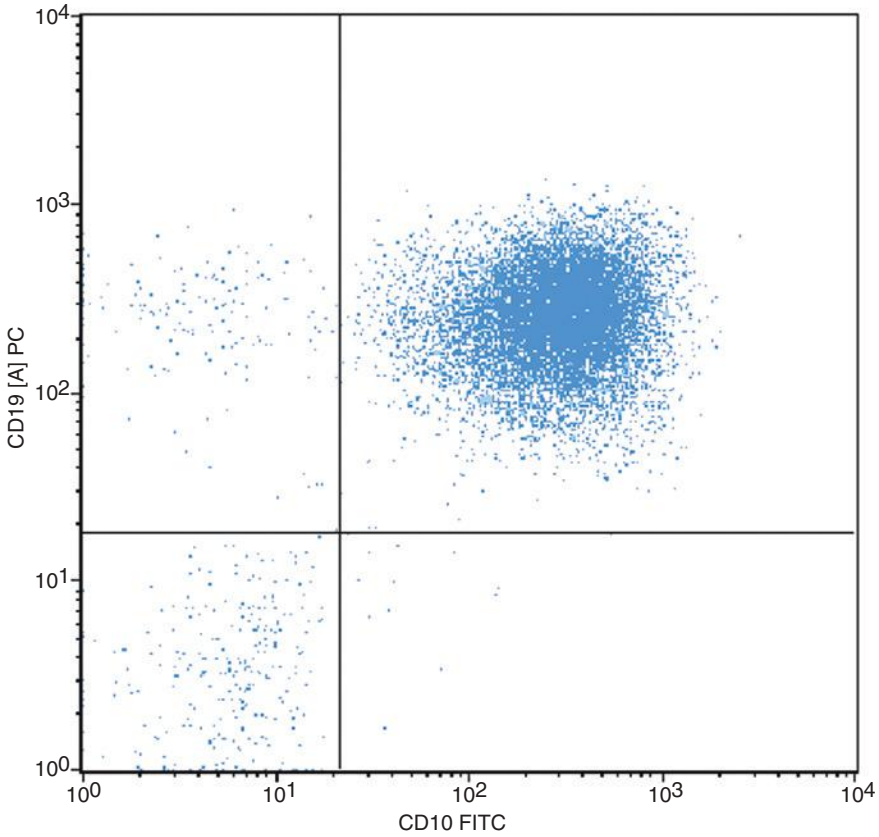


FIG. 15.7

B-ALL, immunophenotypic findings. The blast population (depicted in blue) has a low side scatter similar to the normal lymphocyte population (depicted in pink), and CD45 expression that is weaker than that of the lymphocytes. This blast population expresses CD10, CD19, CD34, cytoplasmic CD79a, and TdT. *(Flow cytometry histograms courtesy Frederick Behm, MD, Memphis, Tenn.)*

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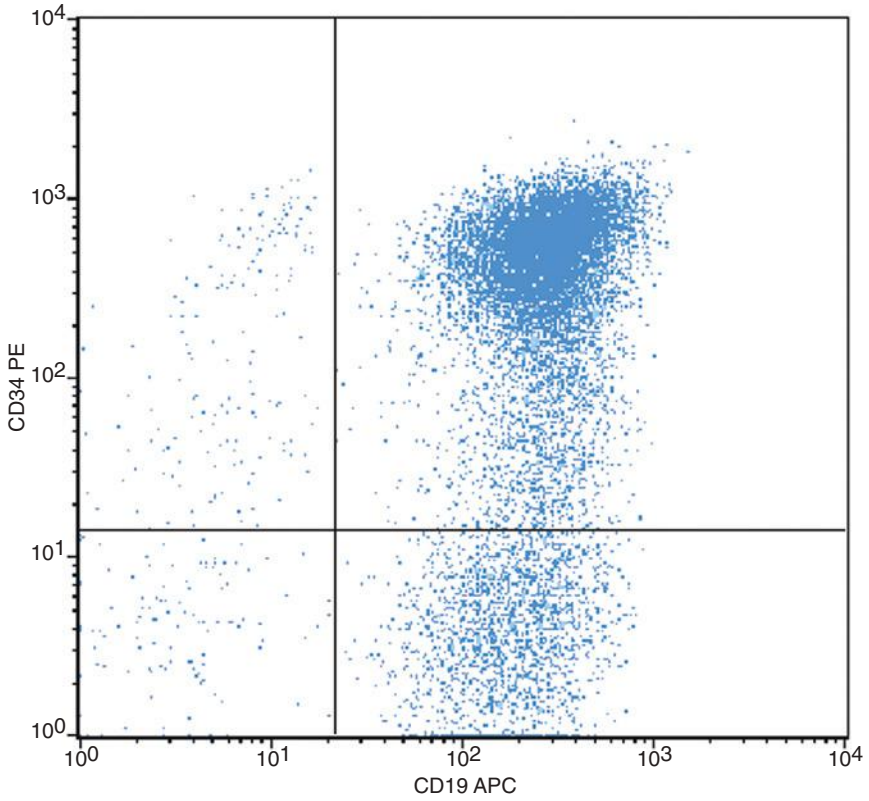


FIG. 15.7, cont'd

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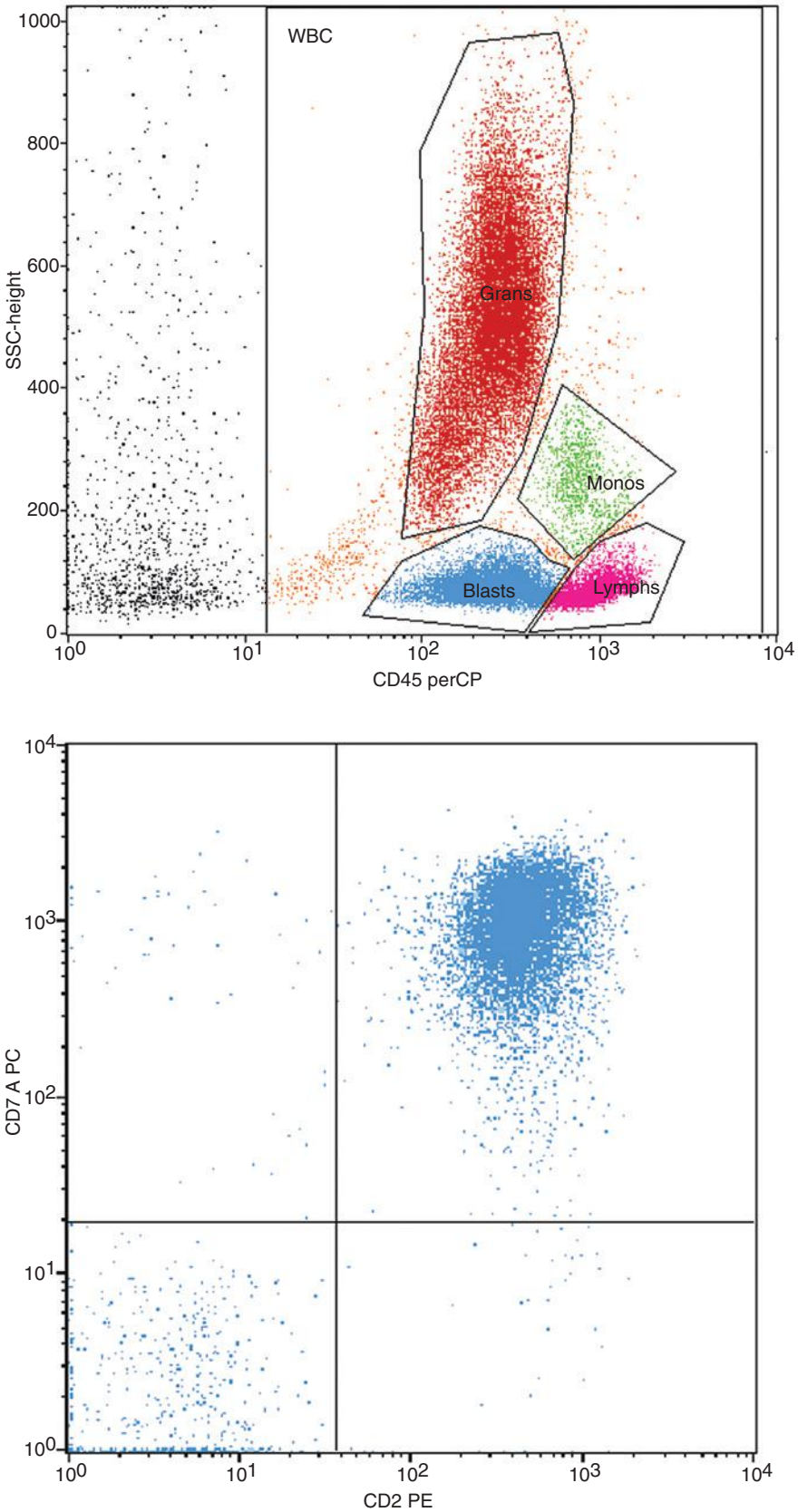


FIG. 15.8

T-cell lymphoblastic leukemia (T-ALL), immunophenotypic findings. The blast population (depicted in blue) has a low side scatter similar to that of the normal lymphocytes (depicted in pink) and CD45 expression that is weaker than that of the lymphocytes. The blasts express CD1a, CD2, cytoplasmic CD3, CD5, CD7, and TdT. The presence of CD1a expression is generally associated with the cortical thymic subtype of T-ALL and leads to potential difficulties in the differential diagnosis with normal thymocytes in tissue obtained from the anterior mediastinum. *(Flow cytometry histograms courtesy Frederick Behm, MD, Memphis, TN.)*

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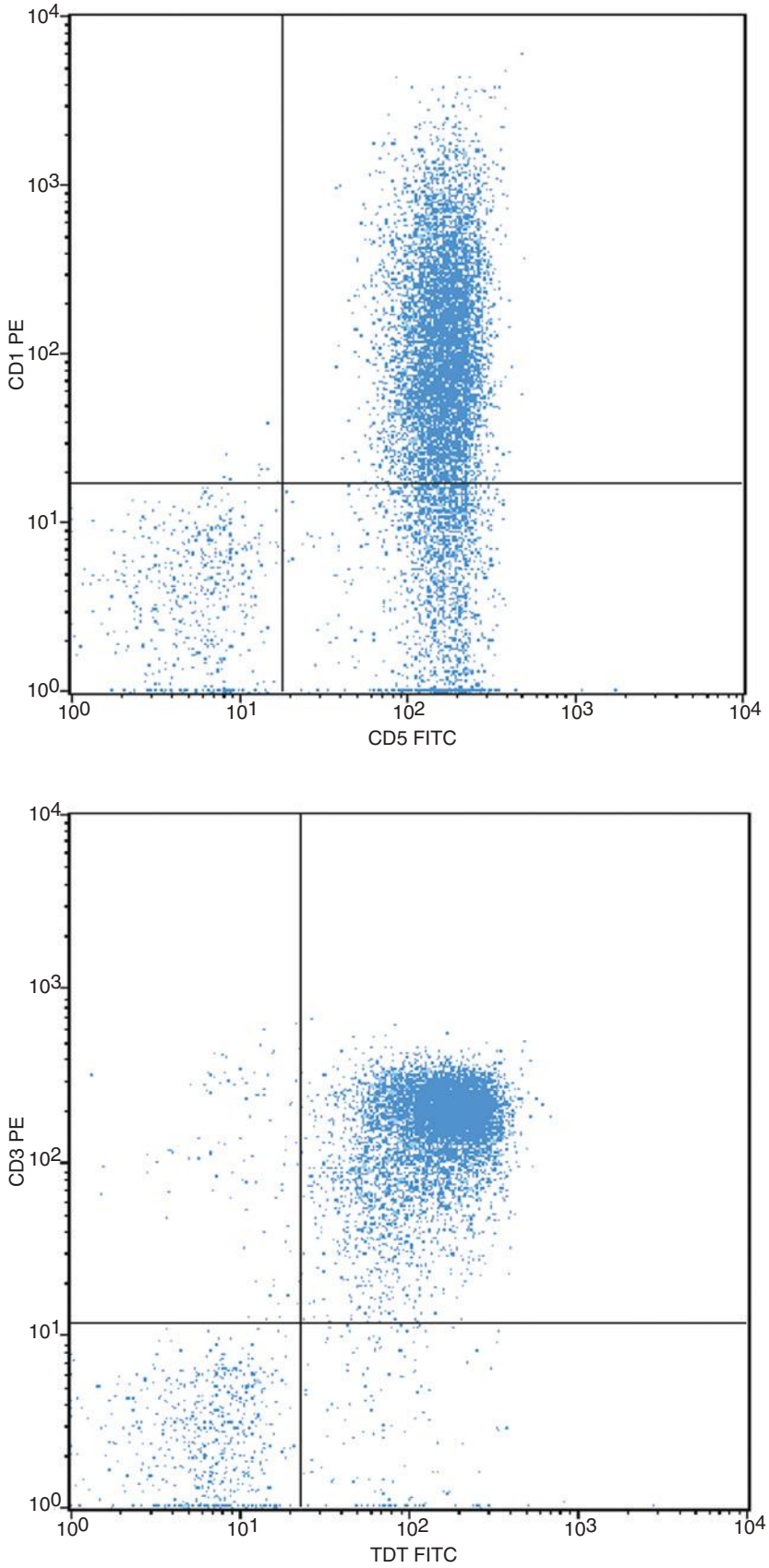


FIG. 15.8, cont'd

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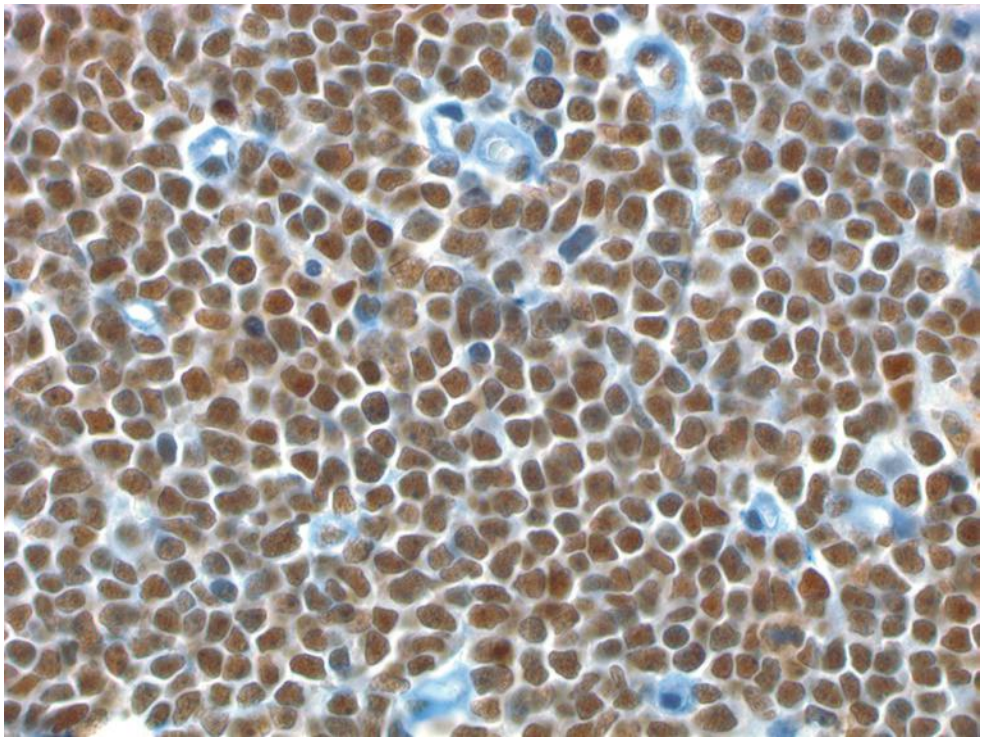


FIG. 15.9

Lymphoblastic lymphoma (LBL), immunophenotypic findings. Most of the lymphoblastic neoplasms express the nuclear antigen terminal deoxynucleotidyl transferase, which can be detected using immunohistochemistry in paraffin-embedded tissue sections, as depicted here in a case of T-LBL. Hematoxylin counterstain; original magnification, ×60; oil immersion.

TABLE 15.1

Molecular and Cytogenetic Subtypes of B-Lymphoblastic Leukemia (B-ALL)

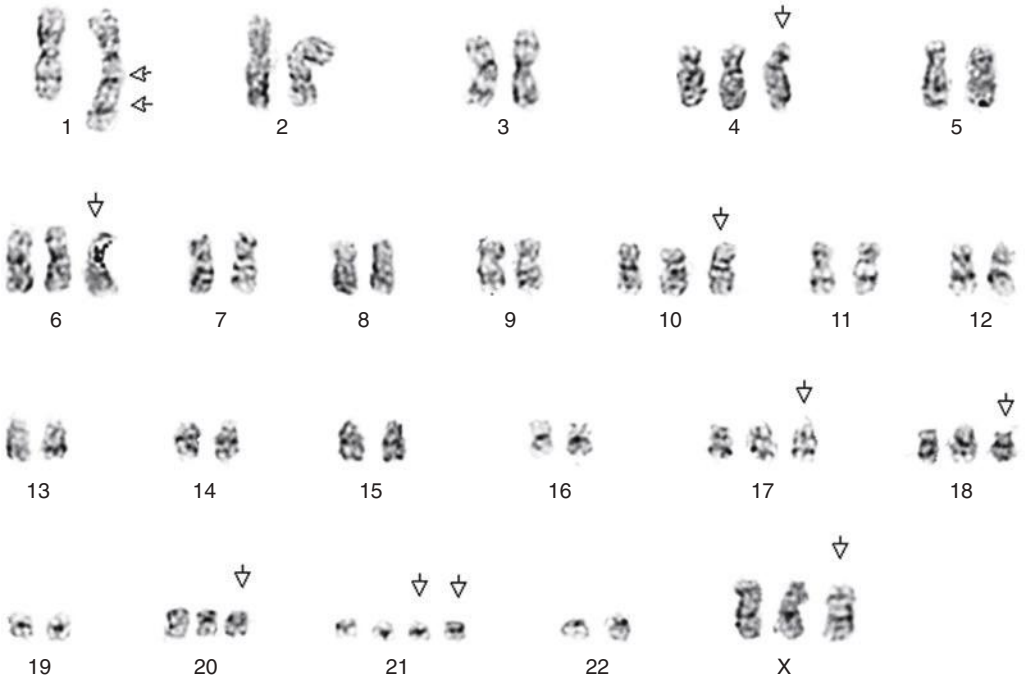
Chromosomal Abnormality	Associated Genetic Mutations	Frequency in Pediatric ALL	Prognostic Significance
Hyperdiploidy >50 (range 51-68) chromosomes	*FLT3*	25% to 30%	Low risk
t(12;21)(p12;q22)	*TEL-AML1 (ETV6-RUNX1)*	20%	Low risk
t(1;19)(q23;p13)	*E2A-PBX1 (TCF3-PBX1)*	5%	Standard risk
t(9;22)(q34;q11) (Philadelphia chromosome)	*BCR-ABL1*	4%	Standard with BCR-ABL1 inhibitor
t(v;11q23) t(4;11)(q21;q23)	*MLL gene* *AF4-MLL*	2%	High risk
Hypodiploidy (<46 chromosomes)[a]	*NF1, IKZF2, IKZF3, TP53, RB1*	4% to 5%	High risk
t(5;14)(q31;q32)	*IL3-IGH*	<1%	Unknown (standard risk)
Ph-like	*ABL1, ABL2,CSF1R, PDGFRB, EPOR, JAK2, CRLF2*	15% to 16%	High risk; probably better with targeted kinase inhibitor
iAMP21	*Intrachromosomal amplification of chromosome 21*	2%	High risk

[a]A heterogeneous group and a low hypodiploidy subgroup (<40 chromosomes) can be identified that has a high incidence of *TP53* mutation and poor prognosis.

required for their detection (Figs. 15.10 and 15.11). A relatively new group of B-ALL cases, Ph-like ALL, has been recognized based on gene expression similarities to Ph+ ALL (but lacking *BCR-ABL1* fusion) that often has rearrangements resulting in activation of other kinases and poor prognosis with conventional therapy. Identification has hinged on evaluation for one of many gene rearrangements so that targeted therapies can be evaluated. It is therefore important to characterize the genetic abnormalities of ALL by more than one technique.

The molecular genetics of T-ALL are less well understood. It appears that abnormalities of genes such as *TAL1, LYL1,* and *HOX11* may correlate with significant disease subtypes and may have prognostic significance, but additional research is required at this time to confirm these results. Activation

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55, XX, +X, dup(1)(q21q42), +4, +6, +10, +17, +18, +20, +21, +21

FIG. 15.10

B-cell lymphoblastic leukemia (B-ALL), conventional cytogenetics. The most common cytogenetic abnormality in pediatric precursor B-ALL is the presence of high hyperdiploid karyotypes (>50 chromosomes), associated with an excellent prognosis. Hyperdiploidy B-ALL (as in this case) commonly have trisomies of the chromosomes 4, 6, 10, 21, and X. *(Courtesy Susana Raimondi, PhD, Memphis, TN.)*

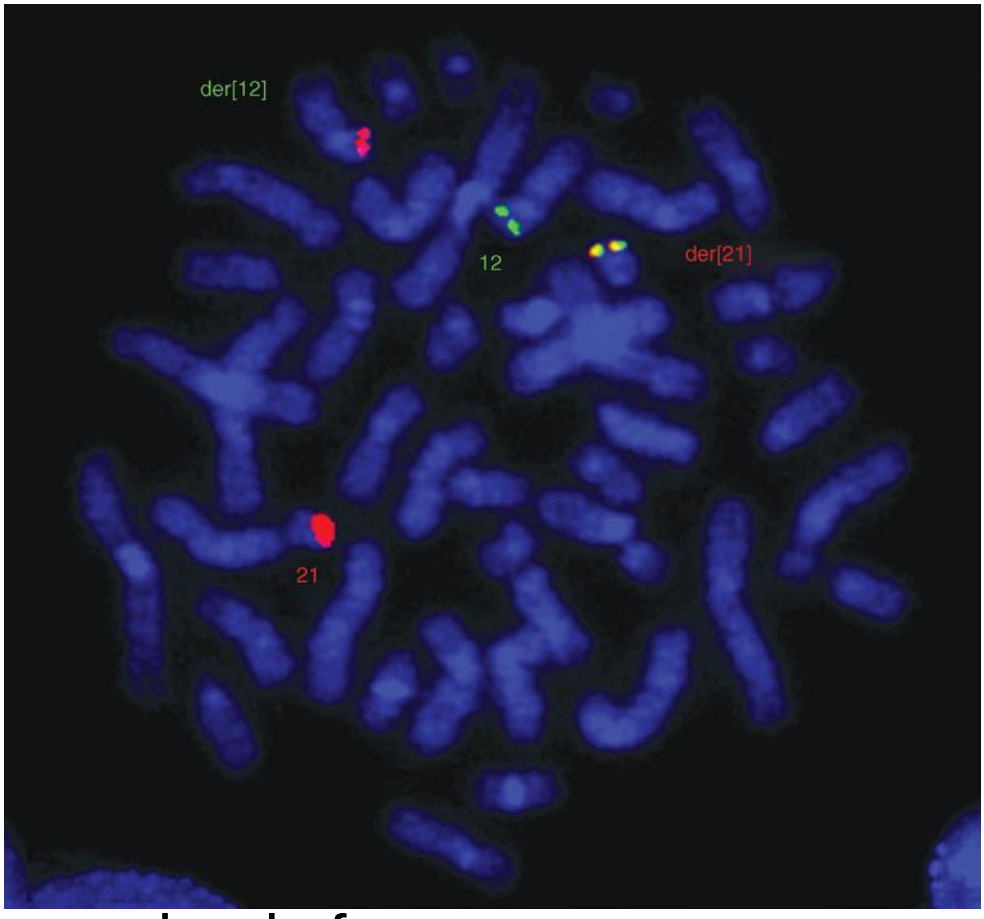


FIG. 15.11

B-cell lymphoblastic leukemia (B-ALL), fluorescence in situ hybridization (FISH) for the *TEL-AML1 (ETV6-RUNX1)* fusion gene associated with the translocation t(12;21)(p13;q22), the second most common genetic abnormality associated with pediatric B-ALL, which portends a very good prognosis. This metaphase FISH assay, using a probe for the TEL gene *(green)* and a probe for the *AML1* gene *(red)* demonstrates a fusion signal *(yellow)* present on the derivative chromosome 21. One copy of each gene is present on the normal chromosomes 12 and 21. This genetic abnormality is often too small to be detected by conventional cytogenetics, and therefore FISH or reverse transcriptase-polymerase chain reaction, or both, should be performed to rule it out in cases that appear diploid by the former technique. *(Courtesy Susana Raimondi, PhD, Memphis, TN.)*

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of the NOTCH1 signaling pathway occurs in perhaps 70% to 80% of cases, either through activating *NOTCH1* mutations in the extracellular heterodimerization domain or C-terminal PEST domain or through inactivating mutations in the *FBWX7* gene (a negative regulator of *NOTCH1*). These mutations are infrequent in early T cell precursor (ETP) T-ALL that more often have genetic mutations often associated with acute myeloid leukemia. ETP T-ALL had a poor prognosis in the initial studies with one chemotherapy regimen, but a larger subsequent study using a different chemotherapy regimen indicates that ETP T-ALL has similar outcome to other T-ALL.

Assays for immunoglobulin and T-cell receptor gene rearrangements are seldom critical in establishing a diagnosis in ALL or LBL but have been used successfully for minimal residual disease monitoring in these patients.

Differential Diagnosis

Acute lymphoblastic leukemia and LBL must be distinguished from benign (reactive) conditions characterized by the presence of normal precursor B-cell or precursor T-cell blasts and from various non-hematopoietic round blue cell tumors. Benign expansions of precursor B cells (so-called hematogones) may be seen in the bone marrow in a variety of reactive states, including infection, extramedullary tumors, and marrow regeneration following chemotherapy and bone marrow transplantation, especially in children. Hematogones have a more heterogeneous morphology and flow cytometry immunophenotype than the leukemic blasts, with a characteristic spectrum of antigen expression, including only partial expression of CD34, CD20, and CD10; unfortunately, there are rare incidences of left-shifted hematogones that are more homogeneous and can mimic residual leukemia without examination of other aberrantly expressed markers. Hematogones lack myeloid antigen expression, clonal immunoglobulin gene rearrangements, and chromosomal abnormalities.

Burkitt lymphoma/leukemia has a characteristic morphology and a mature B-cell immunophenotype that includes CD20 expression and the presence of light chain–restricted surface immunoglobulin and lack of TdT expression. The characteristic chromosomal translocation, t(8;14)(q24;q32) or its variants t(2;8)(p12;q24) and t(8;22)(q24;q11) should be present. The blastic variant of mantle cell lymphoma is seen exclusively in adults and is characterized by a mature B-cell immunophenotype, immunohistochemical expression of cyclin D1, and the t(11;14)(q13;q32) chromosomal translocation.

Benign thymocytes present in the thymic cortex have to be differentiated from T-ALL/LBL when the biopsy material originates in the anterior mediastinum of a child or young adult. Biopsies obtained from mediastinal tumors (e.g., thymoma, germ cell tumor, Hodgkin lymphoma) often include thymic tissue and therefore may lead to an erroneous diagnosis of T-cell LBL. Cortical thymocytes express TdT, CD34, CD1a, CD4, and CD8 (variable intensity), as well as other T-cell antigens. Similar to hematogones, cortical thymocytes lack immunophenotypic aberrancies and evidence of clonality. Benign thymocytes in thymomas often exhibit phenotypic heterogeneity within a single case, reflecting the differing maturational stages of thymocytes as opposed to the relatively uniform phenotype of a T-cell LBL. Demonstration of activated *NOTCH1* signaling using immunohistochemistry with antibodies specific to the cleaved (active) form of the *NOTCH1* intracellular domain appears diagnostically useful in differentiating T-LBL from thymoma. Morphologically, preservation of the normal thymic lobular architecture, including the presence of Hassall corpuscles, favors normal thymic tissue.

Acute myeloid leukemias (AML) often show a spectrum of morphologic differentiation in addition to the presence of blasts, and they may show associated dysplasia in the maturing cells. Myeloid sarcoma often shows associated background immature myeloid elements and eosinophils. Myeloid blasts may contain Auer rods and show cytochemical activity for myeloperoxidase and Sudan black B. The differential diagnosis between ALL, myeloperoxidase-negative AML, and mixed phenotype acute leukemia usually requires extensive immunophenotypic characterization by flow cytometry. Tissue infiltrates of AML (myeloid or monoblastic sarcoma) may be positive for chloracetate esterase (Leder stain) and have immunohistochemical expression of myeloperoxidase, lysozyme, CD34, or CD117. AML are negative for T-cell lineage–specific marker CD3 and are usually negative for B-cell markers CD19, CD79a, and PAX-5 except in cases of AML with t(8;21).

The distinction between T-ALL/LBL and mature peripheral T-cell lymphoma may be challenging, especially in cases of ALL that lack expression of TdT and CD34. Examples of T-cell lymphomas that may involve the bone marrow and peripheral blood in a leukemic fashion include T-cell prolymphocytic leukemia, mycosis fungoides, rare cases of anaplastic lymphoma kinase (ALK)-positive anaplastic large-cell lymphoma (small-cell variant), and hepatosplenic T-cell lymphoma. For details regarding these lymphomas, the reader is referred to the chapters addressing these entities.

Last, small blue cell tumors such as neuroblastoma, rhabdomyosarcoma, or Ewing sarcoma can closely mimic ALL morphologically. This is particularly true on bone marrow aspirate smear or touch imprints when tissue sections are not available, as is common in pediatric marrows. It is important to remember here that some ALL/LBL can be negative for CD45 and most are strongly positive for CD99 (O13), an antigen often used in the diagnosis of Ewing sarcoma. In round blue cell tumors, IHC for TdT, CD3, PAX5, and CD79a/CD19 is recommended to rule out ALL/LBL.

Prognosis and Therapy

The most important prognostic information provided by the pathologist for patients with ALL-LBL includes lineage (B-cell

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versus T-cell) and cytogenetic or molecular abnormalities (see previous discussion, Table 15.1). Therefore, it is crucial that material adequate for the corresponding techniques be obtained at the time of bone marrow or tissue sampling. In addition to these studies, the percentage of bone marrow involvement by blasts and the presence and percentage of blasts in the cerebrospinal fluid at the time of diagnosis may serve in further tailoring of therapy in various treatment protocols.

Detection of minimal residual disease by molecular or flow cytometry methods during therapy has emerged as the best predictor of relapse. Flow cytometry uses the ALL-specific immunophenotypic aberrancies to detect leukemic cells with a sensitivity of 1 in 10,000 cells and to differentiate these cells from normal B-lymphoid precursors. PCR-based methods use leukemic-specific primers for the detection of leukemic-associated immunoglobulin and T-cell receptor gene rearrangements with a sensitivity of 1 in 100,000 cells. The same leukemic-specific rearrangement also can be detected by next generation sequencing methods with a sensitivity of 1 in 1,000,000 cells. Several large-scale studies in pediatric ALL have demonstrated that minimal residual disease measured at specific time points during therapy is an important independent prognostic factor that can be used to adjust risk stratification and, therefore, treatment in these patients.



The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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16

Acute Undifferentiated Leukemia and Mixed-Phenotype Acute Leukemias

John Kim Choi, MD, PhD

INTRODUCTION

Acute leukemias of ambiguous lineage may be divided into acute undifferentiated leukemia (AUL) and mixed-phenotype acute leukemias (MPALs). AULs are leukemias with very primitive phenotypes that lack definitive lineage-specific antigens. MPAL encompasses rare blastic hematopoietic cell neoplasms that express a mixture of myeloid and lymphoid (B- or T-lineage) antigens. Alternative names for these leukemias include hybrid acute leukemias, acute leukemias of indeterminate lineage, and biphenotypic or bilineal acute leukemia. The 2008 World Health Organization (WHO) classification defines several categories of MPALs based on the presence of recurrent cytogenetic lesions or in their absence, on the lineage of the leukemic blasts, defined as B-myeloid, T-myeloid, B-T, or B-T-myeloid. Any of these leukemias can develop as biphenotypic or bilineal (bilineage) acute leukemias. In the biphenotypic leukemias, blasts may be morphologically homogeneous or heterogeneous, but they uniformly express myeloid and lymphoid antigens. In the bilineal acute leukemias, two morphologically and immunophenotypically distinct blast populations (lymphoid and myeloid) can be discerned. The latter category also incorporates acute leukemias that switch lineage during chemotherapy. Some MPALs have both a bilineal and a biphenotypic population; for example, B-lineage clone, myeloid-lineage clone, and a third population that expresess both sets of antigens. The WHO 2016 update will maintain WHO 2008 criteria for MPAL with additional clarifications. In cases of two distinct blast populations (bilineal), each population can be evaluated by the usual leukemia criteria and do not need to fulfill MPAL lineage criteria (Table 16.1). Also, dim MPO staining should be interpreted with caution, especially if no other myeloid-associated antigens are detected.

ACUTE UNDIFFERENTIATED LEUKEMIAS

Clinical Features

Acute undifferentiated leukemias are diagnosed with decreasing frequency due to more stringent criteria; they likely represent less than 1% of acute leukemias. Patients present with signs and symptoms of bone marrow failure, as with other acute leukemias. These include easy bruising, infection, and fatigue due to cytopenias such as thrombocytopenia, leukopenia, and anemia. Leukocytosis with blasts is also commonly seen.

Pathologic Features

Microscopic Features

Blasts, the dominant cell population in bone marrow, are usually intermediate in size with round nuclei and fine chromatin containing visible nucleoli. Blasts may also be smaller, resembling morphologically lymphoblasts. Cytoplasm is scant without azurophilic granules or Auer rods. By definition they are negative for Sudan black B (SBB) and myeloperoxidase (MPO) by cytochemistry. Trephine biopsy generally shows replacement of the marrow cellularity by sheets of blasts with little to no residual hematopoietic elements.

Ancillary Studies

Immunophenotypic Findings

Flow cytometry shows only immature, primitive hematopoietic cells without expression of antigens that define a lineage such as CD19 for B lymphoblasts, cytoplasmic CD3 for T lymphoblasts, myeloperoxidase for myeloblasts, or other myeloid-associated markers with myeloblasts/monoblasts. The last is not precisely defined, leading to contradictory specifications in the 2008 WHO classification. AUL is defined to permit a single myeloid-associated marker, but a single myeloid marker such as CD33 is sufficient to define AML with minimal differentiation (AML-M0). The distinction between AUL and AML-M0 can be difficult and somewhat arbitrary, but a recent hematopathology workshop on MPAL agreed that AML-M0 is the diagnosis based solely on CD33

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TABLE 16.1

Criteria Required by the Most Recent World Health Organization Classification for Assigning More Than One Lineage to a Leukemic Process

Myeloid lineage
Myeloperoxidase expression[a] or
Monocytic antigen expression (at least two of the following: nonspecific esterase, CD11c, CD14, CD64, lysozyme)
T lineage
Cytoplasmic CD3[b] or surface CD3
B lineage
Strong expression of CD19 and strong expression of at least one of the following: CD79a, cytoplasmic CD22, CD10
Weak expression of CD19 and strong expression of at least two of the following CD79a, cytoplasmic CD22, CD10

[a]Myeloperoxidase may be detected by cytochemical or immunologic methods. Cytochemically positive myeloperoxidase should be present in at least 3% of the morphologically identified leukemic blasts. Immunophenotypically positive myeloperoxidase should be seen on a blast population that shows other immunophenotypic aberrancies (in order to exclude residual benign myeloblasts).
[b]Cytoplasmic CD3 expression should be detected by flow cytometry with antibodies to the CD3ε chain (immunohistochemistry using polyclonal anti-CD3 antibodies may detect CD3 zeta chain, which is not specific for T-lineage).

expression provided the absence of any B- or T-cell markers.

Molecular and Cytogenetic Findings

Little is known about the genetic findings in AUL. No specific genetic abnormalities have been associated with these leukemias. Clonal karyotypic abnormalities appear to be detected in most cases, but changes are not specific.

Differential Diagnosis

The main differential diagnosis is with minimally differentiated acute myeloid leukemia. Both lack SBB and MPO. However, minimally differentiated acute myeloid leukemia will express a combination of myeloid-associated markers such as CD13, CD33. Acute erythroid or megakaryoblastic leukemias may appear as AUL on initial work-up until blasts are studied for erythroid markers (glycophorin, hemoglobin A) or megakaryoblastic markers such as CD41 and CD61.

Prognosis and Therapy

Optimal treatment for AUL is not yet known, but most patients are treated with AML-type therapy. Whether myeloablative therapy and stem cell transplantation is beneficial is also unknown. Reports of outcome are few, but some complete and durable remissions have been reported. Additional studies addressing modern therapies are needed but are difficult to undertake due to the rarity of these leukemias.

MIXED-PHENOTYPE ACUTE LEUKEMIAS

Clinical Features

Mixed phenotype acute leukemias represent 3% to 5% of all cases of acute leukemia (patients of all ages) and 8% of all cases of acute leukemia occurring in adults. These leukemias can occur at any age but are slightly more common in adults. In the largest study available, the median age for de novo acute biphenotypic leukemias was 25.5 years. Rare cases of MPAL secondary to chemotherapy for other neoplasms have also been reported.

The clinical presentation is similar to that of other acute leukemias, including symptoms secondary to anemia, thrombocytopenia, and neutropenia, such as fatigue, abnormal bleeding, and infections. Infants with acute bilineal leukemia may exhibit multiple skin lesions similar to those seen in the patients with acute monoblastic leukemia (so-called blueberry-muffin baby).

MIXED PHENOTYPE ACUTE LEUKEMIA—FACT SHEET

Definition
- Malignant blastic hematopoietic neoplasms that show differentiation along more than one lineage (lymphoid and myeloid)

Incidence
- 3% to 5% of all acute leukemias

Morbidity and Mortality
- Reported median survival 6 to 7 months in adult patients
- Probability of survival at 2 years: 39% in adults, 75% in children

Gender, Race, and Age Distribution
- Slight male predominance (male:female = 1.5 : 1)
- May occur at any age but more frequent in adults
- Median patient age, 25 years

Clinical Features
- Symptoms secondary to anemia, thrombocytopenia, and neutropenia (fatigue, abnormal bleeding, infections)
- Multiple skin lesions in some infants with bilineal leukemia

Prognosis and Therapy
- Poor prognosis
- No universally accepted therapy
- Addition of tyrosine kinase inhibitors in MPAL with BCR-ABL fusion transcripts
- Addition of histone-modifying enzyme inhibitor in MPAL with MLL gene rearrangement
- ALL therapy with allogeneic stem cell transplant

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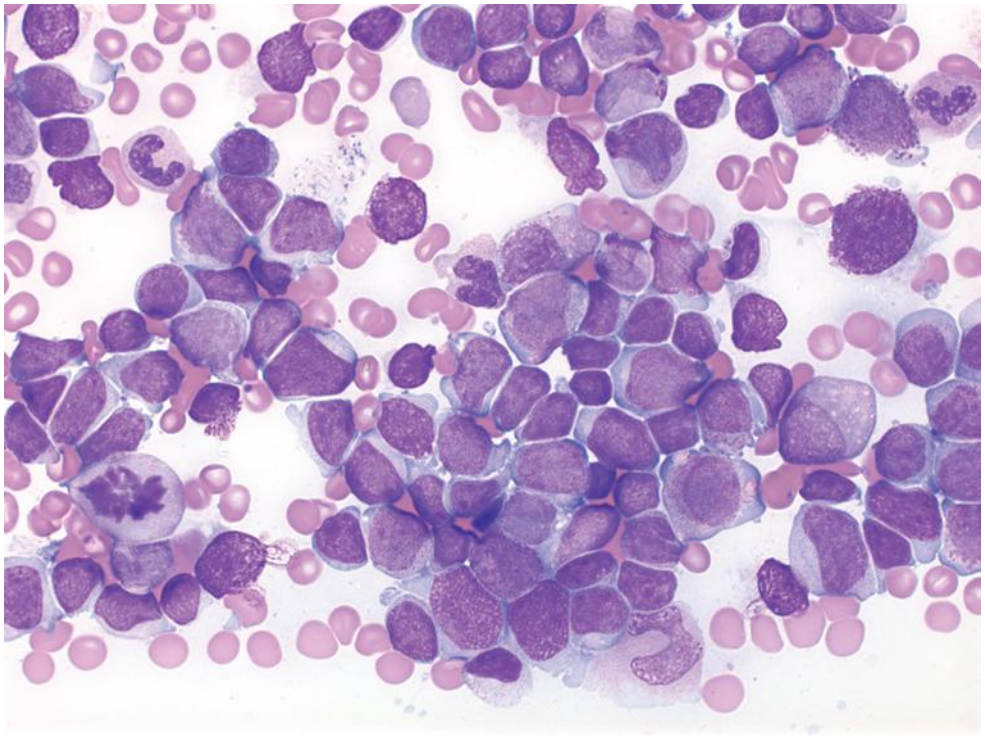


FIG. 16.1
Mixed phenotype acute leukemia (MPAL), biphenotypic type. Cytologic features on smear preparations. This leukemic process is composed of morphologically heterogeneous blasts, with a predominance of large cells with abundant cytoplasm and prominent nucleoli. These leukemic cells co-expressed T-cell and myeloid antigens and contained the Philadelphia chromosome. Wright-Giemsa stain; original magnification, ×60; oil immersion.

Pathologic Features

Microscopic Findings

On Wright-Giemsa–stained smears, the MPALs overlap significantly with other acute leukemias. The acute biphenotypic leukemias and the undifferentiated acute leukemias may consist of morphologically homogeneous or heterogeneous blasts, with a spectrum that includes lymphoid-appearing cells and myeloid-appearing cells (Fig. 16.1). The former cells are small, with scant cytoplasm and condensed nuclear chromatin, often with absent or inconspicuous nucleoli. The latter cells are typically larger, with more abundant cytoplasm, finely dispersed nuclear chromatin, and prominent nucleoli. Some of the cases of acute biphenotypic leukemia may show prominent morphologic differentiation toward myeloid lineage, with cytochemical myeloperoxidase positivity and even Auer rods. These cases may be classified as acute myeloid leukemia (AML) M1, M2, and, more rarely, M4 or M5 in the French-American-British classification. In the bilineal acute leukemias, two morphologically distinct populations of blasts are usually seen. These populations typically include, in variable proportions, small lymphoid blasts and large monoblasts, the latter similar to the neoplastic cells seen in acute monoblastic leukemia (Fig. 16.2).

In hematoxylin and eosin–stained sections of bone marrow or other involved tissues, these leukemias consist of relatively monotonous blasts of intermediate size, with scant cytoplasm, vesicular chromatin, and often prominent nucleoli. Notably, in acute bilineal leukemia, only one of the two blast populations may be seen at a given anatomic site. For instance, leukemias with lymphoblastic and monoblastic differentiation may show only monoblastic infiltrates in the skin, whereas both blast populations are identified at other locations.

Ancillary Studies

Immunophenotypic Findings

Flow cytometric analysis is essential in the diagnosis of acute biphenotypic leukemias (Fig. 16.3), which were initially defined based on their immunophenotypic profile. Careful corroboration of all the antigens expressed is necessary in order to rule out acute lymphoblastic leukemia or AML showing aberrant lineage-inappropriate antigen expression (so-called lineage infidelity). For example, many of the precursor B-cell acute lymphoblastic leukemias may express the myeloid-associated antigens CD13 and CD33. In addition, certain subtypes of AML consistently express lymphoid-associated antigens (e.g., CD19 in AML with AML1-ETO and CD2 in M4Eo AML). In an attempt to better define these neoplasms, several classification systems have been devised. The one most widely used is the scoring system proposed and later revised by the European Group for the Immunological Classification of Leukemia. The WHO 2008 classification and 2016 update have abandoned this scoring system and has proposed a new set of more stringent requirements for assigning more than one lineage to a leukemic process (see Table 16.1).

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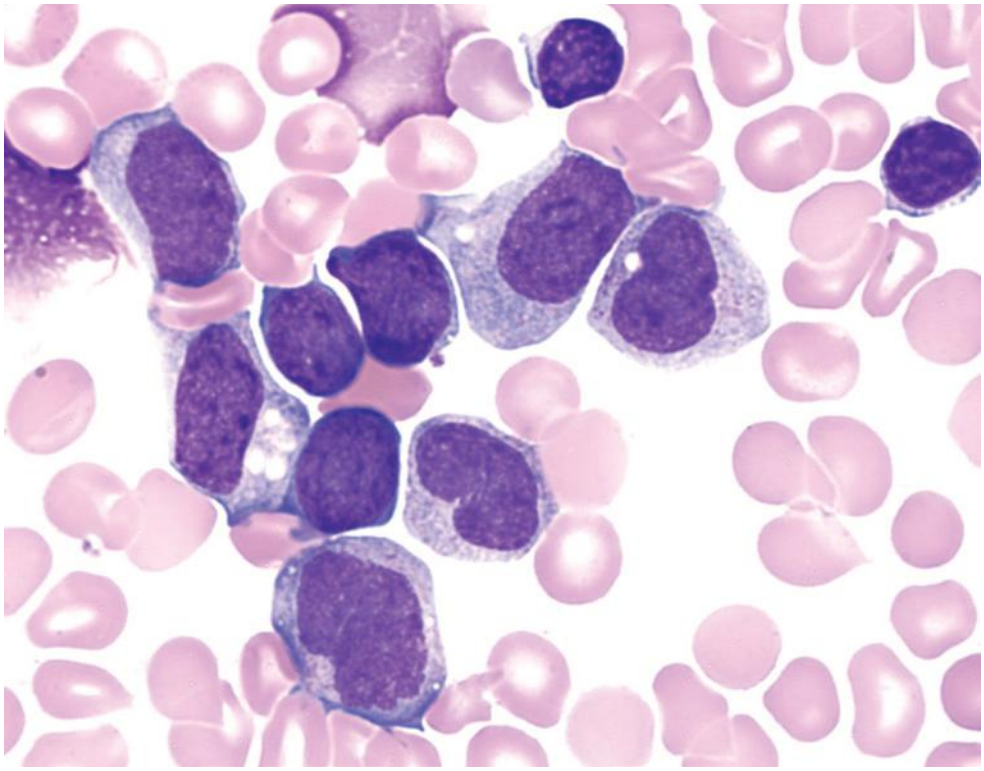


FIG. 16.2

Mixed phenotype acute leukemia, bilineal type. Cytologic features on smear preparations. This leukemia consists of two morphologically distinct blast populations: lymphoid blasts (small, with very scanty cytoplasm and condensed nuclear chromatin) and monoblasts (large, with abundant granular cytoplasm, finer chromatin, and prominent nucleoli). Wright-Giemsa stain; original magnification, ×60; oil immersion.

MIXED PHENOTYPE ACUTE LEUKEMIA—PATHOLOGIC FEATURES

Microscopic Findings

- Blasts may be uniform or heterogeneous in appearance or typically dimorphic (lymphoid, myeloid, monoblastic)
- Lymphoid blasts are small, with scant cytoplasm, condensed chromatin, and inconspicuous nucleoli
- Myeloid blasts are larger with more abundant basophilic cytoplasm, finely dispersed chromatin, and prominent nucleoli
- Some cases may show blasts with Auer rods and myeloperoxidase positivity
- A maturing cell component is usually missing
- In bone marrow biopsy material, the marrow cellularity is increased and consists predominantly of blasts

Immunophenotypic Findings

- Biphenotypic leukemia: a single population of blasts is present that uniformly expresses a mixture of myeloid and lymphoid (B- or T-lineage antigens); diagnosis requires a weighted scoring system (e.g., European Group for the Immunological Classification of Leukemias scoring or requirements of WHO Classification)
- Bilineal (bilineage) leukemias: two morphologically and immunophenotypically distinct lymphoid and myeloid blast populations, usually precursor B-cell lymphoblasts and monoblasts
- Combined biphenotypic and bilineal leukemias can also occur: two distinct single lineage populations and a third biphenotypic population
- Undifferentiated leukemias: poorly differentiated blasts that lack any lineage associated antigens. Hence, they lack CD19, cytoplasmic CD3, MPO, CD33, or CD13

Differential Diagnosis

- Acute lymphoblastic leukemia expressing myeloid antigens
- Acute myeloid leukemia expressing lymphoid antigens

In acute bilineal leukemia, flow cytometry analysis usually shows two distinct or only partially overlapping populations of blasts with respect to CD45 expression and side scatter (Fig. 16.4). On further analysis, each of these populations of blasts has a distinct immunophenotypic profile, similar to that of acute lymphoblastic leukemia and of AML (often acute monoblastic leukemia), respectively. In such cases, one should ensure that the B-lymphoblast population shows immunophenotypic aberrancies compatible with a neoplastic cell population, in order to avoid overcalling benign precursor B-cell (hematogone) expansions associated with an otherwise typical AML.

Lineage criteria in Table 16.1 are to be strictly applied only in immunophenotyping of an MPAL and not for assigning lineage in otherwise typical ALL or AML or in the differential diagnosis of acute undifferentiated leukemia and minimally differentiated AML (AML M0).

Molecular and Cytogenetic Findings

The most common recurring genetic abnormality associated with biphenotypic leukemia is the t(9;22)(q34;q32) translocation resulting in the *BCR-ABL1* fusion transcript (the Philadelphia chromosome), which is present in approximately

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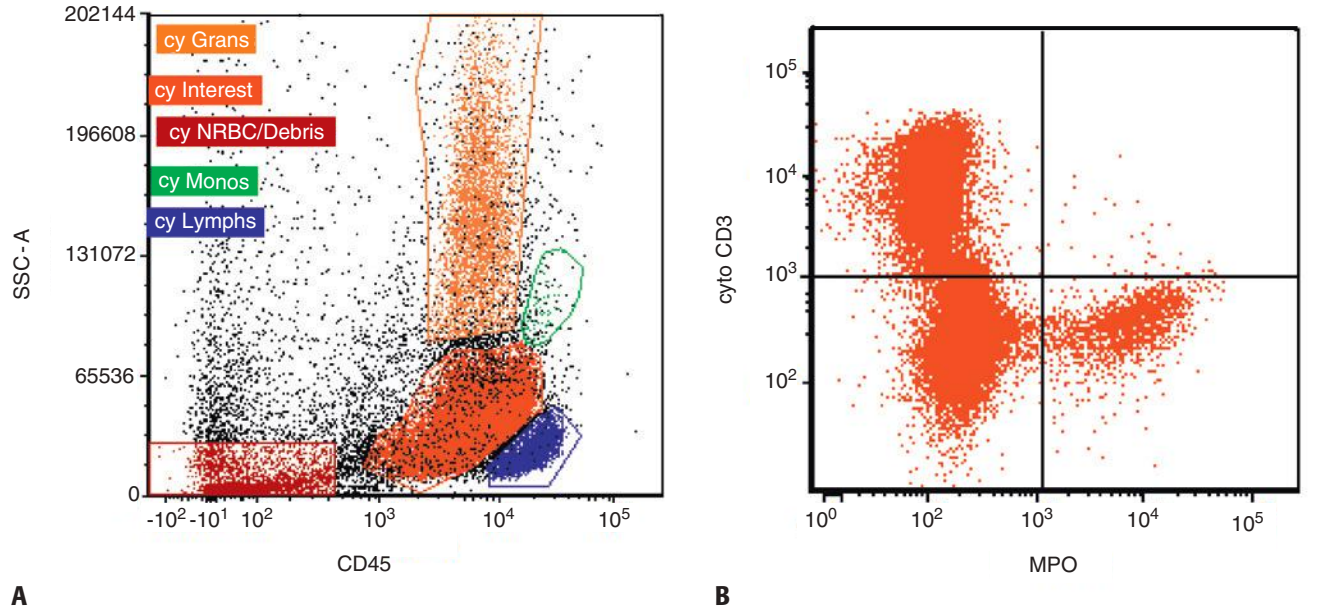


FIG. 16.3

Mixed phenotype acute leukemias, T-myeloid immunophenotypic features. A, The blast population *(red)* has a slightly higher side scatter (SSC) and lower CD45 expression than those of the adjacent normal lymphocyte (blue). B, One subpopulation expresses cytoplasmic CD3, meeting the criterion for T-cell lineage, and another population expresses myeloperoxidase (MPO), meeting one criterion for myeloid lineage. Note that a large subpopulation does not express CD3 or MPO nor does it express any other lineage-specific antigen and could technically be considered acute undifferentiated leukemias (AUL) if not for the other populations.

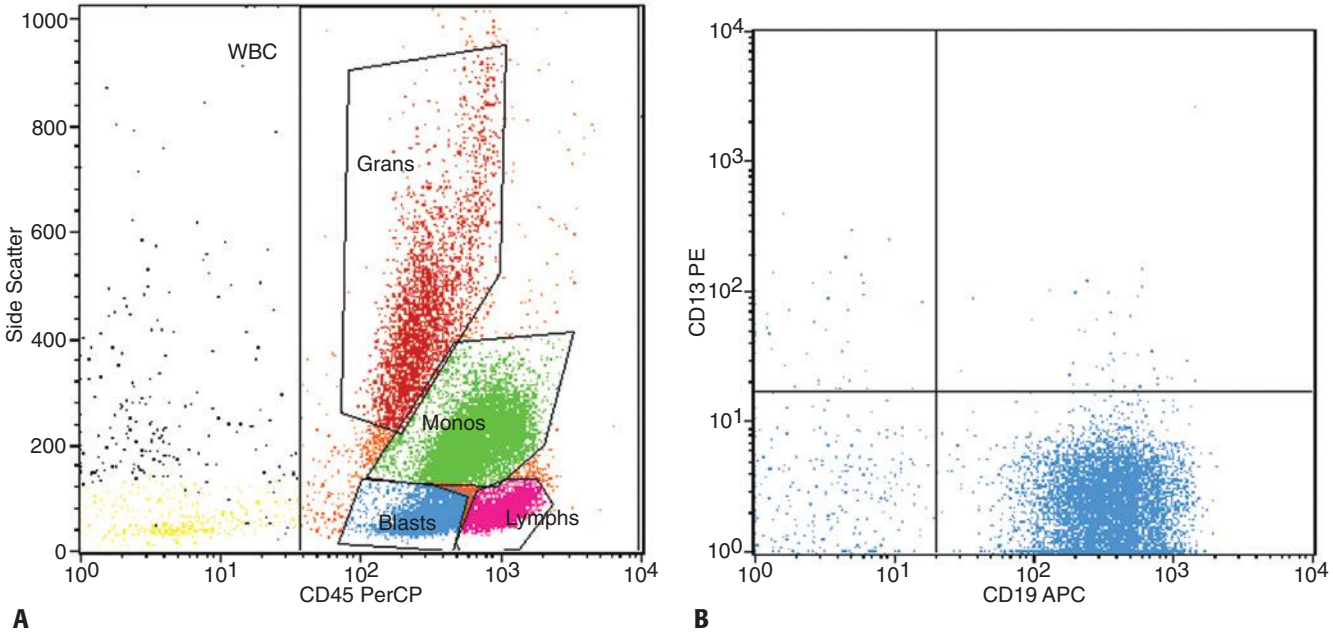


FIG. 16.4

Mixed phenotype acute leukemias, B-myeloid immunophenotypic features in the case with bilineal morphology represented in Fig. 16.2. A, The leukocyte population (WBC) includes two populations of blasts, one with weak CD45 expression and low side scatter *(blue)* and one that has strong CD45 expression, higher side scatter, and overlaps with normal monocytes (monos; *green*). B and C, The first population expresses CD19, lacks CD13, and is B-cell lineage. D and E, The second blast population lacks CD19 and expresses strong CD33. Notably, both populations show partial expression of NG2 (7.1), which is abnormal and has been associated with rearrangements of the *MLL* gene (C and E). *Monos,* Monocyte gate; *WBC,* white blood cells. *(Flow cytometry histograms courtesy Frederick Behm, MD, Memphis, TN.)*

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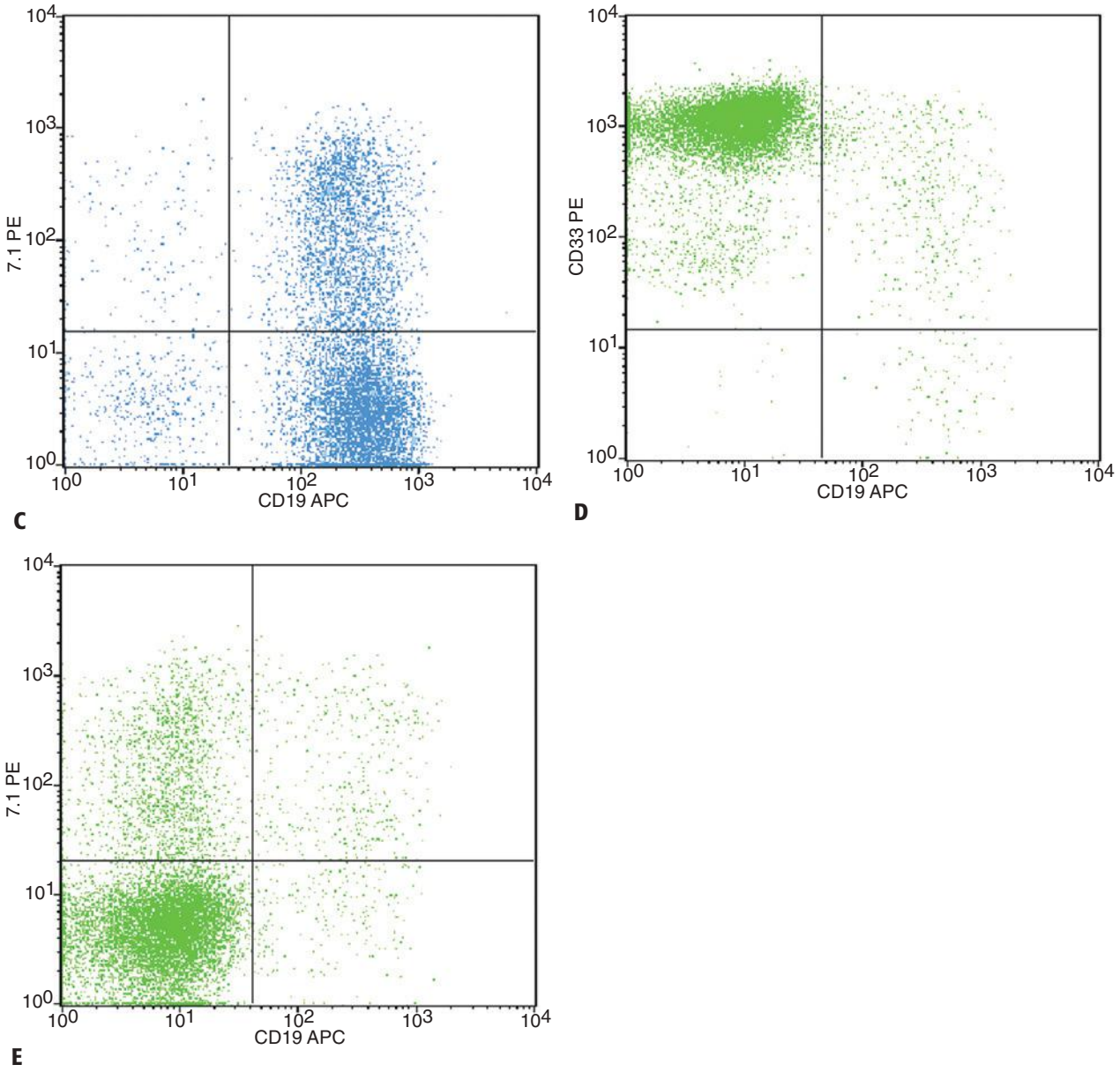


FIG. 16.4, cont'd

one third of cases. MPALs bearing this genetic lesion should be designated as MPAL with t(9;22)(q34;q11.2);*BCR-ABL1*. Most of these cases are of B-myeloid lineage. A much smaller number of cases have 11q23/*MLL* (mixed-lineage leukemia) gene abnormalities as part of the t(4;11)(q21;q23), t(9;11)(p13;q23), or t(11;19)(q23;p13.3) chromosomal translocations or as 11q23 deletions (Figs. 16.5 and 16.6). MPALs containing such genetic lesions should be diagnosed as MPAL with t(v;11q23); *MLL* rearranged. These cases also have predominantly a B-myeloid immunophenotype. In bilineal cases, the B lymphoblasts show an immunophenotypic profile similar to ALL with *MLL* gene rearrangements ($CD19^+$, $CD10^-$, $CD15^+$, $CD65^+$, TdT^+). Other less frequent abnormalities include trisomy 4, deletions of chromosomes 5 and 7, trisomy 8, and rare translocations such as t(9;12), t(7;12), and t(2;7) in which the involved gene partners are unknown. Many of the MPAL have rearrangements or deletions of the immunoglobulin heavy chain gene or T-cell receptor genes.

Differential Diagnosis

The main entities in the differential diagnosis of MPALs are acute lymphoblastic leukemia and AML, especially in cases in which the latter two show lineage-inappropriate antigen expression. Although in some cases the exact distinction between these types of leukemias remains controversial, the use of the recommended stringent diagnostic criteria should allow the accurate diagnosis of MPAL according to the most recent classifications.

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FIG. 16.5

Conventional cytogenetics of myeloperoxidase (MPAL) with t(v;11q23); *MLL* rearranged in the case of bilineal leukemia presented in Figs. 16.2 and 16.4. There is a balanced translocation t(9;11)(p22;q23) that results in the *MLL-AF9* fusion product *(arrows).* Rearrangements and deletions of 11q23 (*MLL* gene) comprise one of the most common genetic abnormalities encountered in MPAL. *(Courtesy Susana Raimondi, PhD, Memphis, TN.)*

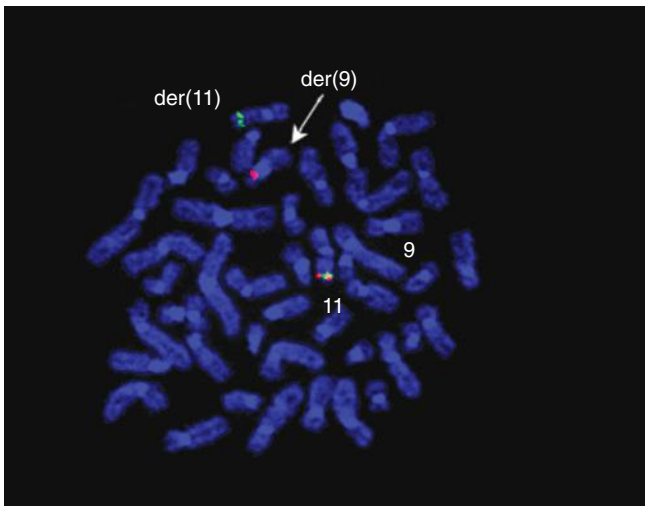


FIG. 16.6

Fluorescence in situ hybridization in myeloperoxidase depicted in Figs. 16.2, 16.4, and 16.5. This assay was performed using a break-apart probe (split-signal fluorescence in situ hybridization) for the *MLL* gene, with green labeling the 5′ end and red labeling the 3′ end of the gene. In this case a fusion signal *(yellow),* here seen on the normal chromosome 11, corresponds to an intact gene, whereas the red signal present on the derivative chromosome 9 demonstrates that the 3′ portion of the *MLL* gene has been translocated onto the short arm of that chromosome, with the 5′ end of the gene *(red)* remaining on the long arm of the derivative chromosome 11. *(Courtesy Susana Raimondi, PhD, Memphis, TN.)*

Prognosis and Therapy

Mixed phenotype acute leukemias are aggressive leukemias that have a poor prognosis. In most series, the median survival of adult patients with acute biphenotypic leukemia is 6 to 7 months, with a probability of survival at 2 years of 39% in adults and 75% in children. Poor prognostic factors include patient age older than 60 years, poor patient performance status, and, in the pediatric age group, the presence of prominent myeloid differentiation (i.e., expression of myeloperoxidase, presence of Auer rods) in the leukemic process. Therapy usually involves a combination of ALL and AML chemotherapy followed by allogeneic stem cell transplant. More recent therapeutic recommendations include targeted therapy and ALL-directed chemotherapy followed by transplant.

The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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17

Myeloproliferative and "Overlap" Myelodysplastic/Myeloproliferative Neoplasms

■ Beenu Thakral, MD ■ John Anastasi, MD ■ Sa A. Wang, MD

■ INTRODUCTION

Myeloproliferative neoplasms (MPNs), formerly referred to as *myeloproliferative disorders,* are a group of clonal multipotential hematopoietic stem cell diseases, proliferative in nature. MPNs are frequently associated with hypercellular bone marrow and with an elevation of one or more cell types in the blood with no ineffective hematopoiesis. These neoplasms are insidious in onset, chronic in course, but have variable tendency to terminate in marrow failure or acute leukemia. The MPNs include the model disease, chronic myelogenous leukemia, now known as chronic myeloid leukemia (CML). CML has become a prototype in medicine because it illustrates how the elucidation of pathways involved in the molecular pathogenesis (specifically in this case the dysregulation of *ABL1* tyrosine kinase signaling) leads to the rational development of targeted therapy for the disease (i.e., imatinib and other tyrosine kinase [TK] inhibitors).

The other more common non-CML MPNs include essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF). These entities are characterized by a multipotential hematopoietic stem cell origin, clonal proliferation, and chronic nature. Although they seem to be distinctive, as there is little transformation from one to another, early in their course they can present a diagnostic challenge and can be difficult to distinguish from one another because more characteristic features have not yet developed. Recent discoveries in the underlying molecular pathology of these entities have demonstrated that, like CML, they too share TK signaling dysregulation, at least to some degree. This dysregulation is due to a mutation in the TK gene *JAK2,* which is present in approximately 50% to 95% of cases. In late 2013, it was demonstrated that exon 9 frameshift mutations in *CALR* are found in 50% to 80% of *JAK2/MPL* unmutated ET and PMF. *CALR* mutation has not been described in PV. Further details of this mutation will be discussed in the section on ET and PMF.

Other less common MPNs include chronic neutrophilic leukemia (CNL) and chronic eosinophilic leukemia, not otherwise specified (CEL, NOS). CNL is a rare entity that is included in the spectrum of MPN and is now known to be pathogenically associated with oncogenic mutations in the gene for the colony-stimulating factor-3 receptor (*CSF3R*). Of patients who present with hypereosinophilia, cases with rearrangements of *PDGFRA, PDGFRB,* or *FGFR1* or with *PCM1-JAK2* are classified as myeloid/lymphoid neoplasms associated with eosinophilia. CEL, NOS is a diagnosis of exclusion and is currently lumped together with idiopathic hypereosinophilic syndrome (HES) owing to the difficulty in proving clonality. The application of next-generation sequencing (NGS) may further refine our ability to diagnose, classify, and manage this group of diseases with hypereosinophilia. Systemic mastocytosis (SM) was classified as a form of MPN in the previous editions of the World Health Organization (WHO) classification scheme. Owing to its unique clinical and pathologic features, SM is no longer considered a subgroup of the MPNs but a separate disease category in the WHO 2016 classification. The cases that do not fit into any specific MPN entity will remain in MPN as MPN, unclassifiable.

The revised WHO 2016 classification of MPN is shown in the fact sheet below. An algorithmic approach to MPN diagnosis can help in appropriate subclassification that will be outlined later in the chapter. It is important to recognize that the diagnosis of the MPNs does not rest solely with the routine microscopic examination of cells and tissues on slides. The diagnostic workup is more far-reaching and must include reviewing the clinical history and pertinent physical findings, as well as obtaining and assessing laboratory values, including recent complete blood cell counts and their trends. Examination of a well-made peripheral blood smear and both bone marrow aspirate and biopsy specimens are still indeed crucial. However, certain ancillary studies, such as cytogenetic and molecular analysis, as well as other more specific laboratory evaluations, such as the presence of leukoerythroblastic blood picture, serum erythropoietin, and lactate dehydrogenase

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CLASSIFICATION OF MYELOPROLIFERATIVE NEOPLASMS (WHO 2016)—FACT SHEET

- Chronic myeloid leukemia (CML), *BCR-ABL1* positive
- Polycythemia vera (PV)
- Primary myelofibrosis (PMF)
- PMF, prefibrotic/early stage
- PMF, overt fibrotic stage
- Essential thrombocythemia (ET)
- Chronic neutrophilic leukemia (CNL)
- Chronic eosinophilic leukemia, not otherwise specified (NOS)
- MPN, unclassifiable

CLASSIFICATION OF MYELODYSPLASTIC/ MYELOPROLIFERATIVE NEOPLASMS (WHO 2016)—FACT SHEET

- Chronic myelomonocytic leukemia (CMML)
- Atypical chronic myeloid leukemia (aCML), *BCR-ABL1* negative
- Juvenile myelomonocytic leukemia (JMML)
- MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)
- MDS/MPN, unclassifiable

(LDH) levels, might be just as important in arriving at the correct diagnosis.

The overlap syndromes (i.e., the myelodysplastic syndromes/myeloproliferative neoplasms [MDS/MPNs]) are a relatively newly devised group of diseases that was created by the WHO committee (2001) writing on the hematopoietic tumors. The current 2016 WHO classification of MDS/MPN is shown in the fact sheet above. This group of diseases was established to recognize the fact that some disorders share features of the MPNs and MDS but do not fit well into either group. The overlap syndromes consist of chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), atypical chronic myeloid leukemia (aCML), MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), and MDS-MPN unclassifiable.

MYELOPROLIFERATIVE NEOPLASMS

CHRONIC MYELOID LEUKEMIA (CML)

The term *chronic myelogenous leukemia* has been changed to *chronic myeloid leukemia* as per the revised WHO 2016 nomenclature. CML holds a unique place among the hematopoietic diseases and especially among chronic myeloid neoplasms. It is remarkably associated with a long list of firsts. It was the first leukemia described and actually is the disease for which the term *leukemia* (meaning "white blood") was coined. CML was the first disorder found to be associated

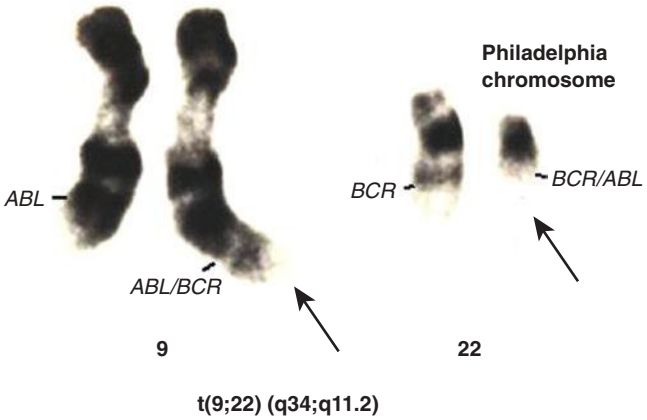


FIG. 17.1

The t(9;22) involving the *ABL1* gene on the long arm of chromosome 9 and the *BCR* gene on the long arm of chromosome 22. The Philadelphia chromosome is the derivative chromosome 22 with the critical fusion gene *BCR-ABL1*.

with a chromosomal abnormality, a smaller than normal G group chromosome that is referred to as the *Philadelphia* (Ph) *chromosome* for the city in which it was recognized. CML was among the first diseases for which a chromosomal abnormality was found to be caused by a reciprocal translocation of genetic material from one chromosome to another. This translocation was the t(9;22)(q34;q11.2), where the derivative chromosome 22, the Ph chromosome, is fused with a portion of the long arm of chromosome 9 (Fig. 17.1). CML was also one of the first diseases in which the chromosomal breakpoints were identified as genes disrupted by the translocation and giving rise to fusion products. These genes are the *ABL1* gene on chromosome 9, the *BCR* gene on chromosome 22, and the critical fusion gene *BCR-ABL1* on the Ph chromosome. CML was also one of the first diseases in which a fusion gene was studied to elucidate the molecular pathogenesis of the disorder, which proved to be increased *ABL1* TK activity of the *BCR-ABL1* gene product. Most spectacularly, CML is the first disease for which an understanding of the underlying molecular pathogenesis has resulted in the development of a drug designed to counteract the molecular abnormality. The success of the drug, the TK inhibitor named imatinib mesylate, has made CML a model for understanding a disease at the molecular level and for developing small molecule therapy to target the abnormal molecular pathway.

CML is notable not just because of the consistent breakthroughs regarding its pathogenesis; it is also considered somewhat unique among the myeloproliferative disorders. It arises in the pluripotent hematopoietic stem cell and essentially affects all the hematopoietic cell lineages, both myeloid (including erythroid and megakaryocytic lineages) and lymphoid (including B, T, and natural killer [NK] cell lineages). It has a chronic phase, which resembles the other myeloproliferative disorders; a blast phase that resembles acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL); and it sometimes has a transitional or accelerated phase, which resembles, to some degree, a myelodysplastic syndrome

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or an MDS/MPN overlap disease. Therefore, CML is a model for hematopoiesis, chronic leukemia, transformation to acute leukemia, and understanding molecular pathogenesis and developing targeted small molecule therapy. CML is also a model for the other MPNs, because it seems that they are also related to the dysregulation of TK signaling.

Clinical Features

Chronic myeloid leukemia is one of the most common leukemias, with an incidence of 12.8 cases per 100,000 persons per year, accounting for approximately 15% of all adult leukemias. The median age is between 46 and 53 years, with a male-to-female ratio of approximately 1.8 : 1. The median age has decreased over the years because of increased incidental diagnosis of early disease and owing to the common use of the routine complete blood cell counts in well-patient examinations. Rare cases can be seen in children.

CML most frequently presents in a chronic phase, and patients are increasingly asymptomatic at diagnosis. When symptoms are present, they include fatigue, lethargy, bleeding, weight loss, and those related to splenomegaly. Less common symptoms include night sweats, bone pain, and symptoms related to hyperviscosity owing to elevated cell counts. Physical findings include pallor, splenomegaly, and occasionally bone tenderness and stigmata of thyrotoxicosis.

CHRONIC MYELOID LEUKEMIA—FACT SHEET

Definition and Other Names

- CML is a chronic myeloid leukemia associated with the *BCR-ABL1* fusion gene, identified at the chromosome level as the Philadelphia (Ph) chromosome or t(9;22)(q34;q11.2). The process has a chronic phase in which the blood and marrow show a prominent proliferation of granulocytes and their precursors, increased small (dwarf) megakaryocytes, and it has accelerated and blast phases
- Chronic myelogenous leukemia, chronic granulocytic leukemia

Incidence, Gender, and Age Distribution

- 12.8 cases per 100,000 population per year, 15% of all adult leukemia
- Median age at diagnosis, 46 to 53 years; occasionally seen in children
- Male:female = 1.8 : 1

Clinical Features

- Frequently asymptomatic
- Symptoms: fatigue, lethargy, bleeding, weight loss, full abdomen
- Physical findings: pallor, splenomegaly, rarely lymphadenopathy

Prognosis and Treatment

Prognosis

- Before imatinib: 4 to 6 years for chronic phase followed by a terminal blast phase
- Since imatinib: prolonged survival, 5-year survival in >70% of patients

Treatment

- TK inhibitor, imatinib (Gleevec)
- Newer, more potent agents (second- and third-generation TK inhibitors); hematopoietic stem cell transplantation for younger patients with matched donor

Pathologic Features

Chronic Phase

The diagnosis of CML requires the evaluation of blood and bone marrow, and ancillary studies, the most important of which is cytogenetic or molecular analysis to identify the Ph chromosome or the *BCR-ABL1* fusion.

Peripheral Blood

Laboratory evaluation plays a critical role in the diagnosis, and the peripheral blood findings are frequently highly suggestive, if not diagnostic in themselves. Patients usually have marked leukocytosis with white blood cell (WBC) count ranging from 20 to 500×10^9/L, with a mean count somewhere between 134 and 225×10^9/L. The peripheral blood smear also shows a characteristic myelocyte bulge, where the myelocytes are greater in percentage than metamyelocytes (Fig. 17.2). This finding is in contrast to the more common reactive granulocytic or leukemoid reaction where there is a progressive decrease in the number of bands, metamyelocytes, myelocytes, promyelocytes, and blasts. Dysplasia in the maturing granulocytic elements is usually absent, and, if present, it is very minimal, and the presence of severe dysplasia should suggest a different diagnosis. Evaluation of the peripheral blood with the leukocyte alkaline phosphate (LAP) test, sometimes referred to as the neutrophil alkaline phosphate (NAP) test, yields a low score. The smear also shows an absolute basophilia in essentially 100% of cases. This finding may be difficult to appreciate because frequently the basophils are slightly hypogranular and not easily recognizable as basophils to the untrained eye (Fig. 17.3). There may also be an eosinophilia, and some of the eosinophils may be immature with basophilic granules; these may resemble the abnormal eosinophils seen in AML with inv(16)/t(16;16) but are usually not as atypical (see Fig. 17.3). Patients frequently have an absolute monocytosis (>1000×10^9/L) owing to a high WBC, but the percentage of monocytes is usually low and less than 3%. Patients also frequently have a moderate normochromic, normocytic anemia, and elevated platelets with counts as high as 1000×10^9/L. This marked thrombocytosis can cause confusion with essential thrombocythemia. Thrombocytopenia is rare, and, if present, another entity should be considered in the differential diagnosis.

Bone Marrow

A bone marrow study is usually performed, and it is important to help exclude other entities and to obtain a

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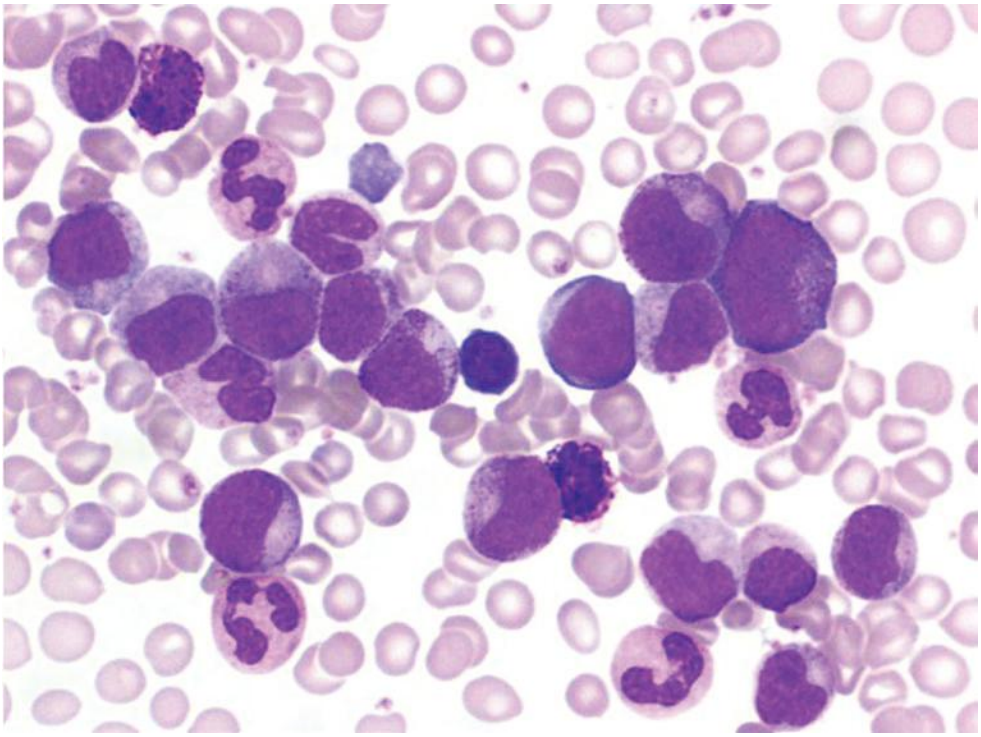


FIG. 17.2

A peripheral blood smear from a typical case of chronic myeloid leukemia in the chronic phase. There is a leukocytosis owing to granulocytes at all stages of maturation. Cells at the myelocyte stage outnumber metamyelocytes resulting in a myelocyte bulge. There is an absolute basophilia and only rare blasts.

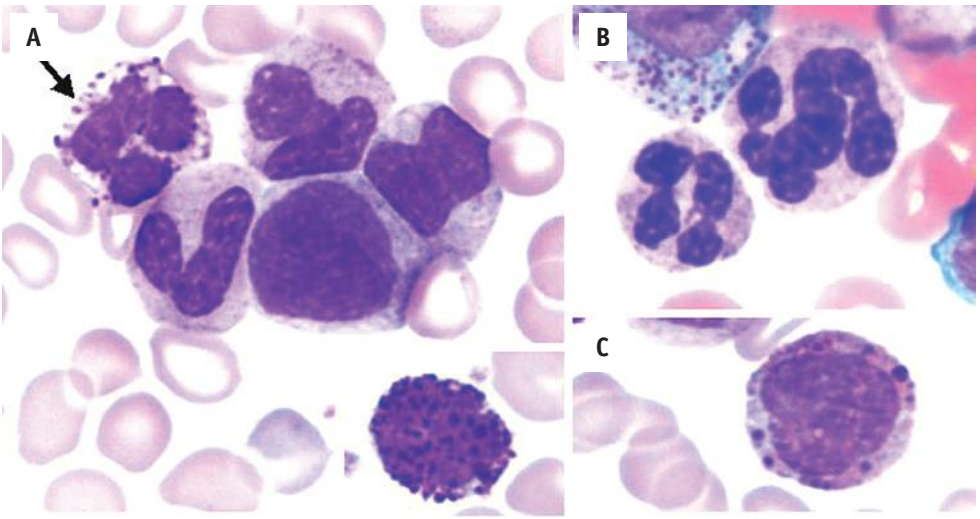


FIG. 17.3

Sometimes the basophils (A) are hypogranular with fewer granules than normal basophils *(inset)*. This can lead to underestimation of basophils, which should be increased in absolute number in all cases. The neutrophils do not show much dysplasia, although some hypersegmentation or nuclear twinning can be seen (B). Some immature eosinophils (C) can resemble the abnormal eosinophils seen in acute myelomonocytic leukemia with abnormal eosinophils, although they are usually less atypical.

specimen for cytogenetic analysis. The marrow is hypercellular, frequently approaching 100%. The marrow shows a marked proliferation of myeloid and megakaryocytic elements, with an elevated myeloid-to-erythroid ratio (≈10:1 to 20:1). Frequently the myeloid elements are expanded along the bony trabeculae, producing an expanded cuff of immature cells (Fig. 17.4). In normal bone marrows, this cuff is approximately three cells thick, but in CML it can be 15 to 20 cells thick. The blast count is low, and the cellular features resemble those seen in the blood with a myelocyte bulge, basophilia, and eosinophilia. Megakaryocytes are frequently increased, although in some cases the megakaryocytic proliferation is not that prominent, whereas in others it is accentuated (Fig. 17.5). The megakaryocytes are characteristically small with hypolobated nuclei, which some refer to as *dwarf megakaryocytes*. They are not large and atypical, nor are they tiny micro-megakaryocytes. This feature is important to recognize, because it helps to distinguish CML from the other MPNs, which have larger than normal megakaryocytes, and from the MDS or MDS/MPNs, in which true micro-megakaryocytes are seen (Fig. 17.6). Numerous micro-megakaryocytes in a suspected case of CML should make one consider another diagnosis. Frequently, in approximately 20% to 40% of cases, the marrow shows histiocytes that resemble Gaucher cells; these are referred to as *pseudo-Gaucher cells*. These cells have the characteristic crumpled tissue paper–like cytoplasm

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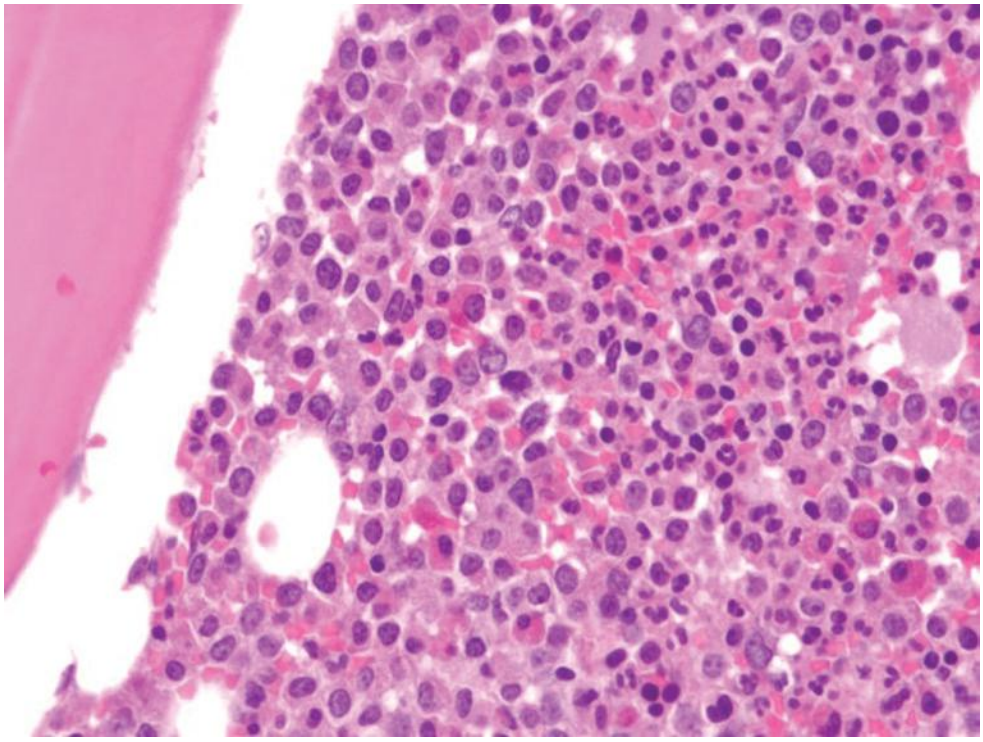


FIG. 17.4

The marrow is hypercellular in chronic myeloid leukemia, and the paratrabecular cuff of immature granulocytic elements is expanded from the normal three to four cells to approximately 15 to 20 cells.

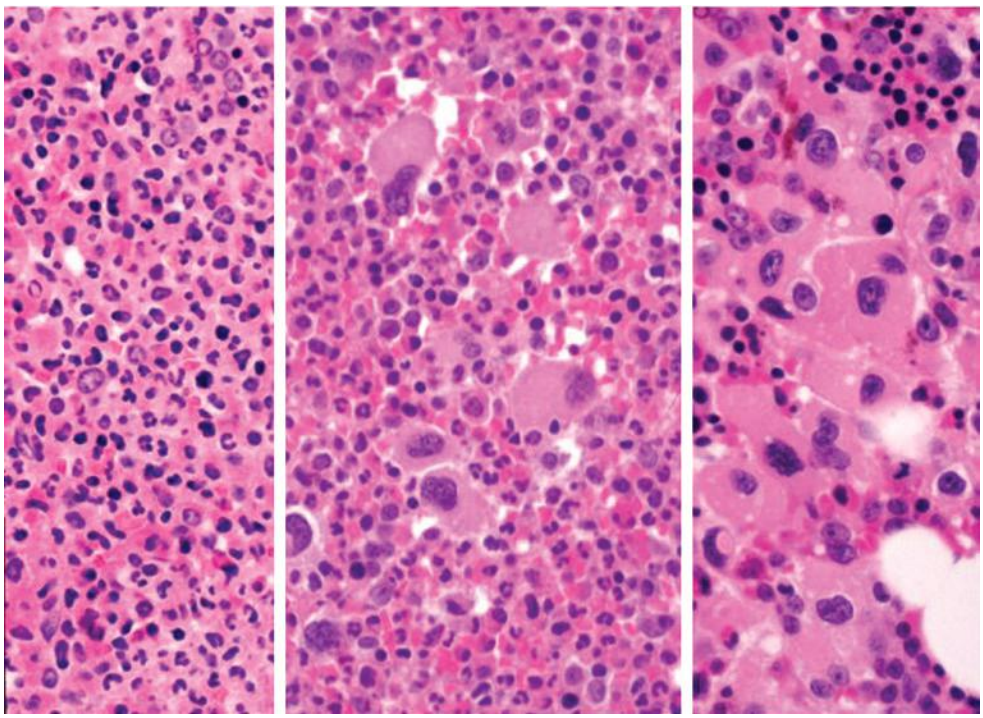


FIG. 17.5

Most cases of chronic myeloid leukemia show a granulocytic and megakaryocytic proliferation in the bone marrow *(center),* whereas rare cases have a granulocytic *(left)* or megakaryocytic *(right)* predominance.

and frequently show hemophagocytosis (Fig. 17.7). The presence of these cells is not diagnostic of CML as they can be seen in any of number of hematologic disorders. However, in CML they are derived from the neoplastic clone, as they have been shown to be *BCR-ABL1*$^{+}$ through fluorescence in situ hybridization (FISH) analysis.

Ancillary Studies

As mentioned previously, the LAP or NAP score is usually below the normal range. Although not commonly used, this screening method is helpful but not absolute, because CML

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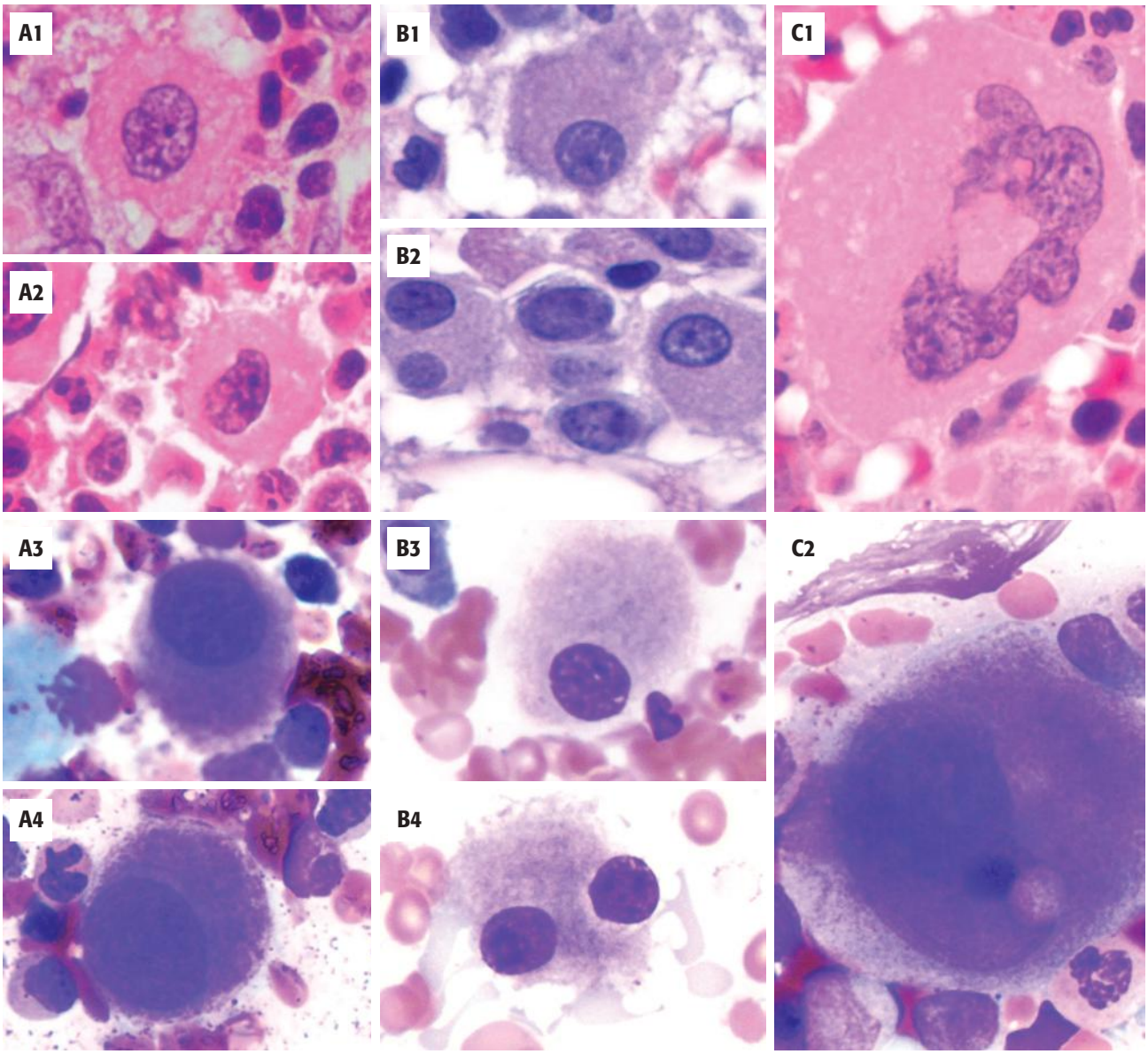


FIG. 17.6

Comparison of megakaryocytes in chronic myeloid leukemia (CML), myelodysplastic syndrome (MDS), and myeloproliferative neoplasm (MPN), *BCR-ABL1*-negative. CML megakaryocytes (from biopsy sections A1, A2, or from aspirate smears A3, A4) are considered "dwarf" forms that are small but not as small as the tiny micro-megakaryocytes seen in MDS (from biopsy sections B1, B2, or on aspirate smears B3, B4). The "dwarf" megakaryocytes in CML (A1, A2, and A3, A4) are also quite distinctive from the huge megakaryocytes seen in the other MPNs (from biopsy section C1 or from aspirate smear C2).

in the accelerated phase can show increased scores. Indeed, the LAP score is being replaced by more specific molecular testing for CML and other MPNs and is being discontinued by many laboratories. Vitamin B_{12} is increased by 10- to 20-fold the normal range, and uric acid is usually elevated.

Although the diagnosis of CML usually can be made with a great degree of certainty from the features in the blood and marrow, confirmation requires the demonstration of the characteristic t(9;22) (or variant) or the associated *BCR-ABL1* fusion gene. This demonstration can be accomplished by conventional cytogenetic analysis, by FISH with probes to *BCR* and *ABL1,* or by polymerase chain reaction (PCR). The t(9;22) is seen in its characteristic form in greater than 95% of cases. In a small number of cases, however, there is a variant translocation involving the 9q34, the 22q11.2, and another involved chromosome (e.g., t[9;14;22]). In slightly less than 5% of cases, there is submicroscopic translocation, which cannot be identified by conventional cytogenetics, and the karyotype appears normal. However, with FISH probes or with specific PCR primers to identify the variant fusion gene, the underlying *BCR-ABL1* can be recognized. These cases are called *Ph-negative CML* and should probably be referred to as *Ph-negative, BCR-ABL1–positive CML* for clarity.

It is important to recognize that the *BCR* gene can be broken into three different regions, giving rise to three different *BCR-ABL1* proteins of different size. Almost all cases of CML are associated with the major breakpoint region, at exon 12-16 (formerly referred to as exon b1-b5) and fusion

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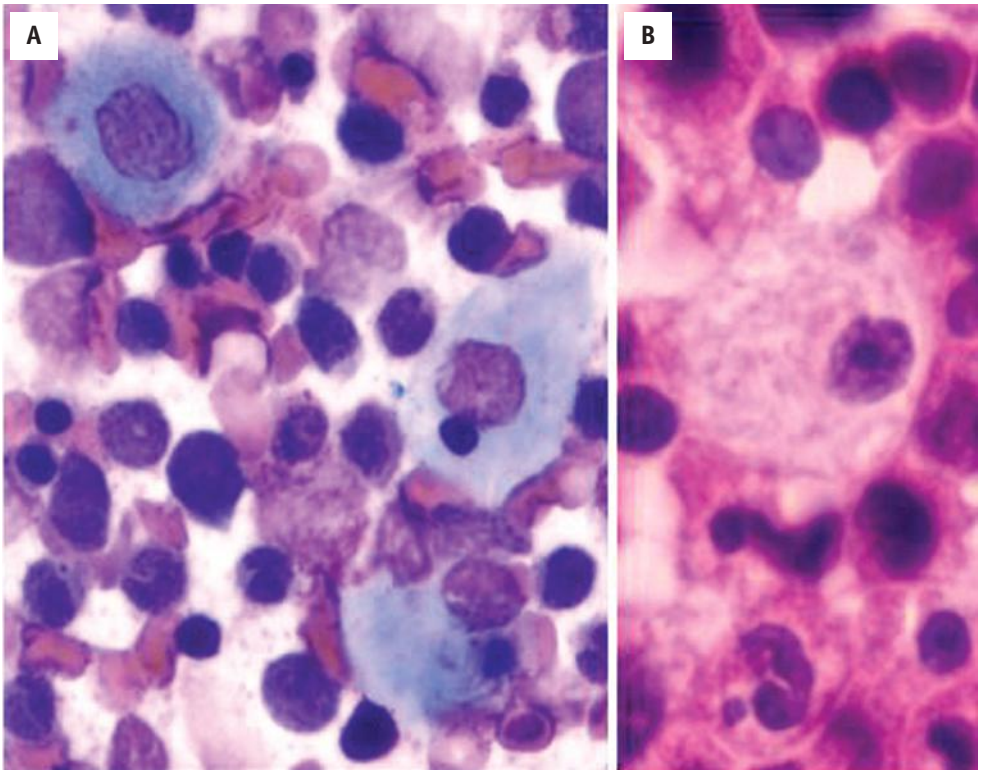


FIG. 17.7

Up to 40% of cases of chronic myeloid leukemia (CML) can show pseudo–Gaucher cells in the aspirate (A) and on the biopsy (B). Sometimes they can be seen undergoing phagocytosis. Although distinctive, they are not specific for CML as they can be seen in other entities.

to the *ABL1* at its exon 2. This fusion is referred to as the major *BCR-ABL1* or fusion associated with the *p210 kilodalton (kd) BCR-ABL1* protein. Rare cases of CML can have a fusion involving the first exon of *BCR*, e1-e2, and this is referred to as the fusion associated with the minor breakpoint and a smaller fusion protein with 190-kd size (p190). This fusion is far more common in Ph+ ALL, but when associated with CML is associated with increased monocytes, which makes it difficult to differentiate from CMML. Last, rare cases of CML can have a fusion of *BCR* involving the regions around exons 17 to 20 (previously referred to as c1-c4) resulting in a μ breakpoint or a larger protein with 230-kd weight (p230). This fusion is also rare but may be associated with CML that has markedly increased platelets or CML with a predominance of mature neutrophils (CML-N). These can mimic either ET or CNL, respectively.

As will be discussed in the latter part of the chapter that *CALR* mutations have primarily been reported in ET and PMF and are exceedingly rare in the setting of CML with two case reports described in the literature. In both these reports of atypical myeloproliferative neoplasms, *CALR* mutation proceeded *BCR-ABL1* fusion. However, the bone marrow morphologic features simulating primary myelofibrosis (*CALR* mutation positive) became apparent only after the Philadelphia-positive clone was eradicated with dasatinib therapy.

Accelerated Phase

Chronic myeloid leukemia sometimes progresses to a blast phase first through an accelerated phase. The accelerated phase is associated with worsening overall performance, fever, night sweats, weight loss, bone pain, progressive splenomegaly, and loss of responsiveness to therapy. Although different criteria had been used to diagnose the accelerated phase, the WHO committee writing on hematologic disorders developed a list of six features, any of which would indicate an accelerated phase. These features include peripheral blood or bone marrow blasts accounting for 10% to 19% of the cells (Fig. 17.8), persistent thrombocytopenia ($<100 \times 10^9$/L) unrelated to therapy or thrombocytosis ($>1000 \times 10^9$/L) unresponsive to therapy, persistent or increasing WBC ($>10 \times 10^9$/L), and spleen size unresponsive to therapy, basophilia greater than 20% (Fig. 17.9), and evidence of clonal cytogenetic evolution (see details under criteria for the accelerated phase). Dysplasia and increased fibrosis are frequently seen in the accelerated phase but in themselves are not considered sufficient for a diagnosis according to the WHO guidelines.

Although almost any additional chromosomal change can be seen in the accelerated phase, the most common changes include an extra Ph chromosome (+Ph or +der[22]), +8, i(17q), and +18. These abnormalities are seen singly or in combination in 81% of cases showing cytogenetic evolution. Other more common abnormalities include −7, −17, +17, +21, and −Y.

Blast Phase

Blast phase, or blast crisis, occurs in virtually all patients with untreated CML. In the era of imatinib and other TK inhibitors it occurs much less frequently, sometimes either following an accelerated phase or suddenly without warning.

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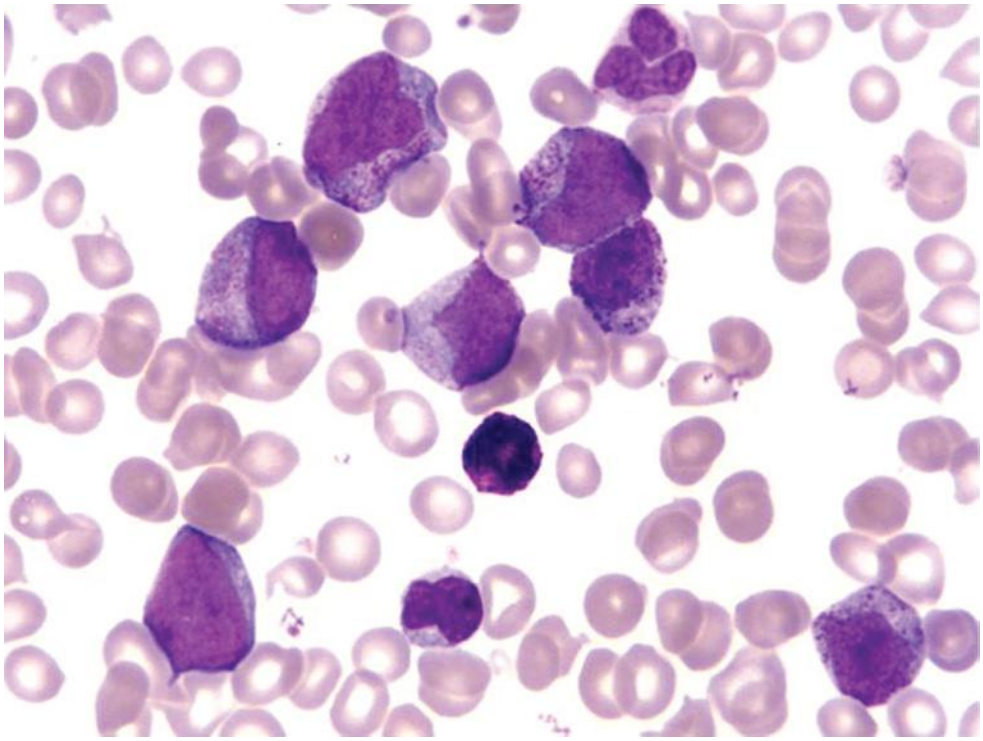


FIG. 17.8
Chronic myeloid leukemia in the accelerated phase showing more prominent left shift with increased immature cells. This patient had greater than 10% circulating blasts.

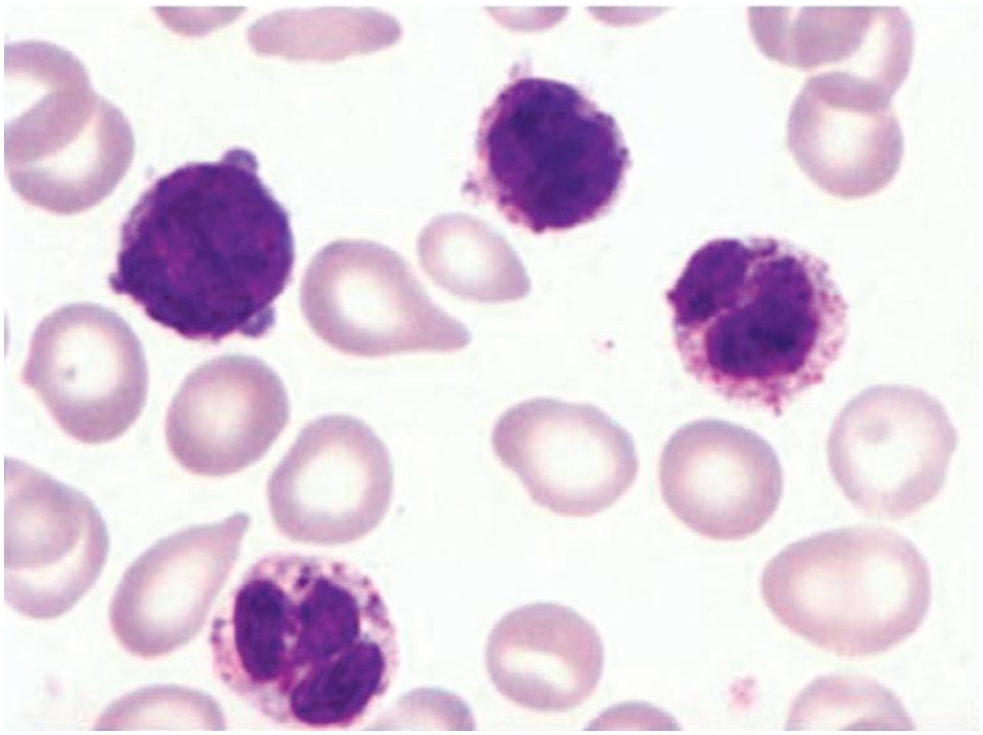


FIG. 17.9
Chronic myeloid leukemia in the accelerated phase. This patient had greater than 20% circulating basophils and also increased blasts.

Blast phase resembles an acute leukemia. Because the CML clone originates in the pluripotent stem cell, blast phase can occur in the myeloid series or the lymphoid series, or it can be biphenotypic or bilineal. Blast phase is diagnosed when there are 20% or more blasts in the blood or marrow, but sometimes it is seen only focally on the biopsy as sheets of blasts (focal intramedullary blast transformation; Fig. 17.10). Although most of the time blast phase is diagnosed from the blood and/or marrow, in some instances it can occur at an extramedullary site. In fact, the development of a mass lesion in a patient with CML should always warrant investigation with a biopsy.

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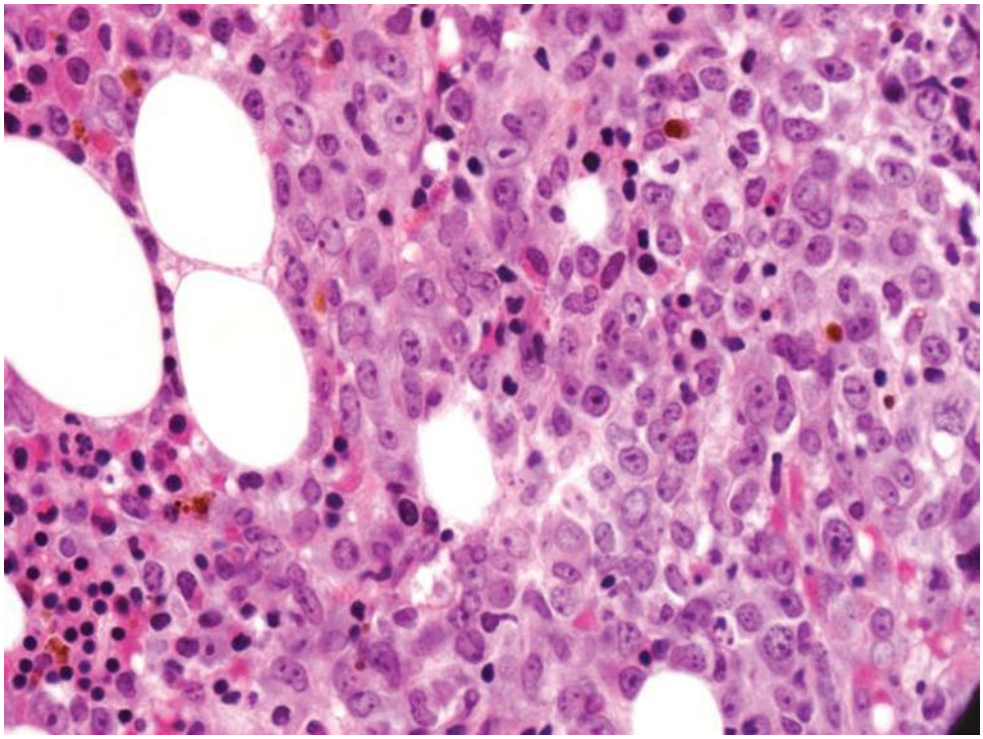


FIG. 17.10
Focal blast phase. Sometimes the blast phase in chronic myeloid leukemia is focal and only recognized in a localized area on the biopsy.

Myeloid Blast Phase

Blast phase is of a myeloid type in approximately 50% to 60% of cases. Before TK inhibitor therapy, it commonly occurred following an accelerated phase and was seen more frequently in older patients with higher blood counts, more severe anemia, and larger spleens. Although blast phase responds to TK inhibitor therapy, it still is refractory to therapy and has a poor survival. The myeloid blast crisis of CML is heterogeneous. In some cases it can resemble a de novo AML without maturation, with maturation, with a monocytic component, or it can even resemble erythroleukemia or megakaryoblastic leukemia (Fig. 17.11). In rare cases, the blast phase can have a t(8;21), inv(16)/t(16;16), or t(15;17) cytogenetic abnormality usually associated with de novo AML. In these latter types, the blast phase component is morphologically and immunophenotypically identical to the de novo leukemia associated with these recurring chromosomal abnormalities (Fig. 17.12). More frequently the myeloid blast phase can be of a mixed myeloid type in which the blastic elements include myeloblasts, monoblasts, megakaryoblasts, and immature basophils (Fig. 17.13). This type of blast phase is distinctive morphologically and does not have a well-known de novo AML counterpart, although a mixed myeloid blast population can be seen in some AMLs arising from MDS.

Lymphoid Blast Phase

Before the use of TK inhibitor therapy, lymphoid blast phase accounted for 16% to 30% of cases of blast crisis and was more uniform morphologically and immunophenotypically than myeloid blast crisis. Clinically, lymphoid blast phase occurs in younger patients with lower counts and less splenomegaly than in patients with myeloid blast phase. Interestingly, the lymphoid blast phase occurs abruptly and is not associated with a preceding accelerated phase. Thus, there is usually no gradual increase in lymphoblasts of lymphoid blast phase. Morphologically the lymphoid blast phase shares features with ALL, although the background of CML is frequently still evident (Fig. 17.14). Most cases are of B lymphoblasts, and, less commonly, the blast phase can be of a precursor T-cell type. Although lymphoid blast phase also responds to second- and third-generation TK inhibitors and other chemotherapeutic agents, the overall survival is still poor, and patients are frequently taken to stem cell transplant.

Bilineal or Biphenotypic Blast Phase

In some cases the blasts in blast phase can be a mixture of lymphoblasts and myeloblasts. These bilineal processes may be associated with two distinct cytogenetic clones that have evolved separately from the Ph^+ clone. The clones likely represent separate lymphoid and myeloid blast phases occurring simultaneously. In the biphenotypic blast phase, the blasts show lymphoid and myeloid markers simultaneously on the same blasts. These blasts may be precursor B/myeloid or precursor T/myeloid. This type of blast phase requires the same diagnostic criteria as de novo acute leukemia with mixed phenotype.

Extramedullary Blast Phase

In approximately 5% to 10% of cases, blast phase can manifest at extramedullary sites; therefore, the development of a mass lesion in a patient with CML should prompt appropriate evaluation. The most common sites of

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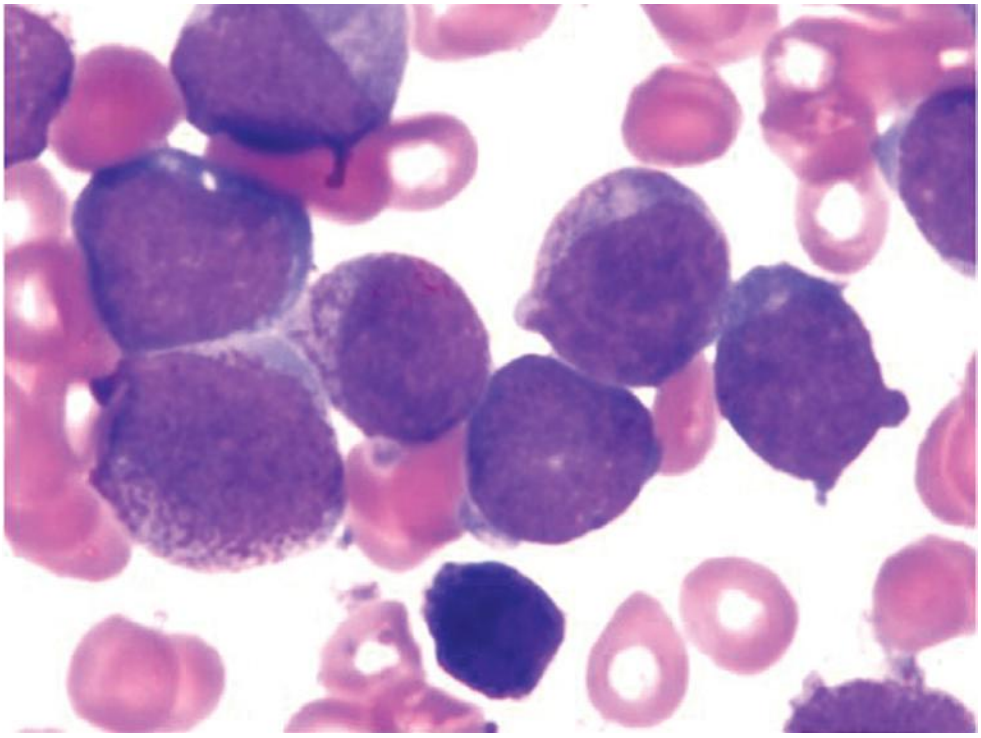


FIG. 17.11
Chronic myeloid leukemia in the myeloid blast phase. This case resembles acute myeloid leukemia without maturation.

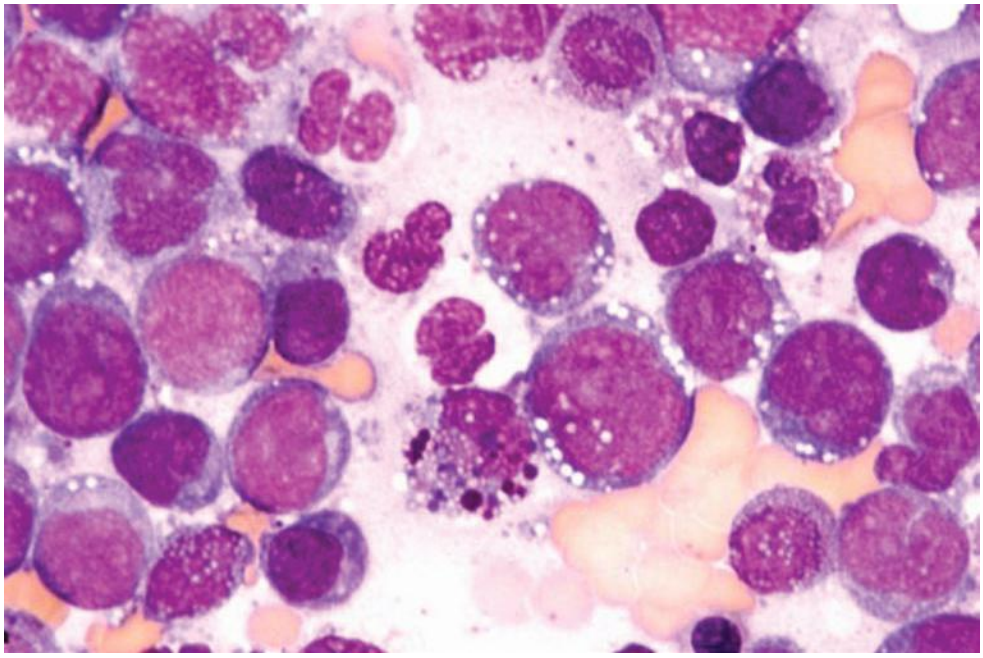


FIG. 17.12
Chronic myeloid leukemia in the myeloid blast phase. This patient had t(9;22) and inv(16) at the blast phase. Myelomonocytic blasts are seen along with an abnormal eosinophil (slightly below center).

extramedullary blast phase include lymph node, soft tissue, and the central nervous system (CNS). Extramedullary disease is usually of the myeloid type, but not always. Bone marrow involvement can be simultaneous; if not, it usually develops in a short time after the extramedullary presentation.

Differential Diagnosis

A discussion of differential diagnosis in CML needs to take into account the stage at presentation. Although most patients present in chronic phase, some initially present in blast phase

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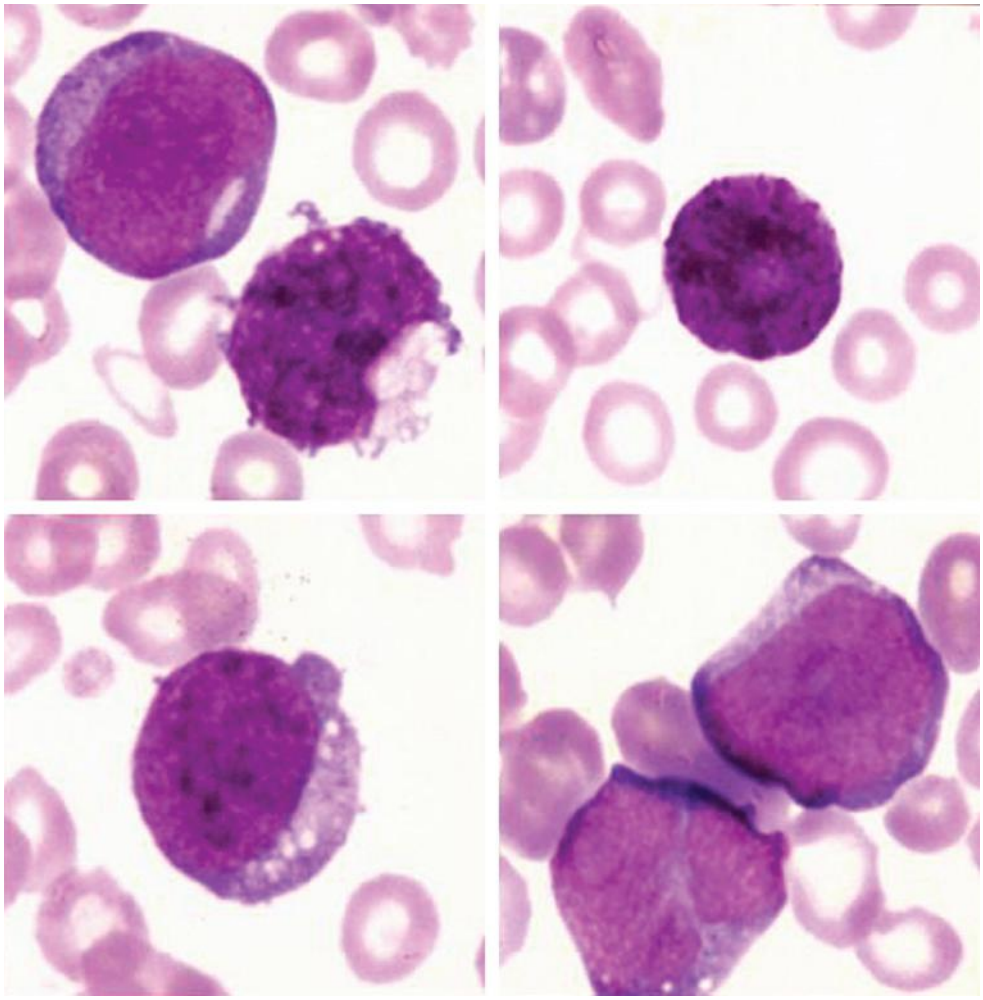


FIG. 17.13

Chronic myeloid leukemia in myeloid blast phase. The myeloid blasts are heterogeneous. Some resemble megakaryoblasts, myeloblasts, monoblasts, or immature basophilic cells.

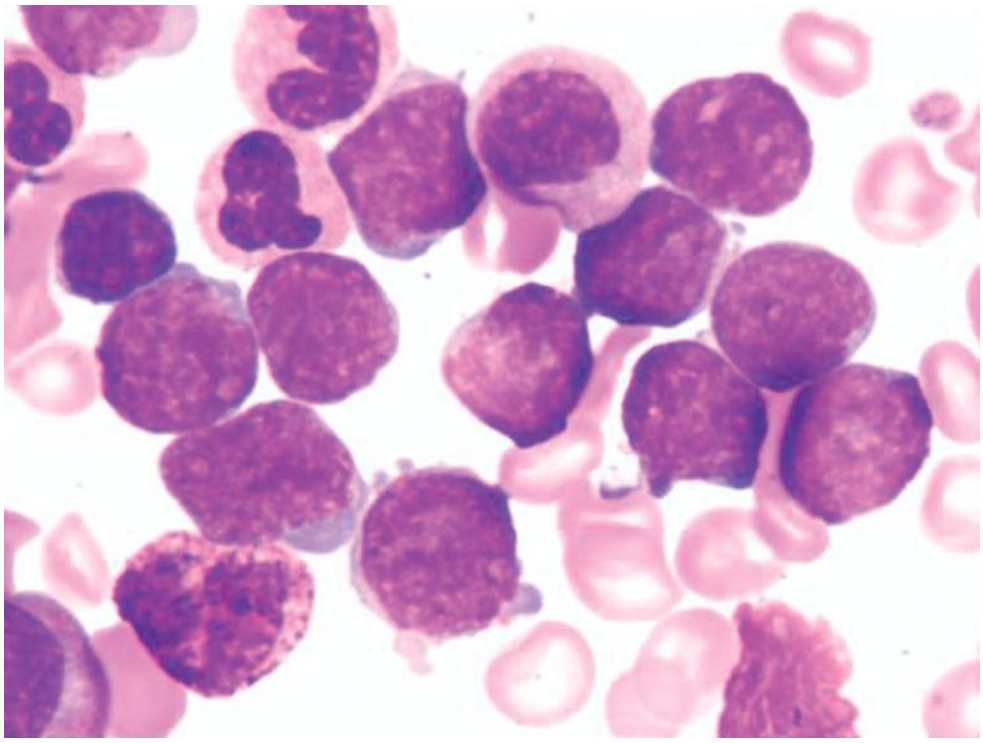


FIG. 17.14

Chronic myeloid leukemia in lymphoid blast phase. Lymphoblasts are present with some residual granulocytic elements of the chronic myeloid leukemia.

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CHRONIC MYELOID LEUKEMIA—PATHOLOGIC FEATURES

Microscopic Features

Blood

- Leukocytosis with granulocytes at all stages of maturation
- Myelocyte bulge, blasts usually <1% to 2%
- Absolute basophilia (100% of cases), absolute eosinophilia (80% of cases)
- Absolute monocytosis common, but monocytes <10%, usually <3% of leukocytes
- Thrombocytosis common, can be prominent; thrombocytopenia very rare

Bone marrow

- Hypercellular due to granulocytic and megakaryocytic proliferation
- M:E ratio: 10–20 : 1
- Widened paratrabecular cuff of immature granulocytes
- Basophilia, blasts usually <10%
- Small hypolobated (dwarf) megakaryocytes
- Mild reticulin fibrosis
- Pseudo-Gaucher histiocytes (in ≈ 20% to 40% of cases)

Ancillary Studies

Peripheral blood

- LAP (NAP) score low; vitamin B_{12} increased

Bone marrow

- Cytogenetic analysis: t(9;22) or variant (>95% of cases)
- Molecular: *BCR-ABL1*$^+$ by FISH or PCR (100% of cases)—may be done on peripheral blood
- Most cases: major *BCR-ABL1*, (e13 or14/a2 or 3), p210 protein
- Rare cases: minor *BCR-ABL1*, (e1/a2 or 3), p190 protein
- Rare cases: mu *BCR-ABL1*, (e19/a2 or 3), p230 protein

- Differential diagnosis
  - Leukemoid reaction
  - CMML
  - Atypical CML (Ph-neg, *BCR-ABL1*-neg)
  - Chronic neutrophilic leukemia or ET

Accelerated Phase Criteria (WHO 2016)

- Any 1 or more of the following hematologic or cytogenetic criteria or response-to-TKI therapy criteria

Hematologic or Cytogenetic Criteria

- 10% to 19% blasts in the PB and/or BM
- 20% or more basophils in the PB
- Persistent thrombocytopenia (<100 × 10^9/L) unrelated to therapy
- Additional clonal chromosomal abnormalities in Ph positive cells at diagnosis that include "major route" abnormalities (second Ph, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype, or abnormalities of 3q26.2
- Persistent or increasing WBC (>10 × 10^9/L), unresponsive to therapy
- Persistent or increasing splenomegaly, unresponsive to therapy
- Persistent thrombocytosis (>1000 × 10^9/L), unresponsive to therapy
- Any new clonal chromosomal abnormality in Ph positive cells that occurs during therapy

"Provisional" Response-to-TKI Criteria

- Hematologic resistance to the first TKI (or failure to achieve a complete hematologic response[a] to the first TKI)
- Any hematologic, cytogenetic, or molecular indications of resistance to 2 sequential TKIs
- Occurrence of 2 or more mutations in *BCR-ABL1* during TKI therapy

Blast phase

- 20% or more blasts in blood or bone marrow
- Myeloid blast phase: 50% to 60% of cases
- Lymphoid blast phase: 16% to 30% of cases
- Bilineal or biphenotypic blast phase: rare
- Extramedullary blast phase: rare; lymph node, soft tissue, central nervous system
- Differential diagnosis
- *De novo* Ph+ acute myeloid leukemia
- *De novo* Ph+ B-ALL

[a]Complete hematologic response = WBC <10^9/L, platelets <450 × 10^9/L, no immature granulocytes in the differential, no palpable splenomegaly.

or accelerated phase, and the entities considered in the differential diagnosis differ widely among these. Key to establishing the differential diagnosis is the evaluation for t(9;22) or *BCR-ABL1*, or both. CML must be shown to have the t(9;22), a variant translocation, or the *BCR-ABL1* by FISH or by molecular techniques.

Differential Diagnosis of Chronic Phase

Included in the differential diagnosis of chronic phase CML are a leukemoid reaction, CMML, atypical CML, and CNL.

A leukemoid reaction is a normal response to infection or another disease process that resembles leukemia with high leukocyte count in the blood. In some cases a leukemoid reaction can have counts as high as 30 to 100 × 10^9/L. Although in a leukemoid reaction the granulocytes can show a significant left shift with circulating metamyelocytes, myelocytes, promyelocytes, and even blasts, the factors that help to distinguish it from CML include the lack of a myelocyte bulge, the presence of toxic granulation and Döhle bodies in the neutrophils, and the lack of absolute basophilia. In addition, there is usually a markedly elevated (not decreased) LAP score. However, identifying a cause of the underlying reactive granulocytosis is most helpful in considering a leukemoid reaction over CML.

Chronic myelomonocytic leukemia is discussed in the overlap syndromes section. It figures prominently in the differential diagnosis of CML. Other than lacking the t(9;22) and *BCR-ABL1*, the key features that help to distinguish it from CML are the increased percentage (>10%) and the absolute number of monocytes (>1 × 10^9/L), the presence of dysplasia involving at least one of the three lineages, absent or fewer circulating granulocytic precursors, and fewer basophils.

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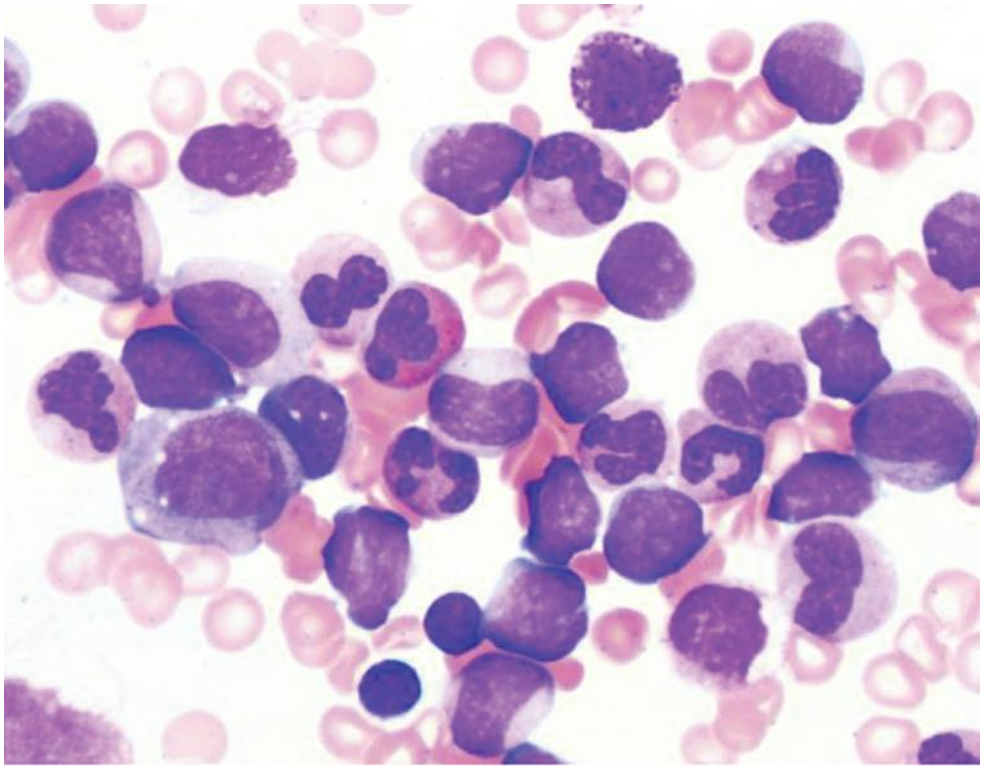


FIG. 17.15

Chronic myeloid leukemia (CML) initially presenting in the lymphoid blast phase. This patient had a WBC count of 400 × 10^9/L. Of the cells, 40% were lymphoblasts with a precursor B phenotype, but there was a significant granulocytic proliferation with left shift and basophilia, indicating CML in the lymphoid blast phase. The Ph+ chromosome and transcripts for the p210 *BCR-ABL1* were identified.

Atypical CML (aCML) is also discussed in the overlap syndromes section. It can be distinguished from CML by the presence of marked dysplasia in the granulocytic, megakaryocytic, and erythroid series; by the presence of thrombocytopenia; and, of course, by the lack of t(9;22) and *BCR-ABL1*. Interestingly, a subset of cases of aCML or CMML has isochromosome 17 as a sole abnormality.

Chronic neutrophilic leukemia, a rare disorder, is discussed later. It must be considered in the differential diagnosis, especially in cases of CML with prominent neutrophilia. Although CML-N is also rare, evaluation for t(9;22) associated with the μ breakpoint (p230) would be necessary to distinguish it from CNL, as this breakpoint can be seen in CML-N.

Essential Thrombocythemia

Rare cases of CML can present with marked thrombocytosis and mimic ET. Part of the diagnostic criteria for ET requires excluding the presence of the *BCR-ABL1* fusion.

Differential Diagnosis of Accelerated Phase and Blast Phase

Chronic myeloid leukemia does not commonly manifest initially in accelerated phase; in the rare case in which it does, the differential considerations would include entities in the MDS/MPN category as well as some of the MPNs, particularly PMF. Morphologic distinction from CML might be difficult, because the finding of dysplasia characteristic of MDS and MDS/MPN can also be seen in accelerated phase of CML. Cytogenetic and molecular studies are key to the correct diagnosis.

Patients with CML can initially present in the lymphoid blast phase. In some, the chronic phase may have gone unnoticed, but in others there may not have been a chronic phase at all. In either of these cases, the patient usually exhibits leukocytosis with lymphoblasts and the background typical of CML (Fig. 17.15). In other cases in which there is no recognizable CML in the background of the blastic process, the diagnosis can be made only after treatment because many patients revert to a chronic phase after therapy. Identification of t(9;22) or *BCR-ABL1* does not necessarily help in the initial evaluation because ALL can be t(9;22) and *BCR-ABL1* positive. If a minor *BCR-ABL1* (p190) is present, then CML would be unlikely, but if the major *BCR-ABL1* (p210) is seen, either Ph+ ALL or CML presenting in lymphoid blast phase is possible. Identification of the *BCR-ABL1* specifically in the granulocytic, erythroid, or megakaryocytic components might be helpful to distinguish between these, because CML is a stem cell disorder involving all cell lineages, whereas Ph+ ALL is believed to be a lymphoid-restricted process.

Rarely patients present with an AML that is shown to be t(9;22) positive or *BCR-ABL1* positive. Many of the patients have an aggressive course with no reversion to chronic phase CML after therapy. Whether these are truly Ph+ AML or just CML presenting in the myeloid blasts phase is difficult if not impossible to determine. Currently, Ph+ AML is considered as a provisional category per WHO 2016. Some of the features that are suggested in the literature can help to differentiate between the two entities is highlighted in Table 17.1. In addition, in some patients the blastic proliferation is a mixed population of myeloblasts, monoblasts, erythroblasts, and megakaryoblasts similar to the mixed-myeloid blast phase of

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TABLE 17.1
Clinicopathologic Features Distinguishing Ph+ Acute Myeloid Leukemia[a]

Features	Ph+ acute myeloid leukemia[a]	Myeloid blast phase of chronic myeloid leukemia (CML)
Presentation	Lack of history of chronic phase of CML, acute presentation, lack of splenomegaly	Previous history of chronic phase of CML, presence of splenomegaly
Peripheral blood and BM basophilia	Absent	Present
Bone marrow findings	Usually lower cellularity; decreased myeloid-to-erythroid (M:E) ratio; presence of dwarf megakaryocytes is uncommon	Usually higher cellularity; increased M:E ratio; presence of dwarf megakaryocytes
Cytogenetic findings	Additional chromosomal abnormalities less common (25% to 59.9%) Deletion 7q/-7 abnormality is seen in a few studies	Additional chromosomal abnormalities more common (60% to 80%) including extra Ph chromosome, trisomy 8, trisomy 19, or isochromosome 17q
Molecular findings	*NPM1* mutation in 25% of cases No *ABL1* mutation Deletion of antigen receptor genes (*IGH, TCR*), *IKZF1* and/or *CDKN2A*	Usually absent *ABL1* mutations are common No *IGH* and *TCR* gene deletion or antigen deletions present

[a]Provisional category as per WHO 2016 from myeloid blast phase of chronic myeloid leukemia.

CML. This clue can be a strong indication for CML myeloid blast phase rather de novo Ph+ AML. Other patients may have t(9:22) in addition to a common recurring cytogenetic abnormality in AML, such as inv(16) or t(8;21), whereas other patients will exhibit a mixed or bilineal acute leukemia. Some reports have noted increased incidence of the p190 *BCR/ABL1* in cases presenting as AML or mixed lineage leukemia.

Prognosis and Therapy (Box 17.1)

The development of imatinib (Gleevec) in the late 1990s has revolutionized the treatment of CML and provides successful management for most CML cases. After trials of imatinib—first in patients who failed interferon therapy, then in patients with accelerated or blast phase, and finally in an up-front comparison to interferon—imatinib has become the treatment of choice because of its superiority to other therapies. A controversy that still exists is in the treatment of young patients with a suitable stem cell donor and whether first to treat with imatinib or whether to transplant immediately. Currently, three tyrosine kinase inhibitors (imatinib, nilotinib, and dasatinib) are approved by the U.S. Food and Drug Administration for initial treatment of chronic-phase CML. Box 17.1 summarizes the National Comprehensive Cancer Network (NCCN) 2015 guidelines that define complete hematologic, cytogenetic, and molecular response in CML at 3 months, 12 months, and 18 months, respectively. Results with nilotinib and dasatinib as frontline therapy in single-institution trials at MD Anderson Cancer Center have shown very high rates of complete cytogenetic response (CCyR) and major molecular response (MMR), which are superior to those in historical populations treated with standard-dose imatinib.

Box 17.1 Definition of Response Rate in CML (Based on NCCN Guidelines Version 1.2015)

Complete Hematologic Remission
- WBC <10 × 10^9/L; basophils <5%; platelets <450 × 10^9/L; lack of immature granulocytes; non-palpable spleen (achieved at 3 months of start of therapy)

Partial Cytogenetic Remission
- 1% to 35% Ph+ metaphases in bone marrow (achieved at 3 months of start of therapy)

Complete Cytogenetic Remission
- 0% Ph+ metaphases in bone marrow (achieved at 12 months of start of therapy)

Major Molecular Response
- *BCR-ABL1* international scale ≤0.1% (achieved at 12 months of start of therapy)

Complete Molecular Response
- Undetectable *BCR-ABL1* (achieved at 12 months of start of therapy)

Imatinib inhibits the constitutive phosphorylation activity of the *BCR-ABL1* TK. The drug sits in a pocket of the *ABL1* portion of the *BCR-ABL1* fusion protein and blocks the adenosine triphosphate from binding. Usually after

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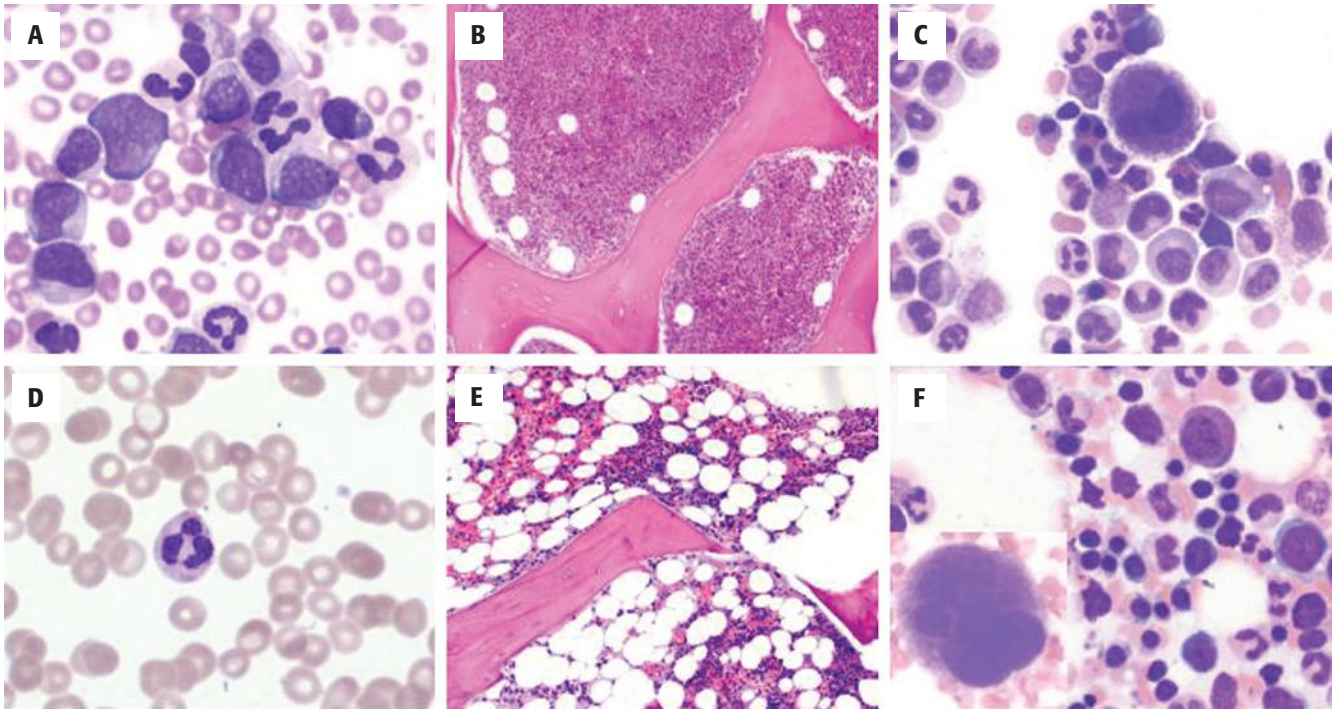


FIG. 17.16

A comparison of before (A-C) and after (D-F) 3 months of imatinib therapy. There is a reduction in the number of circulating granulocytes (A and D), and bone marrow cellularity (B and E) and a reversion to normal megakaryocyte size (C and F) in the post-therapy specimen.

approximately 3 months of therapy there is normalization of blood counts, reduction of bone marrow cellularity with correction of the myeloid-to-erythroid (M:E) ratio, and normalization of megakaryocytes (Fig. 17.16). Frequently there are lymphoid aggregates composed of mostly small lymphocytes, which are a mixture of B and T cells (Fig. 17.17). These lymphocytes are reactive in nature. At the 3-month mark, many patients achieve a complete cytogenetic remission, but a molecular remission by PCR analysis for *BCR-ABL1* is relatively uncommon, occurring in only 10% to 15% of all patients. Resistance to imatinib can occur because of mutations, overexpression of *BCR-ABL1,* or reduced cellular uptake of the drug. Although newer drugs with more powerful TK inhibitor activity are being used, these still have little effect in cases in which clonal evolution resulted in a stimulation of leukemogenic pathways that are independent of the *BCR-ABL1*–associated constitutive activity of TK. The presence of *T315I ABL1* kinase domain mutation shows a poor response to all TKIs except the third-generation TK inhibitor ponatinib. Currently, mutational testing is not recommended at the start of treatment for patients in chronic phase, as they are present at very low allele frequencies that may not be clinically pertinent. However, the *T315I ABL1* kinase domain mutation testing is recommended in patients in accelerated or blast phase or in those who fail first-line TK inhibitor therapy.

The development of clonal chromosomal abnormalities in *BCR-ABL1*–negative (Ph negative) cells can occur in patients undergoing TK inhibitor therapy. Studies suggest that the incidence of these abnormalities is low (3% to 9%), some are transient in nature, and there usually is no adverse clinical consequence in the majority of patients on imatinib therapy. The median interval from starting TK inhibitor therapy to emergence of clonal chromosomal abnormality is about 12 to 18 months. The most common cytogenetic abnormality includes loss of chromosome Y, trisomy 8, and monosomy 7. Balanced translocations, structural abnormalities, and a complex karyotype are also reported. The emergence of clonal karyotypic abnormalities usually does not affect patient response to TK inhibitor therapy, as confirmed by molecular and cytogenetic studies. Although the majority of these clonal karyotypic abnormalities are not associated with MDS or AML and have no clinical consequences, a small number of patients may develop MDS and AML at follow-up. The latter is often associated with a complex karyotype, inv(3) or monosomy 7. Thus, to summarize, the presence of clonal chromosomal abnormalities can occur in patients with CML on TK inhibitor therapy, but only rarely can these clonal abnormalities indicate an underlying or impending MDS/AML; thus, close follow-up of peripheral blood counts and monitoring of cytogenetic clone on follow-up bone marrow specimens may be helpful.

Successful therapy has now led to the idea that TK inhibitor therapy may at some point be discontinued, and monitoring for minimal residual disease at levels lower than that defined for a major molecular response (*BCR-ABL1* international scale ≤0.1%) will be required (levels <0.01%, so-called deep molecular response). However, further studies will be required to better define criteria for discontinuing or restarting TK inhibitor therapy.

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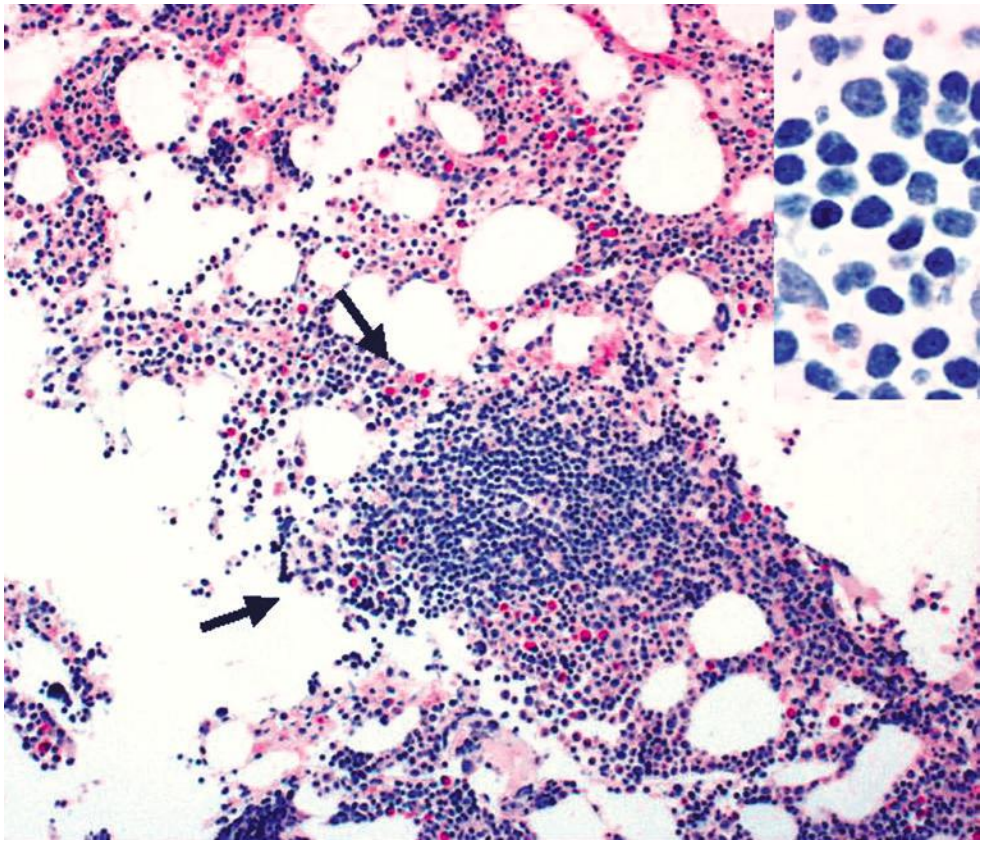


FIG. 17.17
Atypical lymphoid infiltrate seen after imatinib therapy. The infiltrate is composed of mostly small lymphocytes, which on immunophenotyping were a mixture of B and T cells.

BCR-ABL–NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

POLYCYTHEMIA VERA

Polycythemia vera is a myeloproliferative neoplasm that is characterized by a proliferation of erythroid cells that leads to marked erythrocytosis in the blood. As a stem cell disease, PV can involve other myeloid elements and, in some instances, might involve the lymphoid lineage as well. The finding of a mutation in tyrosine kinase *JAK2* in a large percentage of cases of PV, as well as in lesser numbers of PMF and ET, has linked PV and the other common MPNs as a group of diseases driven by dysregulated TK signaling.

In PV, the *JAK2* mutation at V617F occurs in approximately 95% of cases, and mutations in exon 12 occur in another 3% to 4%. These findings have greatly simplified the diagnostic workup for PV. Thus, the finding of a *JAK2* mutation rules out a reactive process for erythrocytosis.

PV has two distinct phases that include the polycythemic phase and the so-called spent phase or post-polycythemic myelofibrotic phase, which is a terminal event. There is likely a pre-polycythemic phase as well, although this is difficult to recognize. Some patients with PV develop dysplasia and cytopenia(s) or progress to AML. The progression of PV is more frequent (3% to 5%) in patients treated previously with cytotoxic therapy administered for PV than in patients treated with phlebotomy alone (1% to 2%). Whether some cases might represent a therapy-related process is difficult to prove.

Clinical Features

The incidence of PV is approximately 1 to 3 per 100,000 persons per year. The incidence increases with age, with the median age at diagnosis being 60 years. There is a slight male predominance and an increased incidence in Ashkenazi Jews. Rare familial cases have been described, but the genetic basis for most of these is not known. Some familial cases have been shown to be associated with mutations in the erythropoietin (EPO) receptor that results in hypersensitivity to EPO.

Most of the presenting symptoms in PV are related to the increased red cell mass that is central to the disease process. Patients may exhibit a hyperviscosity-related headache and blurry vision or arterial thrombosis and hemorrhage. There may also be symptoms related to gastrointestinal ulcers or bleeding. Many patients will have splenomegaly and pruritus, which is frequently provoked by warm water; this is referred to as *aquagenic pruritus*. Other common symptoms are those related to gout from hyperuricemia and erythromelalgia, which is reddening and painful swelling usually of the lower extremities.

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POLYCYTHEMIA VERA—FACT SHEET

Definition

- PV is a myeloproliferative neoplasm arising in a pluripotential hematopoietic stem cell that is characterized by increased red blood cell production resulting in an elevated red blood cell mass. The process has a polycythemic phase and a spent phase characterized by marrow fibrosis. There may also be a pre-polycythemic phase (masked PV), but this is difficult to recognize. Occasionally it may transform to acute leukemia

Incidence, Gender, and Age Distribution

- 1 to 3 cases per 100,000 population per year
- Slight male predominance
- Median age at diagnosis, 60 years; <5% younger than 40 years, rare cases in children
- Increased incidence in Ashkenazi Jews

Symptoms

- Hyperviscosity-related headache, blurry vision
- Arterial thrombosis
- Hemorrhage
- Pruritus provoked by warm water
- Erythromelalgia
- Symptoms related to gout

Physical Findings

- Splenomegaly, hepatomegaly
- Plethora

Prognosis and Therapy

- Treatment: phlebotomy with or without myelosuppression; *JAK2* inhibitor
- Survival: 15-year survival is 65%
- Prognosis: poor prognosis with history of thrombosis

POLYCYTHEMIA VERA—PATHOLOGIC FEATURES

Diagnostic Criteria

- All 3 major criteria or the first 2 major and minor criteria (WHO 2016)

Major Criteria

- Hemoglobin >16.5 g/dL in men; hemoglobin >16.0 g/dL in women OR hematocrit >49% in men; hematocrit >48% in women OR increased red cell mass (RCM) >25% above mean predicted value
- Hypercellular bone marrow for age with panmyelosis with pleomorphic mature megakaryocytes (differences in size)
- Presence of *JAK2 V617F* or *JAK2* exon 12 mutation

Minor Criteria

- Subnormal serum EPO levels

Pathologic Features

The discovery first of an elevated red blood cell count, hemoglobin, or hematocrit through clinical signs and symptoms and then through laboratory studies is the usual starting point in the diagnosis of PV. The major difficulty in distinguishing PV from reactive or spurious polycythemia has been overcome by *JAK2* mutation testing. *JAK2 V617F* mutation is by far the most common variant seen in 95% of PV cases, whereas the rest are due to *JAK2* exon 12 mutation. Very rarely, *MPL* and *CALR* mutations have been described in PV. To establish polycythemia, the value of hemoglobin is lowered in the 2016 WHO classification from more than 18.5 g/dL to more than 16.5 g/dL in men and from more than 16.5 g/dL to more than 16 g/dL in women to capture masked PV cases. This change in a lower Hb level requirement from the 2008 WHO criteria may make a distinction from other MPNs challenging, since other MPNs can have mild to moderately elevated Hb. For that caveat, bone marrow morphology is now considered one of the major criteria to diagnose PV besides erythrocytosis and *JAK2 V617F* or *JAK2* exon 12 mutations. Of note, endogenous erythroid colony formation in vitro is no longer one of the minor criteria, leaving subnormal EPO level as the only minor criterion in the diagnosis of PV. A bone marrow evaluation is usually obtained as a baseline for comparison to future evaluations, in addition to diagnostic purpose. It should be noted that *JAK2* mutation–negative cases are extremely rare but can still be diagnosed as PV by meeting the other two major criteria and one minor criterion, highlighting the importance of bone marrow morphologic evaluation at the time of diagnosis.

Blood and Bone Marrow: Polycythemic Phase

In the polycythemic phase, the peripheral blood shows erythrocytosis, and the red blood cells are normochromic and normocytic with little poikilocytosis (Fig. 17.18). Neutrophils may be elevated and there is often a basophilia. A significant left shift in the granulocytic elements is not common, although some immature cells may be seen. Platelets are elevated in at least half of the patients. The bone marrow cellularity is usually elevated, but some patients could have a normocellular marrow. When the marrow is hypercellular there is usually a panmyelosis, but the increase in erythroid precursors and megakaryocytes is most prominent. The erythropoietic cells are fairly unremarkable; however, the megakaryocytes are atypically large, but show variability in size, and are sometimes clustered around sinuses and close to the bone (Fig. 17.19). They do not exhibit the bizarre features of the megakaryocytes seen in PMF, nor the prominent lobulated nature of those in ET, and they are clearly different from the small hypolobated (dwarf) megakaryocytes in CML. Fibrosis is usually not increased, and stainable iron is absent in many if not most patients (95%). Patients with *JAK2* exon 12 mutations have a somewhat different appearance in their bone marrow. These patients have been reported to show more prominent erythroid proliferation without involvement of the megakaryocytic and granulocytic cell lines.

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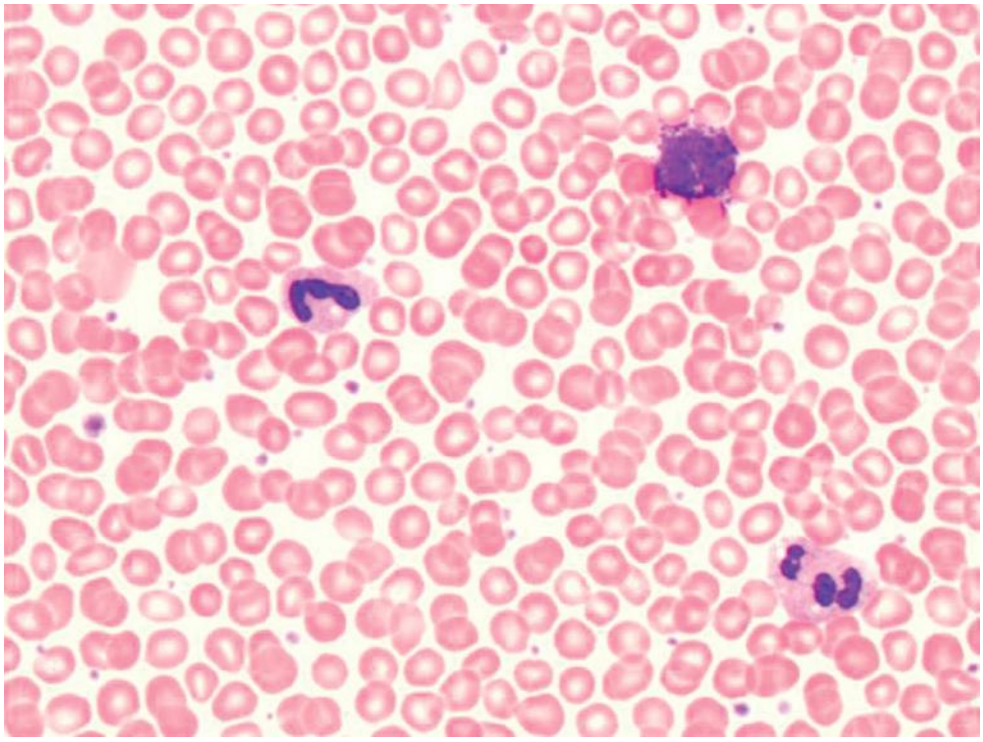


FIG. 17.18

Peripheral blood from patient with polycythemia vera. Note the density of the red blood cells.

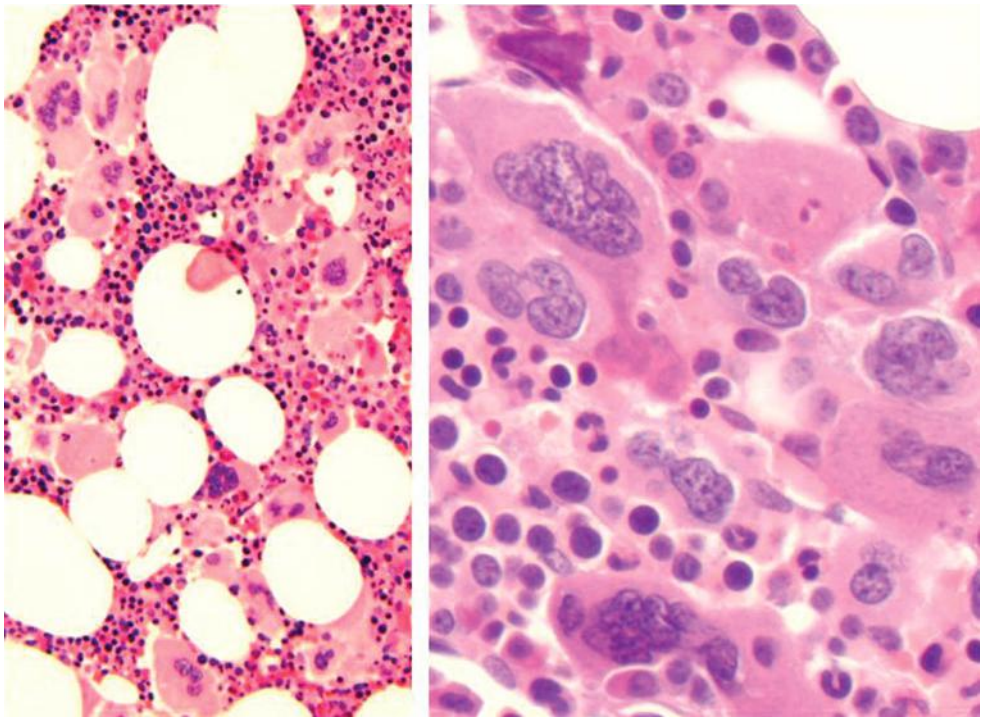


FIG. 17.19

Polycythemia vera. Note the clustered and variably sized megakaryocytes.

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Molecular

As noted previously, *JAK2* mutation is critical to the diagnosis in most cases because the *JAK2* V617F mutation is seen in approximately 95% of cases, and exon 12 mutations are seen in approximately 80% of the remaining cases. A number of methods are available to assess the mutations, including allele-specific PCR and the NGS method. Care must be taken not to overinterpret cases with low levels of *JAK2 V617F,* which can be associated with age-related clonal hematopoiesis. In fact, in PV the mutation is commonly homozygous, and much higher values would be expected (variant allele frequency often >50%).

Cytogenetics

Although no specific cytogenetic changes are diagnostic of PV and although they occur in only 10% to 20% of cases at the time of diagnosis, their presence confirms clonality of the hematopoietic elements and essentially rules out a reactive condition. Trisomies 8 and 9 (sometime seen together) del(20q), del(13p), and del(1p) are the most frequent findings.

Blood and Marrow, Spent Phase, Post-Polycythemic Myelofibrosis

In a later stage of the disease, the red cell mass normalizes and even sometimes decreases. A leukoerythroblastic process is seen in the blood, resembling that associated with PMF (see PMF section below). There are teardrop red blood cells and immature granulocytic forms with nucleated red blood cells in the blood. The marrow becomes increasingly fibrotic and sometimes progresses to collagen fibrosis. Sinusoidal hematopoiesis is common, and osteosclerosis may develop. Immature elements also become more prominent. When patients initially present in the post-polycythemic phase, hemoglobin levels have often become normalized or even reduced. At this stage and without a history of PV, distinction from a *JAK2* mutation–positive PMF or other MPN in fibrotic phase may not be possible. Some patients may develop an acute leukemic transformation; however, this is seen more frequently in patients treated with chemotherapies than without.

Differential Diagnosis

With the association of *JAK2* mutations in such a high percentage of cases of PV, there are fewer differential diagnostic considerations than in the past when a host of apparent (spurious), secondary and congenital causes of polycythemia had to be considered. These diagnoses must still be considered in patients with polycythemia to avoid costly workup. Apparently polycythemia occurs because of hemoconcentration; secondary causes are hypoxia driven, owing to abnormal production of erythropoietin or to medications, and the congenital form is sometimes caused by mutations in the erythropoietin gene, but more commonly it is of unknown causes. Hemoglobinopathies may also result in polycythemia. The *JAK2 V617F* mutation occurs in significant numbers of the other MPNs and in some MDS/MPNs, but these are much less likely to manifest with elevated hemoglobin or hematocrit, which are the key features for considering PV.

Prognosis and Therapy

Most patients with PV are treated with phlebotomy with or without myelosuppressive agents. The 15-year survival for PV is approximately 65%, and an independent prognostic indicator is whether the patient has a history of thrombosis. Life expectancy is reduced, particularly when a diagnosis is made before the age of 50 years. This reduction is possibly caused by the longer disease course and more time for complications and natural evolution to the fibrotic stage or to acute leukemia. Thus the goals of therapy in PV are to improve disease-related symptoms, splenomegaly, to prevent the occurrence or recurrence of thrombosis, and to delay or prevent the progression to myelofibrosis or AML and increase survival. Cytoreductive therapies (hydroxyurea or IFN-α) have been the standard treatment for older patients with polycythemia vera or those with a history of prior thrombosis. Recently, the JAK2 inhibitor ruxolitinib, an FDA-approved drug in this setting, has demonstrated significant reduction in hematocrit and splenomegaly especially in PV patients who have failed or are intolerant to hydroxyurea therapy.

ESSENTIAL THROMBOCYTHEMIA

Essential thrombocythemia (ET) is a myeloproliferative disorder that is largely characterized by a pronounced proliferation of megakaryocytes, resulting in sustained thrombocytosis, which is also referred to as *thrombocythemia*.

Clinical Features

Essential thrombocythemia is an uncommon disorder with an incidence of approximately 1 to 2.5 cases per 100,000 persons per year. The median age is approximately 60 years, and as in PV there may be a slight female predominance. In addition, there is an increased incidence in Ashkenazi Jews. Familial cases are extraordinarily rare, and some have been found to be caused by mutations in the *TPO* gene that results in increased production of thrombopoietin.

Clinically, patients are usually asymptomatic and usually come to be evaluated for ET because of an elevated platelet count found on a routine complete blood cell count performed for a well-patient check-up. Some patients exhibit symptoms

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ESSENTIAL THROMBOCYTHEMIA—FACT SHEET

Definition

A chronic myeloproliferative neoplasm characterized by pronounced proliferation of megakaryocytes resulting in severe thrombocytosis (thrombocythemia)

Incidence, Gender, and Age Distribution

- 1 to 2.5 cases per 100,000 population per year
- Slight female predominance (male to female = 2:1)
- Median age, 60 years
- Increased incidence in Ashkenazi Jews
- Rare familial cases

Clinical Features

- Many patients are asymptomatic (one quarter to one third)
- Symptoms
  - Headache, lightheadedness, blurry vision, scotomata, palpitations, chest pain, distal paresthesias, erythromelalgia, symptoms related to large vessel thromboses
  - Spontaneous abortions
- Physical findings
  - Splenomegaly (20% to 50%), hepatomegaly

Prognosis and Therapy

- Very good prognosis; generally does not lower life expectancy
- Treatment aims are to lower platelet count and risk for thromboses
- Rare transformation to acute leukemia (1% to 2%)

ESSENTIAL THROMBOCYTHEMIA—PATHOLOGIC FEATURES

Diagnostic Criteria

Diagnosis requires all four major criteria or first three major criteria and minor criterion (WHO 2016)

Major Criteria

- Platelet count ≥450 × 10^9/L
- Bone marrow biopsy showing normocellular bone marrow with megakaryocytic proliferation with increased number of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase in left-shifted neutrophilic granulopoiesis, or erythropoiesis, and no or rarely minimal increase in reticulin fibrosis (grade 1)
- Not meeting WHO criteria for *BCR-ABL1*-positive CML, PV, PMF, MDS or other myeloid neoplasms
- Presence of *JAK2, CALR,* or *MPL* mutation

Minor Criteria

- Presence of a clonal marker or absence of evidence of reactive thrombocytosis

believed to be related to thrombotic occlusion of the microvasculature. Such symptoms include headaches, lightheadedness, blurring vision and scotomata, palpitations, chest pain, and distal paresthesia. Erythromelalgia, characterized by erythema, warmth, and pain in the distal extremities is another symptom that is unusual, but not entirely specific for ET, as it is seen with some frequency in PV. More severe clinical features of ET include large vessel thromboses of either arterial or venous circulation. Pulmonary embolism and deep venous thromboses occur, but thrombotic events can also develop in less common sites such as hepatic or portal vein or retinal vein. Bleeding is another serious clinical manifestation and complication. Despite the high platelet count, patients are at risk for bleeding, which may be due to an acquired von Willebrand factor deficiency related to platelet absorption. Hemorrhage can occur in mucocutaneous areas and more seriously in the gastrointestinal tract. Splenomegaly is not as prominent as in the other MPNs, but it occurs in approximately 3% to 50% of cases. Hepatomegaly is less common.

Pathologic Features

The diagnosis of ET is made by identifying a sustained elevation in the platelet count (≥450 × 10^9/L); examining bone marrow and identifying marrow findings consistent with the disease; excluding other MPN, MDS, or other myeloid neoplasm associated with elevated platelets; and showing *JAK2* or *CALR, MPL* mutations, cytogenetic clonality or in the absence of these, ruling out reactive thrombocytosis.

Blood and Bone Marrow

The peripheral smear in ET shows marked thrombocytosis with a significant size variation (anisocytosis) of the platelets. Some giant platelets may be present (Fig. 17.20). The platelets are usually fairly normally granulated. White blood cells are usually normal in number, with no left shift, and there is no dysplasia. There is usually no absolute or relative basophilia. Red blood cells are normocytic and normochromic, except in patients with significant hemorrhage and iron deficiency, in which case they may be hypochromic and microcytic. Red cell morphology should be otherwise unremarkable. A rare teardrop form and a mild leukoerythroblastic picture are reasons to consider PMF rather than ET.

The bone marrow is generally moderately hypercellular. Megakaryocytes are prominent and have a particular morphology that can help to distinguish them from those seen in PV, PMF, and CML. The megakaryocytes are generally large and have abundant cytoplasm frequently with cells within the cytoplasm (emperipolesis); however, the latter finding is not diagnostic, because it is typical even in normal megakaryocytes. The nuclei are highly lobulated, a feature that has led some to refer to them as *staghorn-like* (Fig. 17.21). Bizarre nuclear forms with hypercondensed chromatin and tight clustering (typical of PMF) and small megakaryocytes with hypolobated nuclei (typical of CML) are not seen. Granulocytic proliferation is usually absent or only minimal, and there is

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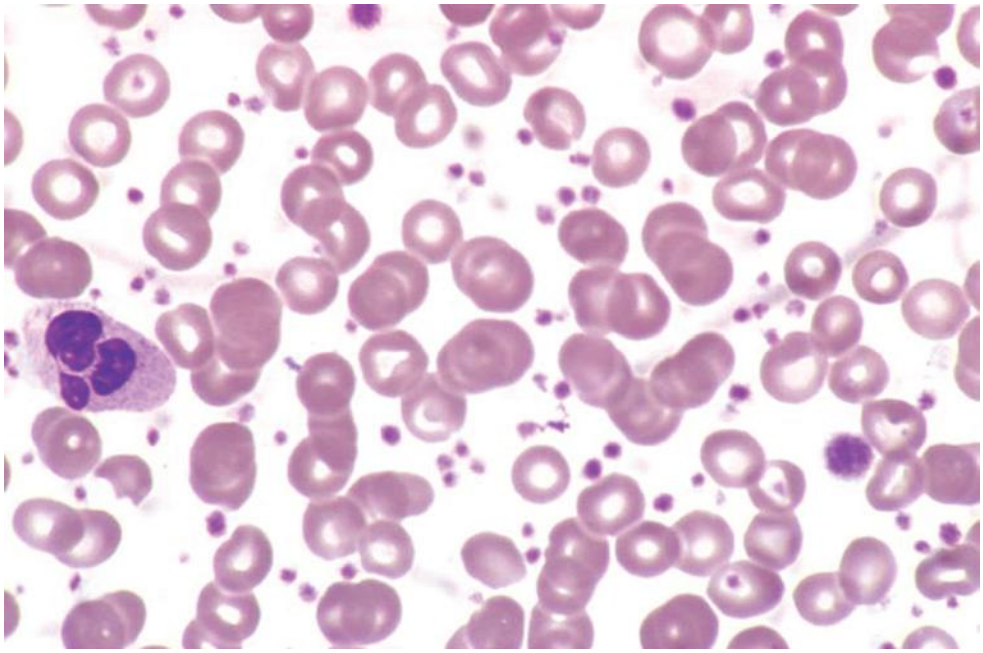


FIG. 17.20

Elevated platelet count in essential thrombocythemia. The platelets exhibit moderate anisocytosis with occasional giant forms.

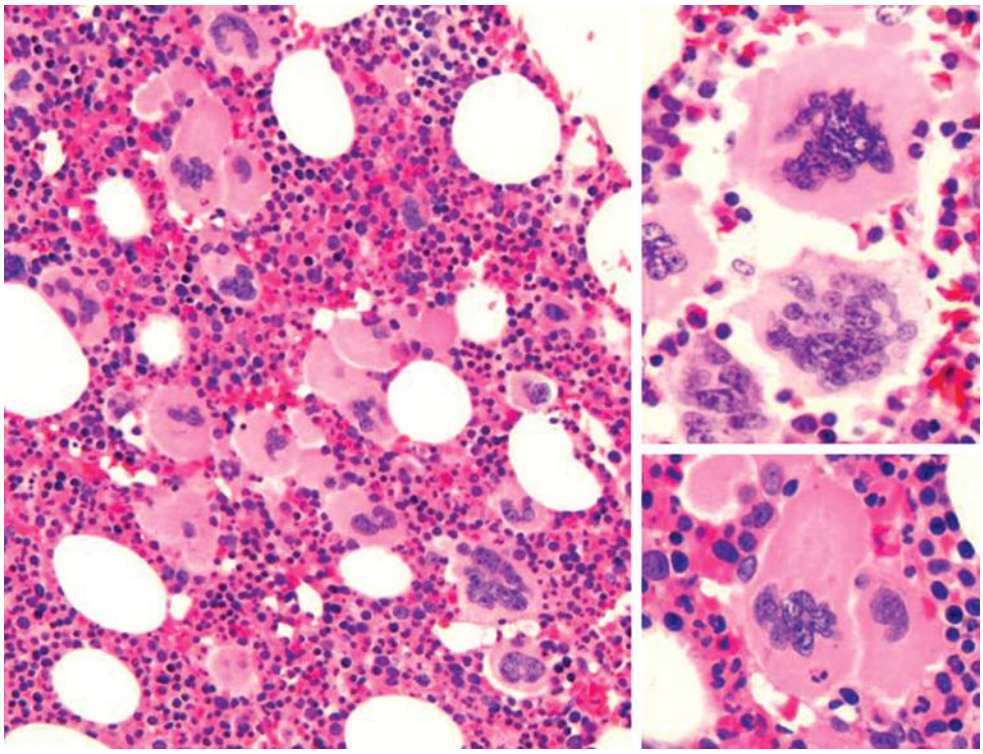


FIG. 17.21

Essential thrombocythemia bone marrow. Note the large megakaryocytes with abundant cytoplasm and prominent nuclear lobulation.

no left shift or increase in blasts. The mature granulocytes do not show dysplasia. Erythroid activity may be increased. Reticulin fibrosis may be minimally increased, but significant fibrosis should suggest another diagnosis. Stainable iron is usually present. Absent or decreased marrow iron is a common feature in ET and should not be used as a finding to favor an alternative diagnosis of PV over ET.

Molecular and Ancillary Studies

In approximately 50% to 60% of ET cases there is a *JAK2 V617* mutation, followed by mutated *CALR* (calreticulin gene) in 25% to 30% of cases and mutated *MPL W515 L/K* in 3% to 5% of cases. These mutations are mutually exclusive and

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lead to constitutive activation of JAK/STAT pathways that stimulate megakaryocyte proliferation and platelet production. Molecular analyses for *JAK2 V617F, CALR*, and *MPL* M515K/L are particularly helpful in ruling out a reactive process. It is noteworthy, in 5% to 10% of ET cases, the underlying driver proliferation of ET is unknown and such cases are known as triple-negative cases. In ET, the allele burden of the *JAK2 V617* mutation is lower than that of PV, and it is believed to be a more lineage-restricted mutation. Nevertheless, cases of ET with the *JAK2* mutation are believed to have some similarities to PV, in that there is a more panmyelosis with increased granulopoiesis and erythropoiesis compared to the mutation-negative cases in which thrombopoiesis is most prominent. On the other hand, *CALR*-mutated ET shows some unique features, not only at the molecular level but also with respect to clinical presentations and outcomes. CALR-mutated ET is an MPN that affects relatively young individuals and is characterized by markedly elevated platelet count but relatively low thrombotic risk as compared to *JAK2* V617F and *MPL* M515K/L-mutated ET. Patients with *CALR*-mutated ET more frequently progress to the accelerated or blast phases compared with patients with *JAK2* mutations. *CALR* mutations lead to an alteration of the C-terminal of the protein that results in the loss of an endoplasmic reticulum retention motif and activates STAT signaling. There are two types of mutations, including a 52-bp deletion in exon 9 (type 1) and a 5-bp insertion (type 2 mutation), with type 1 mutation being more common than type 2 mutations. Mutation subtypes in *CALR* contribute to a patient's clinical phenotype and outcomes. Type 1-*CALR* mutations are mainly associated with a myelofibrosis phenotype and a significantly higher risk of myelofibrotic transformation in ET. Type 2-*CALR* mutations are preferentially associated with a low risk of thrombosis despite very high platelet counts and an indolent clinical course. These mutations result in a novel epitope that can be detected by immunohistochemistry in fixed tissues using a mutation-specific monoclonal antibody (CAL2). Thus, presence of *CALR* mutations can now be easily tested in routine biopsy material.

Besides these driver mutations, mutations in epigenetic regulatory genes, such as *TET2, ASXL1, IDH1/2*, or *DNMT3A*, are also found in ET. The exact molecular pathology in ET is not well understood, but it is believed that there is loss of control of the proliferative activity in the megakaryocytes, leading to autonomous platelet production. The megakaryocytes are believed to be hypersensitive to stimulation by one of a number of growth factors, such as interleukin 3, or the megakaryocytic growth factor, thrombopoietin (TPO). However, it should be noted that TPO levels are not sufficiently different among ET, other myeloproliferative disorders, and reactive conditions; therefore, they cannot be used for diagnostic purposes. Mutations in TPO have not been found except in rare familial cases.

Cytogenetic analysis does not add much in resolving a differential diagnosis. Cytogenetic clones are rare in ET, as they are found in only 5% to 10% of cases. The cytogenetic findings can be used to support the diagnosis of a clonal MPN over a reactive thrombocytosis, but the genetic changes are nonspecific and do not help with cases that are difficult to distinguish from PV or early PMF. The abnormalities seen in ET include +8, +9, and del(13q).

Differential Diagnosis

As mentioned previously, the differential diagnosis includes reactive conditions leading to elevated platelet counts, other MPNs with markedly elevated platelet counts, and rarely AML, MDS, or MDS/MPNs, associated with increased platelets (most notably AML with t[3;3] or inv[3], the 5q- syndrome, and MDS/MPN-RS-T).

Reactive conditions causing thrombocytosis include infection, inflammatory diseases, blood loss and chronic iron deficiency, malignancy, trauma and surgery (especially splenectomy), and rebound following chemotherapy or replacement therapy for vitamin B_{12} or folate deficiency. Reactive conditions are more frequently associated with elevated acute phase reactants like C-reactive protein. The reactive conditions should not be persistent or associated with splenomegaly, and they are not likely associated with a history of thrombotic episodes.

The myeloid disorders with thrombocytosis that are *JAK2* mutation negative are common, but they usually have some features that allow them to be recognized when considering ET in the differential diagnosis. For example, CML can frequently develop with thrombocytosis, but it shows the full spectrum of myeloid proliferation, with a myelocyte bulge and basophilia in the peripheral blood that is usually quite distinctive and not to be expected in ET. In addition, the small, dwarf megakaryocytes in the bone marrow are also distinguished easily from the larger staghorn-like megakaryocytes in ET. Even in cases with prominent thrombocytosis and less easily distinguished blood or marrow morphology, the t(9;22) or *BCR-ABL1* will clarify any dilemma. Some patients with CML with markedly elevated platelets can have the p230 *BCR-ABL1* protein.

MDS 5q- syndrome can present with increased platelets, but typically the megakaryocytes are small, hypolobated, and distinctive from the large, hyperlobulated megakaryocytes seen in ET. In addition, the del(5q) would unlikely be seen in ET.

AML can sometimes present with elevated platelets; this is a feature of AML with inv(3)(q21;q26.2) or t(3;3)(q21;q26.2) or in some megakaryoblastic leukemias. In addition to the abnormal cytogenetic finding, these patients will have elevated blasts and usually highly dysplastic megakaryocytes, including the classic micro-megakaryocytes that make them easily distinguishable from ET.

The other MPNs or MDS/MPNs with *JAK2* mutations that must be considered in the differential diagnosis include PV, PMF, and MDS/MPN-RS-T; however, even these have features that make the distinction possible. The high hemoglobin–hematocrit of PV, the dysplasia and ring-sideroblasts of MDS/MPN-RS-T, and the fibrosis of the fibrotic

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TABLE 17.2

Comparison of Features in Distinguishing Prefibrotic PMF from ET

Features	Prefibrotic PMF	ET
Peripheral blood WBC count Platelet count	Variable, often increased with left-shifted granulocytes Often ≥450 × 10^9/L, may be normal or decreased	Usually normal, may be mildly increased; however, left-shifted granulocytes not seen Increased (≥450 × 10^9/L)
Bone marrow cellularity M:E ratio	Increased, often increased	Usually normal, often normal
Megakaryocyte clustering	Tighter clusters	Loose clustering
Megakaryocyte size, maturation	Variable, small to large, immature to mature, with hyperchromatic forms	Mostly large, mature
Megakaryocyte nuclear shape	Variable, bizarre	Hyperlobulated, staghorn

PMF, Primary myelofibrosis; *ET,* essential thrombocythemia.

phase of PMF all provide ample clues for a differential diagnosis. The most difficult differential is between ET and the prefibrotic phase of PMF, and features distinguishing the two entities are highlighted in Table 17.2.

Prognosis and Therapy

The prognosis of ET is good. In the first decade the disease does not lower life expectancy. Overall median survival from the time of diagnosis is approximately 20 years. Risk factors that predict a worse overall survival in ET include age more than 60 years, WBC count more than 11 × 10^9/L, and a history of previous thrombosis. Treatment is aimed at lowering both platelet counts and the risk for thromboses. Acute leukemic transformation in ET is rare, occurring in approximately 1% at 10 years and 2% at 15 years. A post-ET myelofibrotic transformation has been described, occurring in approximately 5% at 10 years and 10% at 15 years.

PRIMARY MYELOFIBROSIS

Primary myelofibrosis (PMF) has been previously referred to by a number of terms, the most common of which include agnogenic myeloid metaplasia, myelosclerosis with myeloid metaplasia (MMM), and idiopathic myelofibrosis. The WHO committee writing on hematopoietic tumors in 2001 developed the term *chronic idiopathic myelofibrosis* (CIMF) but, in keeping with the frequent name changing, altered it again in 2008 to PMF. The disorder is the most aggressive of the three common *BCR-ABL1*–negative MPNs (PV, ET, and PMF), because it is characterized by a bone marrow that becomes progressively fibrotic and even osteosclerotic; this results in a leukoerythroblastic process in the blood, marked extramedullary hematopoiesis with extensive hepatosplenomegaly, and marrow failure. Sometimes there is also a transformation to AML. Transformation at 10 years occurs in approximately 5% to 30% of cases, whereas the burned-out phase of marrow fibrosis occurs in the majority (~100%). Although best characterized by this progressive fibrosis and osteosclerosis, the process begins with a more cellular phase with a proliferative process including a prominent megakaryocytic proliferation, which can be difficult or impossible to distinguish from ET and sometimes from PV. This cellular or prefibrotic phase frequently goes unnoticed as more patients (~60% to 70%) are seen in the fibrotic phase.

The pathogenesis of PMF is probably the least understood when compared to the other types of MPN. Hematopoiesis (including lymphoid lineage cells in some cases) is clonal, but the proliferating fibroblasts, which play a major role in the pathology, are not. The exact nature of what is believed to be a "cytokine storm" responsible for the fibroblastic, osteoblastic, myeloid, and even vascular proliferation (leading to a neoangiogenesis), is not known. In about 50% to 60% of cases there is a *JAK2* V617F mutation, 20% to 25% have *CALR* exon 9 gene mutation, 5% to 10% have a mutation of *MPL* exon 12 mutation (*MPL* W515K/L), and the remaining 5% to 10% are "triple negative." Some of the growth factors responsible for the disease are from the abnormal megakaryocytes, but others are likely from monocytes and macrophages. Growth factor pathway abnormalities have been detected, but they are not unique to PMF. CD34 cells, which are increased in the blood, may have a point mutation in the stem cell factor *KIT*. Serum VEGF is increased in most patients. Expression of the basic fibroblastic growth factor is increased, and transforming growth factor β, a negative regulator of hematopoiesis, is decreased. In addition, MPL is incompletely glycosylated and poorly expressed on platelets, megakaryocytes, and the stem cells. As mentioned previously, reduced expression of MPL is also seen in ET and in PV, and it is not unique to PMF. The extramedullary hematopoiesis in PMF is probably derived from marrow progenitor cells taking residence in the spleen and not from reactivation of fetal splenic hematopoiesis as believed previously.

Clinical Features

The incidence of PMF is approximately 0.5 to 1 per 100,000 persons. The average age is between 54 and 62 years, with an equal sex distribution. Occurrence is rare in children, and there is an increased incidence in Ashkenazi Jews.

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PRIMARY MYELOFIBROSIS—FACT SHEET

Definition and Other Names
- A progressive myeloproliferative neoplasm arising in a pluripotential hematopoietic progenitor, which is characterized by a proliferation of megakaryocytic and granulocytic elements and associated with marrow fibrosis
- Marrow fibrosis results in prominent extramedullary hematopoiesis and is commonly a progressive neoplasm with resulting marrow failure, in addition to transformation to acute leukemia
- Myelofibrosis with myeloid metaplasia, agnogenic myeloid metaplasia, chronic idiopathic myelofibrosis

Incidence, Gender, and Age Distribution
- 0.5 to 1 per 100,000 population per year
- Equal sex distribution
- Median age, 54 to 62 years
- Increased incidence in Ashkenazi Jews

Clinical Features
- Symptoms
- 30% to 40% of patients are asymptomatic
- Weight loss, constitutional symptoms
- Symptoms related to anemia, splenomegaly, gout, renal stones

Physical Findings
- Splenomegaly, often massive

Prognosis and Therapy
- Progressive disease
- Poor prognostic factors: age (>70 years), low hemoglobin level, abnormal karyotype
- Accelerated phase when blasts are 10% to 19%
- Transformation to acute leukemia in 5% to 30% of cases
- Mean survival, 3 to 5 years from diagnosis
- Driver mutational profile has been associated with overall survival (OS) with *CALR*-mutated cases having the longest and "triple-negative" (lacking *JAK2/MPL/CALR* mutations) cases having the shortest
- Hematopoietic stem cell transplantation for patients predicted to have a poor survival (≤5 yr). *JAK1/2* inhibitors improve symptoms, decrease spleen size, and improve quality of life but are not curative.

Clinically, many patients are asymptomatic (30%). For those with symptoms, complaints are related to anemia, splenomegaly, or constitutional symptoms. Other symptoms, such as weight loss, gouty arthritis, or renal stones from hyperuricemia may also be present.

Pathologic Features

Diagnosis, General

The diagnosis of PMF is fairly straightforward in the fibrotic phase, because the blood findings together with the bone marrow findings are distinctive and rarely seen in other entities. The prefibrotic phase is diagnostically difficult, but some morphologic clues have been developed. The WHO 2016 criteria are given in the pathologic features box that follows. The features include meeting three major criteria (including appropriate bone marrow findings, excluding other MPNs and other myeloid neoplasms, and demonstrating clonal markers or excluding other causes of marrow fibrosis) and one of four minor criteria (including leukocytosis $\geq 11 \times 10^9$/L, increased LDH, anemia, or palpable splenomegaly). As with ET, the criteria are partially exclusionary in nature, indicating the importance of excluding other myeloid neoplasms (most notably CML, PV, and MDS) and excluding other causes of fibrosis.

Differential Diagnosis: Prefibrotic Phase

Blood

A few patients are first seen in the prefibrotic phase, which can present a diagnostic challenge. There will be a modest anemia, and the WBC count will be moderately elevated. Platelets are often significantly elevated, with a mean platelet count of approximately 900×10^9/L. The more classic features of teardrop red blood cells and leukoerythroblastosis are usually absent, although if seen they can be of great help diagnostically.

Bone Marrow

The bone marrow is usually hypercellular, and reticulin fibrosis is minimal if present at all. There is a proliferation of granulocytes and megakaryocytes (Fig. 17.22). Blasts are not increased. The megakaryocytes might give a clue to the diagnosis because they are clustered and, more important, atypical or even bizarre. The megakaryocytes are of variable size and have abnormal nuclei with disorganized lobulation and hypercondensed nuclear chromatin. Bare megakaryocyte nuclei might also be seen. Vascular proliferation may be present, and some cases may have reactive lymphoid nodules.

Some morphologic clues to help distinguish prefibrotic PMF from ET include cellularity (higher in PMF); megakaryocyte clustering (more prominent in PMF with tighter clusters); megakaryocytic size, shape, and maturation (more varied and defective in PMF); and background hematopoiesis (more granulocytic predominant in PMF (Fig. 17.23; see Table 17.2).

Differential Diagnosis: Fibrotic Phase

Blood

Most patients (~70%) present in the fibrotic phase, at which time both the marrow fibrosis and extramedullary

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PRIMARY MYELOFIBROSIS—PATHOLOGIC FEATURES

Diagnostic criteria of prefibrotic PMF (WHO 2016): Requires meeting all three major and one of minor criteria confirmed in two consecutive determinations

Major Criteria

- Presence of megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1, accompanied by an increased age-adjusted bone marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis
- Not meeting WHO criteria for PV, CML, ET, MDS, or other myeloid diseases

  Presence of *JAK2 V617F, CALR*, or *MPL* mutations or in the absence of these mutations, presence of other clonal marker or no evidence that the marrow fibrosis or other changes are secondary to infection, autoimmune, chronic inflammation, hairy cell leukemia, other malignancies including metastatic tumor, or are due to toxic myelopathies

Minor Criteria

- Leukocytosis $\geq 11 \times 10^9$/L
- Increased serum lactate dehydrogenase
- Anemia not attributed to a comorbid condition
- Palpable splenomegaly

Diagnostic Criteria of Fibrotic PMF (WHO 2016)

- Requires meeting all three major and one of minor criteria

Major Criteria

- Presence of megakaryocytic proliferation and atypia, with either reticulin and or collagen fibrosis grade 2 or 3
- Not meeting WHO criteria for PV, CML, ET, MDS, or other myeloid diseases

  Presence of *JAK2 V617F, CALR*, or *MPL* mutations or in the absence of these mutations, presence of other clonal marker or no evidence that the marrow fibrosis or other changes are secondary to infection, autoimmune, chronic inflammation, hairy cell leukemia, other malignancies including metastatic tumor, or due to toxic myelopathies

Minor Criteria

- Leukoerythroblastosis
- Leukocytosis $\geq 11 \times 10^9$/L
- Increased serum lactate dehydrogenase
- Anemia not attributed to a comorbid condition

Differential Diagnosis

- Prefibrotic stage: difficult, may not be resolvable from early PV, early ET
- Fibrotic stage
  - Myeloid disorders causing marrow fibrosis: post-PV and ET myelofibrosis, CML in accelerated phase, MDS with fibrosis, MDS/MPN with fibrosis, AML with fibrosis (including acute panmyelosis with myelofibrosis), mast cell disease
  - Other hematopoietic disorders causing marrow fibrosis: Hodgkin lymphoma, non-Hodgkin lymphoma, hairy cell leukemia
  - Other conditions causing marrow fibrosis: metastatic disease, certain infections, autoimmune myelofibrosis

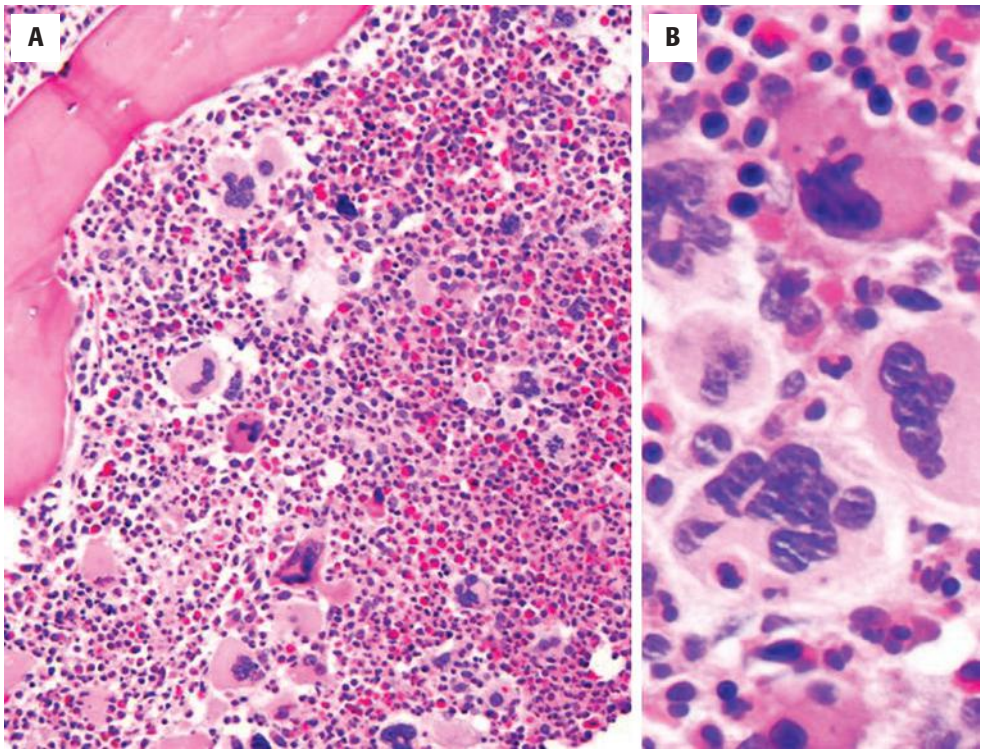


FIG. 17.22

Primary myelofibrosis in the cellular phase. There is a granulocytic and megakaryocytic proliferation but no fibrosis. Note the atypical megakaryocytes with hypercondensed nuclear chromatin. Diagnosis is difficult in such cases because the distinction from early polycythemia vera and essential thrombocythemia is difficult, if not impossible.

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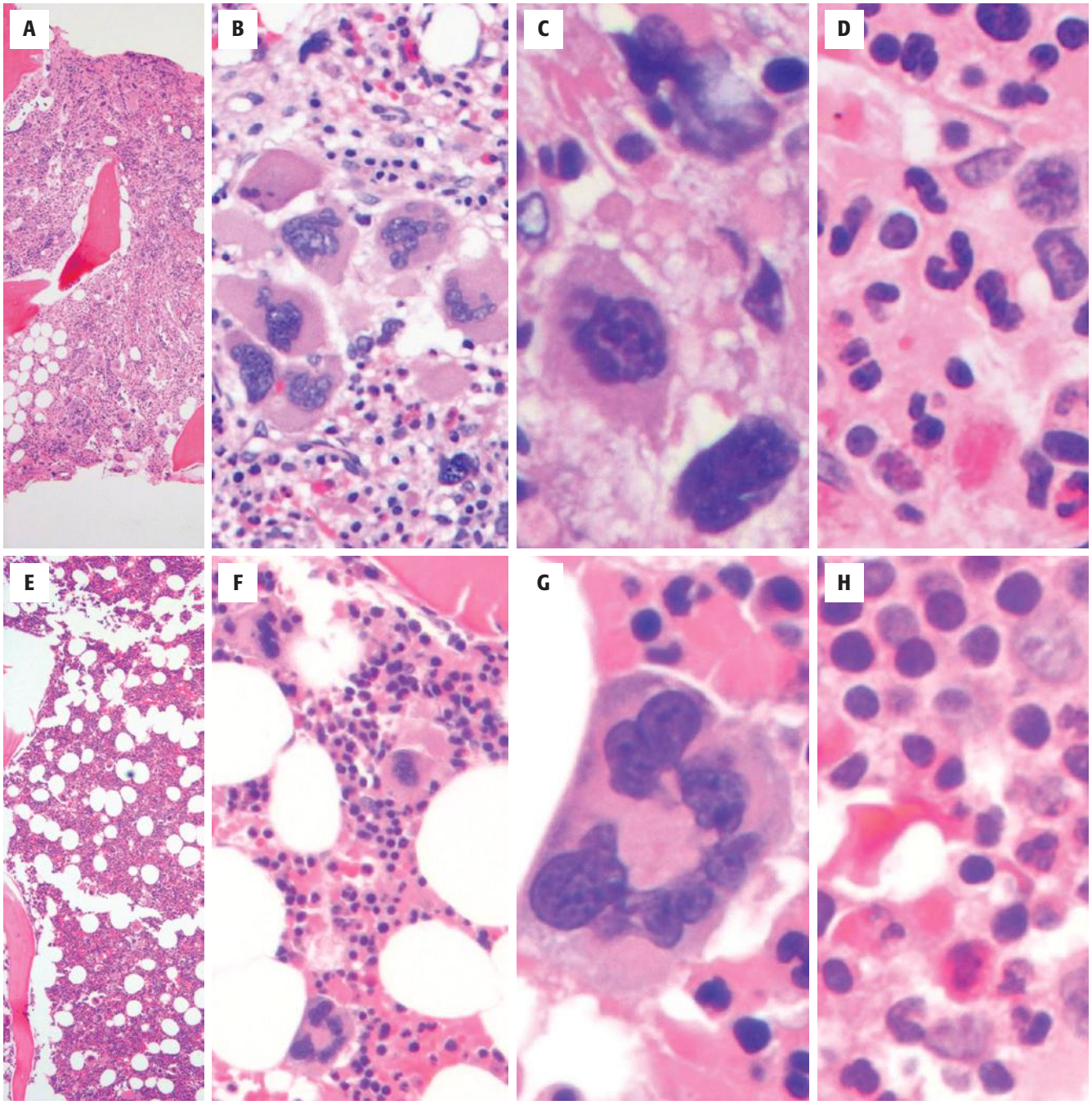


FIG. 17.23

Some morphologic clues to distinguish the prefibrotic phase of primary myelofibrosis (A-D) from essential thrombocythemia (E-H). In prefibrotic primary myelofibrosis (A-D), the cellularity is usually high, the megakaryocytes are more tightly clustered, and they exhibit maturation abnormalities and nuclear atypia. The background hematopoiesis in prefibrotic primary myelofibrosis is also usually more granulocytic.

hematopoiesis are significant enough to produce findings that are characteristic and diagnostic of the process. Patients often have mild leukocytosis, anemia, and normal to moderate thrombocytosis. In some patients, WBC may be normal and some may have mild thrombocytopenia. However, pancytopenia(s) is very uncommon and should raise a differential diagnosis of MDS with fibrosis. The peripheral blood findings are fairly classic, with a leukoerythroblastic picture showing numerous teardrop red blood cells, immature granulocytes, and nucleated red blood cells (Fig. 17.24). The red blood cells can show significant poikilocytosis with numerous ovalocytes in addition to the dacryocytes or teardrop forms. Circulating myeloblasts are not uncommon and usually account for a low percentage of the cells. When blasts increase to greater than 10%, the process can be considered in the accelerated phase. Platelets can be significantly elevated, but the range is wide. Some of the platelets may be large giant forms.

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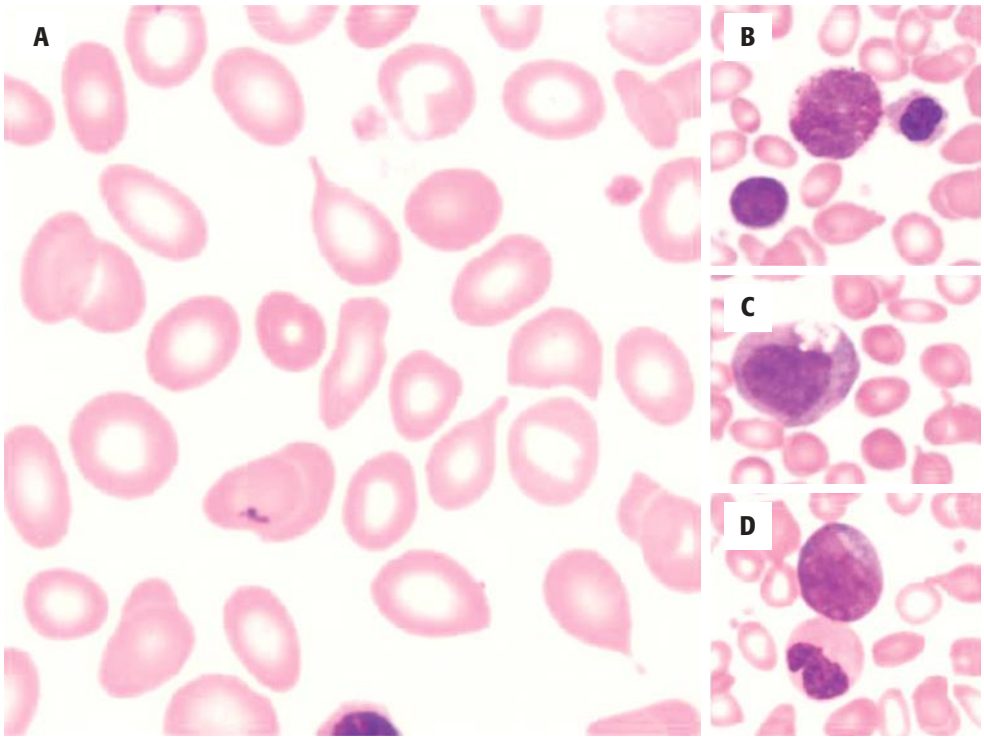


FIG. 17.24

Peripheral blood smear from a patient with primary myelofibrosis. Numerous teardrop forms (A) are seen, and there is a leukoerythroblastic picture with nucleated red blood cells (B), immature granulocytes (C), including occasional circulating blasts (D).

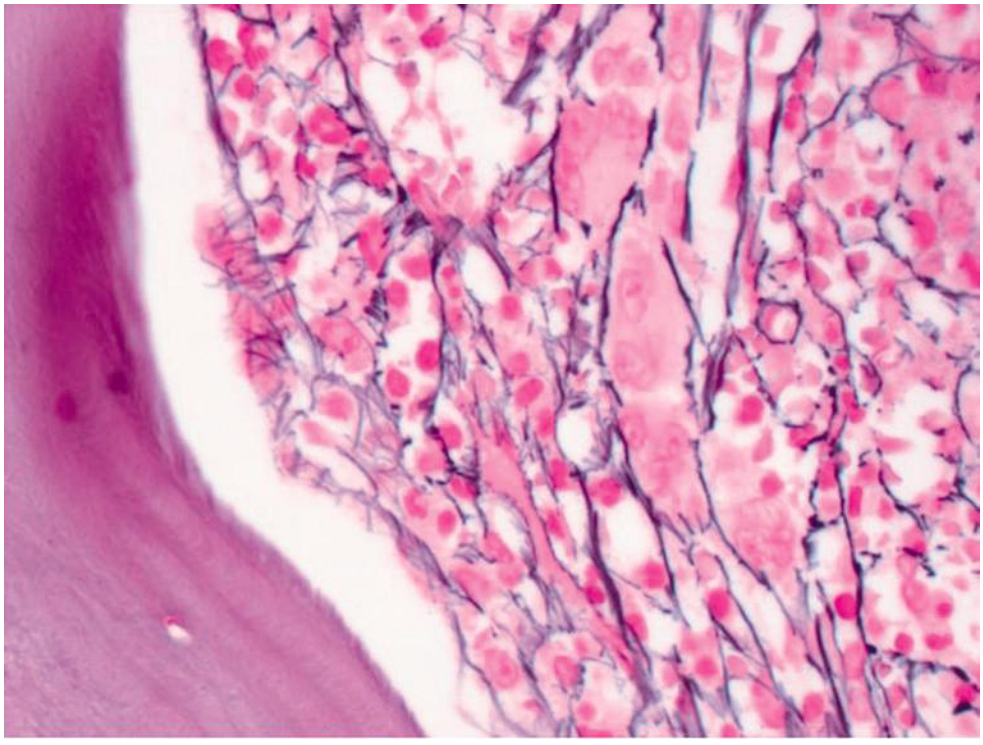


FIG. 17.25

Reticulin stain in a case of fibrotic stage of primary myelofibrosis. Note the markedly increased reticulin fibrosis, particularly around megakaryocytes.

Bone Marrow

In the fibrotic phase, the bone marrow is usually inaspirable because of increased reticulin fibrosis (Fig. 17.25) and possibly collagen fibrosis. The cellularity varies from more cellular to hypocellular as the disease progresses. Atypical megakaryocytes are prominent and are present in sizable clusters; they are large and have large, close to trabecular bone, bizarre nuclei frequently with hypercondensed nuclear chromatin (Fig. 17.26). Naked megakaryocyte nuclei may be prominent. Myeloid and erythroid elements are fairly unremarkable but may be decreased as the disease progresses. Blasts can be increased but should be less than 20%. Sinusoidal hematopoiesis is present and is a feature that some say should be

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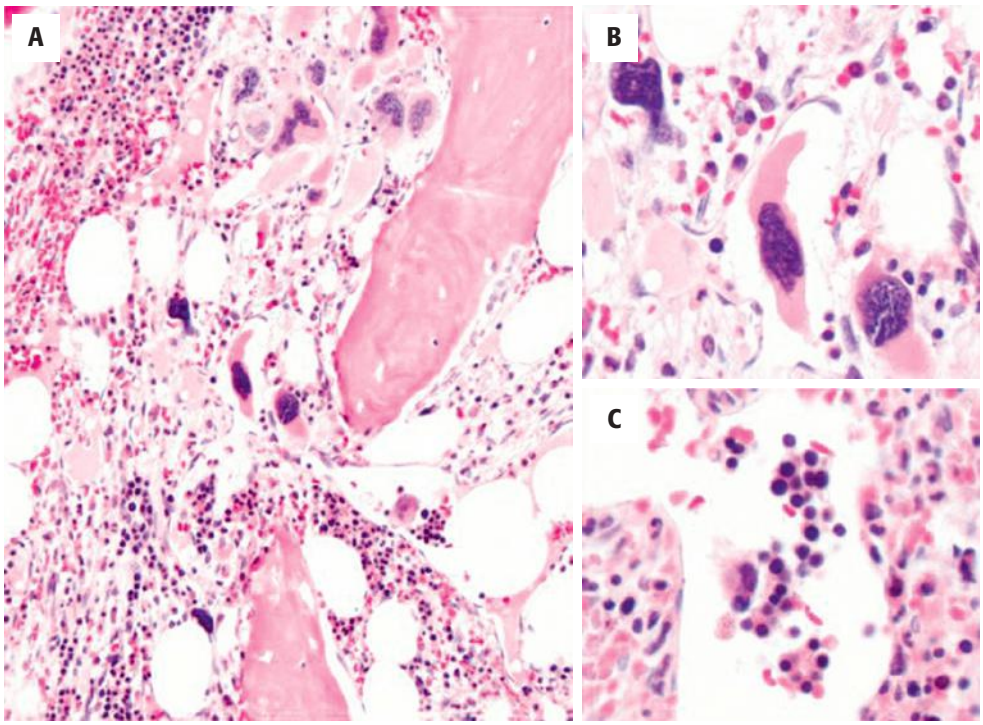


FIG. 17.26

Fibrotic stage of primary myelofibrosis, bone marrow biopsy. A, Note the swirling effect of cells owing to underlying fibrosis. B, Megakaryocytes are atypical with hyperchromatic, almost pyknotic nuclei. C, Sinusoidal hematopoiesis is frequently if not always seen.

present in all cases. It is characteristic but not diagnostic in itself. It is identified by megakaryocytes or other hematopoietic elements within dilated sinuses.

Ancillary Studies

Molecular analysis for *JAK2 V617F, CALR* exon 9 mutations, and *MPL W515K/L* is useful because the finding can be used to rule out a reactive cause of marrow fibrosis. These mutations are seen in approximately 50% to 60%, 20% to 25%, and in 5% to 10% of cases, respectively. Of PMF cases, 5% to 10% are triple negative. In addition, the presence of frequent accompanying mutations (ranging from 4% to 26% of cases depending on the gene) involving *ASXL1, EZH2, TET2, SRSF2, SF3B1,* and *IDH1/2* are helpful in determining the prognosis in PMF. Cytogenetic abnormalities are detected in 35% to 45% of cases of PMF, but they are not diagnostic and overlap with those seen in the other common non-CML MPNs. Although not diagnostic, they too can be important in ruling out reactive causes of marrow fibrosis. The more common findings include 20q-, 13q-, +8, +9, 12p-, abnormalities of chromosomes 1 and 7, and a complex karyotype. 13q- is frequently associated with mutant *CALR*, +9 with mutant *JAK2*, and 20q- with mutant *SRSF2*.

Differential Diagnosis

The diagnosis in the prefibrotic phase can be difficult, especially if there are few abnormal megakaryocytes. The diagnosis of an MPN can usually be suggested because the cellularity is high for the patient's age, and there is a granulocytic and megakaryocytic proliferation. A high platelet count might suggest ET, but the megakaryocyte morphology differs, in that the megakaryocytes in ET are consistently larger, whereas those of PMF are variable in size and more bizarre.

Differential considerations of the prefibrotic stage include ET and PV, as discussed previously. Frequently the differential diagnosis cannot be resolved, and only time and the development of fibrosis can resolve the issue. Until more successful therapy is developed, the inability to resolve this differential diagnostic problem remains more important for prognosis. Differential diagnostic considerations of the fibrotic stage include any disorders that cause marrow fibrosis, including post-PV and ET myelofibrosis, CML in accelerated phase, MDS with fibrosis, MDS/MPN with fibrosis, mast cell disease, and AML with fibrosis, including acute panmyelosis with myelofibrosis. The latter can be distinguished from PMF. In acute panmyelosis with myelofibrosis, there is an absence of both teardrop cells and a leukoerythroblastic picture in the blood because of the abrupt onset of the process. The most challenging differential diagnoses include MDS and MDS/MPN with fibrosis. There is substantial overlap in peripheral blood (PB) and bone marrow (BM) features among these entities. *JAK2* mutations can be seen in up to 20% of MDS and MDS/MPN with fibrosis. Anemia is common in fibrotic stage PMF, even mild thrombocytopenia; however, severe cytopenia or pancytopenia is very uncommon. MDS and MDS/MPN often exhibit more pronounced dysplasia involving granulocytic and erythroid lineages and show predominant small dysplastic megakaryocytes, in contrast to a mixture of small and large megakaryocytes with clustering in PMF. PMF

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may show some dysplasia in erythroids but is often mild, with no significant ring sideroblasts, and shows a myeloid instead of erythroid hyperplasia. PMF often has large spleen and high LDH, which is not so frequent or pronounced in MDS and MDS/MPN with fibrosis. Other causes of marrow fibrosis must also be considered, including non-Hodgkin lymphoma, Hodgkin lymphoma, hairy cell leukemia, metastatic disease, and certain infections and autoimmune myelofibrosis. These causes are usually not a significant challenge because other malignant cells are usually present within the fibrosis, or large atypical megakaryocytes and sinusoidal hematopoiesis are lacking and make PMF less likely.

Prognosis and Therapy

Survival times in PMF vary significantly; however, mean survival is usually 3 to 5 years from the time of diagnosis. Poor prognostic indicators include age greater than 65 years, anemia, leukocytosis (>25 × 10^9/L), thrombocytopenia, type of mutation, and possibly an abnormal karyotype. Patients with *CALR*-positive PMF have the longest survival of approximately 15 years, whereas *JAK2* and *MPL* mutation–positive patients have an intermediate survival, and "triple-negative" PMF patients have the worst outcome of only 2.3 years in a recent study by Tefferi and colleagues. Karyotypic abnormalities other than -20q and -13q, such as a complex karyotype or a single or two abnormalities including +8, -7/7q-, i(17q), -5/5q-, 12p-, inv(3), or 11q23 rearrangement, might be associated with a poor outcome. Other poor prognostic indicators for patients at fibrotic phase PMF include the presence of 1% or more PB blasts and red cell transfusion dependence.

In addition to the driver mutations mentioned above, studies have evaluated the prognostic significance of other mutations. *ASXL1*, in particular, and also the number of additional mutations (>1 of the following 5: *ASXL1, EZH2, SRSF2*, and *IDH1/2*) have been shown to be a poor prognostic factor independent of the clinical scoring systems such as the International Prognostic Scoring System (IPSS) and dynamic IPSS.

Whether the patient is in the prefibrotic or fibrotic stage, the disease is chronic and progressive. Over time, the marrow becomes more fibrotic and can become osteosclerotic with a marrow space "wiped out" by proliferating bone (Fig. 17.27). The cellularity tends to reduce as the fibrosis increases, and this can be prominent and lead to a burned-out appearance. Some patients will develop progression with myelodysplasia and an increase in blasts. When the blast count in the blood or marrow reaches 10% to 19%, the process should be considered in accelerated phase, which can signify impending acute disease. A transformation to acute leukemia ensues in approximately 5% to 30% of cases; this is higher than in PV or ET. The acute process is always myeloid and sometimes resembles acute megakaryoblastic leukemia, although acute disease with features of AML with minimal differentiation or AML with maturation is also common. Acute transformation in PMF is associated with dismal outcome and a life expectancy of only a few months.

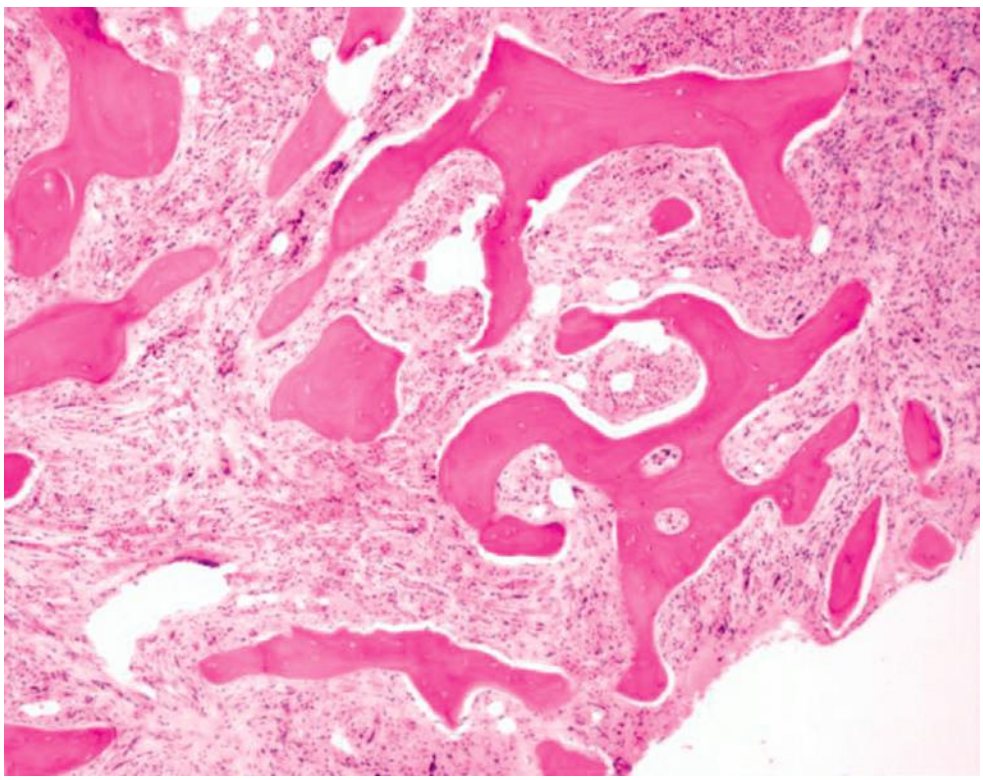


FIG. 17.27

End-stage primary myelofibrosis with prominent osteosclerosis. Note the fibrotic marrow space with depletion of marrow elements.

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CHRONIC NEUTROPHILIC LEUKEMIA

Clinical Features

Chronic neutrophilic leukemia (CNL) is an uncommon MPN with approximately 200 cases reported to date. This MPN is characterized by persistent neutrophilia in blood and bone marrow hypercellularity caused by granulocytic proliferation and hepatosplenomegaly in the absence of identifiable causes of physiologic neutrophilia. In view of a subset of reported cases of CNL and monoclonal gammopathy of undetermined significance and multiple myeloma, the WHO recommends that bone marrow of all possible cases of CNL be examined for evidence of plasma cell dyscrasia. If the latter is present, clonality of the neutrophil lineage should be supported by cytogenetic or molecular techniques (*CSF3R*) before making the diagnosis of CNL.

Clinically, the leukemia occurs in older patients with a 2:1 male-to-female distribution. Patients frequently have splenomegaly, and some also have enlarged liver. Bleeding from mucous membranes is common, but bleeding elsewhere can also occur, as can infectious complications. Rarely, patients have been reported with chloromas or extramedullary tumor of mature neutrophils.

CHRONIC NEUTROPHILIC LEUKEMIA—FACT SHEET

Definition

A chronic leukemic process characterized by a proliferation of mature clonal neutrophils in blood and marrow and often infiltrating into tissues

Incidence, Gender, and Age Distribution

- Rare, with ~200 cases reported
- Occurs in elderly
- Male to female = 2:1

Clinical Features

- Splenomegaly
- Bleeding
- Infectious complications

Prognosis

- Median survival <2 years
- Transformation to acute leukemia uncommon

Pathologic Features

Patients with CNL must have leukocytosis 25×10^9/L or greater in peripheral blood. The segmented neutrophils and band stages account for 80% or greater of the white blood cells, and neutrophilic precursors (promyelocytes, myelocytes, and metamyelocytes) are less than 10% of WBC. The neutrophils characteristically have toxic granulation with Döhle bodies and some tendency for hypersegmentation (Fig. 17.28). Myeloblasts are not increased (<5% of marrow cellularity), monocyte count less than 1×10^9/L or 10% and no dysgranulopoiesis is noted. Red blood cells and platelets are relatively normal. The bone marrow is hypercellular because of a proliferation of mature neutrophils. Erythropoiesis and megakaryopoiesis are usually normal, and reticulin fibrosis is not present. Evaluation for increased and clonal

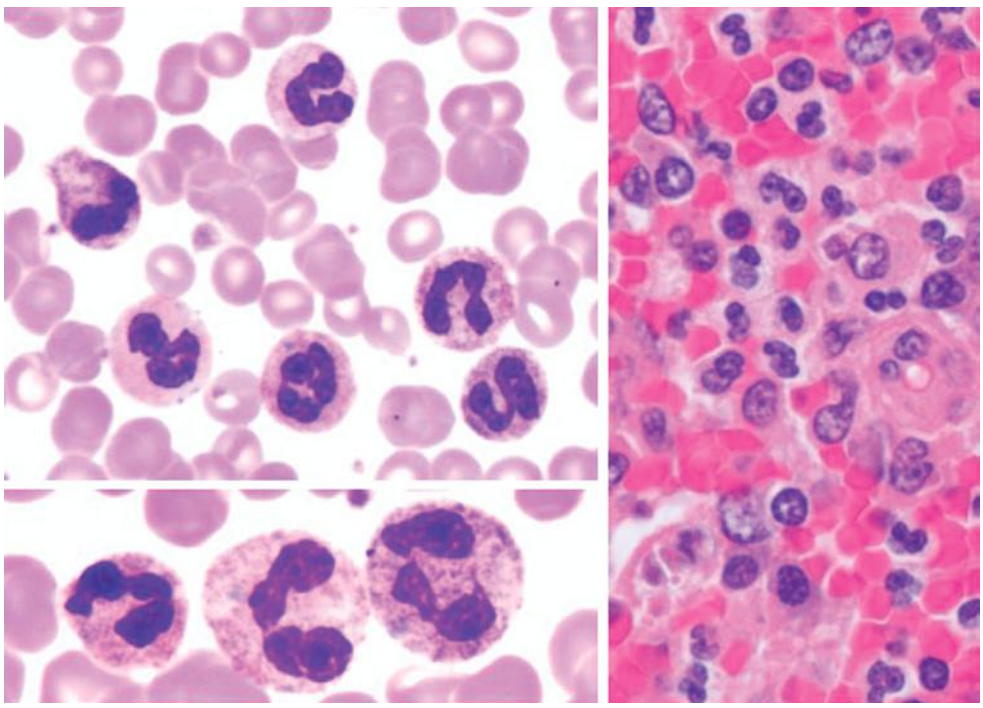


FIG. 17.28

Chronic neutrophilic leukemia with leukocytosis in the blood *(right)*. The neutrophils show toxic granulation and Döhle bodies *(bottom left)*. The patient underwent a splenectomy (2200 g), and the neutrophils were found to be clonal using the human androgen receptor assay (HUMARA).

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CHRONIC NEUTROPHILIC LEUKEMIA—PATHOLOGIC FEATURES

Diagnostic Criteria (WHO 2016)

- Peripheral blood: Leukocytosis with WBC ≥25 × 10^9/L; segmented neutrophils and band stages account for ≥80% of the WBCs and neutrophilic precursors (promyelocytes, myelocytes, and metamyelocytes) <10% of WBC; myeloblasts are rare, monocyte count either <1 × 10^9/L or 10% and no dysgranulopoiesis is noted
- Bone marrow: hypercellular bone marrow with increased neutrophilic granulocytes, <5% myeloblasts, a normal pattern of neutrophil maturation, and normal megakaryocytes
- Not meeting the WHO criteria for *BCR-ABL1+* CML, PV, ET, or PMF
- No rearrangement of *PDGFA, PDGFB, FGFR1, or PCM1-JAK2*
- Ancillary study: Presence of *CSF3R T618I* or other activating *CSF3R* mutation or in the absence of *CSF3R* mutation, persistent neutrophilia (at least 3 months), splenomegaly and no identifiable cause of reactive neutrophilia including absence of a plasma cell neoplasm or if present, demonstration of clonality of myeloid cells or cytogenetic or molecular studies

Differential Diagnosis

- Reactive neutrophilia, including leukemoid reaction and secondary neutrophilia due to plasma cell proliferation
- CML and CML-neutrophilic variant
- Atypical CML

plasma cells should be undertaken. WHO 2016 criteria are listed in the pathologic features box and in large part are exclusionary.

Differential Diagnosis and Ancillary Studies

Chronic neutrophilic leukemia must be distinguished from a leukemoid reaction, CML, and other MPNs or MDS/MPNs. The search for a cause of neutrophilia must be undertaken with secondary causes ruled out. The presence of dysplasia should make the pathologist consider the diagnosis of a disorder with a myelodysplastic component rather than CNL. Cytogenetic and molecular studies should be undertaken to rule out CML. There should be no t(9;22) or *BCR-ABL1* and particularly no p230 *BCR-ABL1,* which is associated with CML with neutrophilia (CML-N).

Over the recent few years, great progress has been made in understanding the molecular basis of CNL, especially with the discovery of disease-defining somatic-activating mutations in *CSF3R*, the gene for receptor for colony-stimulating factor 3 (CSF3) on chromosome 1p34.3 that encodes the primary growth factor for neutrophil production. The reported mutation frequency in *CSF3R* in CNL varies from 89% to 100% in different studies. Two types of mutations are found in *CSF3R*; most occur in the extracellular domain (membrane proximal point mutations), and a small number occur in the cytoplasmic portion of the receptor (non-sense or frameshift mutations) leading to truncation of the cytoplasmic domain. The most common membrane proximal mutations include *T618I* and *T615A*. These mutations result in ligand-independent activation of *CSF3R* that initiates downstream signaling through *JAK2*. The point mutation is usually present in isolation or can be seen along with compound frameshift or non-sense mutations. *CSF3R* mutations have not been reported in patients with reactive neutrophilia. Cases of CNL in which *CSF3R* was commutated with cooperating mutations in either *SETBP1* or *ASXL1* mutations have been reported. The presence of coexisting *ASXL1* and *CSF3R* mutations may indicate a worse prognosis.

Cytogenetic findings occur rarely (10%) in CNL, including +8, +9, del(20), and del(11q). These findings would be important as a proof of clonality but do not help in ruling out other clonal myeloid disorders. If no cytogenetic or molecular clone is identified then a clonality assay, such as the human androgen receptor assay (HUMARA), may be necessary to prove that a clone is present; this is particularly the case when there is an associated plasma cell dyscrasia, in order to rule out a reactive neutrophilia secondary to the plasma cell proliferation (Fig. 17.29).

Prognosis and Therapy

The overall survival in CNL is variable but usually low, with a median survival of less than 2 years according to some studies. A recent study evaluated the role of various factors for prognostication including age, LDH levels, splenomegaly, hemoglobin, thrombocytopenia, total bilirubin levels, *SETBP1* mutation, *ASXL1* mutation, and *T618I* versus other *CSF3R* mutation in a group of 14 cases of *CSF3R*-mutated CNL. On a multivariate analysis, only *ASXL1* mutation and thrombocytopenia were found to be independently predictive of a shorter survival. The median survival in this group was 23.2 months. A trend of short survival has also been reported in patients with coexisting *CSF3R* and *SETBP1* mutations. A transformation to AML can occur but is not common.

MYELOID/LYMPHOID NEOPLASMS ASSOCIATED WITH EOSINOPHILIA AND REARRANGEMENTS OF *PDGFRA, PDGFRB,* OR *FGFR1* OR WITH *PCM1-JAK2*

Myeloid and lymphoid neoplasms with eosinophilia and rearrangements of *PDGFRA, PDGFRB, FGFR1,* or *PCM1-JAK2,* according to the WHO classifications (2008 and 2016), are segregated from MPN classification as a stand-alone category.

After CML, the myeloid neoplasms with eosinophilia were the next group of MPNs discovered to be related to TK signaling dysfunction. Compared to the hard-won inquiry in CML

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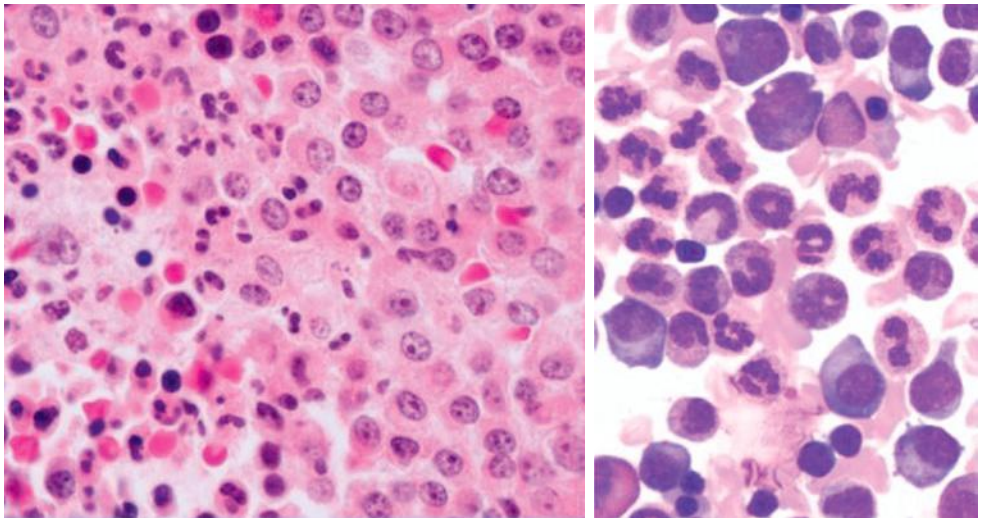


FIG. 17.29
Suspected chronic neutrophilic leukemia in which the neutrophil proliferation was shown to be associated with a plasma cell myeloma.

with its decades-long, logical scientific progression of knowledge, the discovery in the eosinophilic disorders came somewhat indirectly. After imatinib was approved for use, it was tried in a number of disorders other than CML; in these trials, some of the eosinophilic disorders were found to be exquisitely sensitive. This finding implied that some of the eosinophilic diseases were due to overactivity of an inhibitable TK; these were later realized to be the receptor TKs, *PDGFRB,* or *PDGFRA* (platelet-derived growth factor receptor, β and α genes, respectively). These genes have been shown to be altered in rare chromosomal translocations involving 5q31-3 (*PDGFRB*) and deletions of 4q (*PDGFRA*). Another disease added to this group was a rare disorder with eosinophilia and associated with rearrangement of the *FGFR1* (fibroblast growth factor receptor 1 gene), yet another TK receptor. Uniquely, the 8p11 usually develops as a myeloid proliferation but subsequently can develop into or can initially present as a precursor lymphoid neoplasm, more frequently the precursor T-cell type than precursor B. As such, the 8p11 myeloid neoplasm resembles CML, in that it seems to be a stem cell disorder that can transform to or sometimes present in a lymphoid blast crisis. This stem cell nature of the process has led to the separation of these entities (as mentioned previously) and the use of the term *myeloid and lymphoid neoplasm*. Unlike cases with rearrangement of *PDGFRA* or *B* that are responsive to imatinib, myeloid or lymphoid neoplasms associated with the 8p11/*FGFR1* rearrangement are not responsive to the first- and second-generation TKI. Ponatinib, a third-generation TKI with a high affinity to *FGFR1*, has shown treatment efficacy in individual cases. Recently, this group also incorporates myeloid/lymphoid neoplasms associated with t(8;9)(p22;p24.1) *PCM1-JAK2* as a new provisional entity. The prognosis of patients with *PCM1-JAK2* is poor, and targeted therapy with *JAK2* inhibitors offers the possibility of benefit. Last, it is recognized that myeloid/lymphoid neoplasms with these specific molecular/genetic changes have heterogeneous presentations, and some may not present with eosinophilia.

Clinical Features

The myeloid neoplasms associated with eosinophilia (as defined above) are uncommon, and the exact incidence is not known. In general these diseases can occur at any age, but some are more common in the fourth decade and are more frequent in men. The eosinophilia is in the blood and bone marrow, but there is also tissue infiltration that frequently results in end-organ damage due to cytokines, enzymes, and other proteins released from the eosinophils. The most serious complication of this is endomyocardial fibrosis that can result in a constrictive cardiomyopathy. Many patients also have splenomegaly. Transformation to acute leukemia is not common in most of the eosinophilic disorders, except in the *FGFR1* rearranged cases. Patients with the 8p11/*FGFR1* rearrangement frequently have lymphadenopathy, splenomegaly, or sometimes mediastinal masses. The disease is frequently aggressive with transformation to an acute process within 1 or 2 years. In patients with *PCM-JAK2* rearrangement, about one half to two thirds of the patients present with marked eosinophilia. Other patients can present like MPN or MDS/MPN.

Pathologic Features

General, Blood, and Bone Marrow

In patients with *PDGFRA* rearrangement, the presentation is generally similar to chronic eosinophilic leukemia but can present as AML, T-lymphoblastic leukemia (T-LBL), or both simultaneously. Similar to CEL, NOS the BM is hypercellular with markedly increased eosinophils with or without increased precursors. Blasts are generally not increased. There may be necrosis and Charcot-Leyden crystals. Bone marrow mast cells are often increased on trephine biopsy, which is especially

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MYELOID/LYMPHOID NEOPLASMS ASSOCIATED WITH EOSINOPHILIA AND REARRANGEMENTS OF *PDGFRA, PDGFRB,* OR *FGFR1* OR WITH *PCM1-JAK2*—FACT SHEET

Definition

- A myeloid/lymphoid neoplasm characterized by the presence of rearrangements of *PDGFRA, PDGFRB,* or *FGFR1* or with *PCM1-JAK2,* often associated with a proliferation of eosinophils and their precursors in the blood and marrow, and infiltration into tissue
- *PDGFRA* on 4q12
- *PDGFRB* on 5q33
- *FGFR1* on 8p11
- *PCM1-JAK2 or* t(8;9)(p22;p24.1)- Provisional entity

Incidence

- Rare, unknown

Clinical Features

- Mean age in fourth decade, more common in men
- Splenomegaly common
- End-organ damage owing to infiltration by eosinophils is common
- Endomyocardial fibrosis, most serious
- Lymphadenopathy in cases with *FGFR1* (8p11)

Prognosis and Therapy

- Chronic disease, rare transformation to acute leukemia, except for *FGFR1* (8p11) and *PCM-JAK2*–associated cases, which can present in or transform to acute leukemia of myeloid or lymphoid type
- Subset of patients respond to imatinib but not those with *FGFR1* (8p11) or *PCM1-JAK2* or t(8;9)(p22;p24.1)
- *FGFR1* (8p11) rearranged cases: Chemotherapy for ALL/AML with *FGFR1* inhibitor monoclonal antibody, followed by allogeneic stem cell transplant
- *PCM1-JAK2 or* t(8;9)(p22;p24.1)- *JAK2* inhibitor may be beneficial

a common feature of *FIP1L1-PDGFRA*–associated MPN. The mast cells are often scattered or in loose non-cohesive clusters, but they are often spindle with aberrant CD25+ expression, and only occasionally indistinguishable from those of systemic mastocytosis. Patients presenting with AML or T-LBL often have a pre-phase or coexisting PB eosinophilia.

PDGFRB can have multiple partner genes. Rearrangement of t(5;12)(q31~33;p12) with formation of an *ETV6-PDGFRB* is the most common. The hematologic features are most often those of CMML with eosinophilia, but some patients can show hematologic features of atypical chronic myeloid leukemias (aCML) with eosinophilia, CEL, or other MPN with increased eosinophils. AML is very rare. Rare cases with coexisting T-lymphoblastic lymphoma have been reported.

In the 8p11/*FGFR1* rearranged myeloid/lymphoid neoplasms, blood counts are variable, but 85% of patients have eosinophilia, and another 75% have an absolute monocytosis. The bone marrow is typically hypercellular, frequently with increased eosinophils in addition to MPN features. When blasts are increased sufficient to diagnose an acute leukemia, the blasts are more frequently myeloid (in two thirds of cases) than lymphoid. The lymphoid blasts are usually of precursor T type, but some may be of precursor B-lymphoblast type. The latter is often associated with t(8;22)/*BCR-FGFR1* rearrangement. In patients who have lymphadenopathy, the majority have a T-lymphoblastic process, with or without myeloblasts, whereas the remainder have a myeloid process.

Myeloid/lymphoid neoplasms associated with t(8;9)(p22;p24.1) or *PCM1-JAK2* rearrangement is a new provisional entity under WHO 2016. This rare entity are characterized by a combination of eosinophilia with BM findings of left-shifted erythroid predominance, lymphoid aggregates, and often myelofibrosis mimicking PMF. It may also rarely present as T-cell or B-cell lymphoblastic leukemia. Other *JAK2* rearranged neoplasms like *ETV6-JAK2*: t(9;12)(p24.1;p13.2) and *BCR-JAK2*: t(9;22)(p24.1;q11.2) may have similar features but are currently best classified as Ph-like B-ALL, another provisional entity under B-ALL as per the WHO 2016.

Molecular and Genetic Analyses

Molecular and genetic analyses play an important role in the definitive diagnosis and sub-classification of the myeloid neoplasms with eosinophilia. When the clinical workup and blood and marrow evaluation have ruled out reactive causes (infectious- or malignancy-related), and when they have also ruled out other myeloproliferative disorders such as CML associated with eosinophilia, an assessment for alterations of *PDGFRA, PDGFRB, FGFR1*, or PCM1-JAK2 by cytogenetic, molecular, or FISH analysis should be undertaken. Although abnormalities of *PDGFRB* and *FGFR1* are usually detected at the cytogenetic level with chromosomal abnormalities—t(5;12)(q31-q33;p12) and t(8;13)(p11;q12)—the abnormalities of *PDGFRA* involving 4q are cryptic. The molecular fusion between *PDGFRA* and another gene at 4q, *FIP1L1,* is usually due to a tiny deletion of an intervening gene called *CHIC2,* and this deletion cannot be readily seen microscopically at the level of the chromosomes. The deletion of *CHIC2* that can be identified by FISH has become a surrogate marker for the *FIP1L1-PDGFRA* fusion.

Differential Diagnosis

As mentioned previously, the myeloid neoplasms associated with eosinophilia must be distinguished from disorders in which there is a reactive or secondary eosinophilia or from a clonal eosinophilia associated with other myeloid diseases. Frequent causes of reactive eosinophilia include allergy, parasitic disease, other infections, and hypersensitivity or autoimmune disorders. Reactive eosinophilia seen in association with neoplastic disorders, in which the eosinophils are not part of the neoplastic clone, must also be excluded. Some disorders frequently associated with eosinophilia include T-cell lymphoma, Hodgkin lymphoma, acute lymphoblastic leukemia (especially precursor B-cell acute lymphoblastic leukemia

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MYELOID/LYMPHOID NEOPLASMS ASSOCIATED WITH EOSINOPHILIA AND REARRANGEMENTS OF *PDGFRA, PDGFRB,* OR *FGFR1* OR WITH *PCM1-JAK2*—PATHOLOGIC FEATURES

Microscopic Findings

Blood

- Eosinophil count ≥1.5 × 10^9/L common but may not be invariably present
  - Abnormal eosinophils (hyogranulation, abnormal nuclear lobation, immature forms) are frequently observed (Fig. 17.30)
  - Neutrophilia or basophilia in some cases
  - Monocytosis in some cases
- Blasts usually <2%

Marrow

- Hypercellular owing to infiltration by mature eosinophils, some may have increased neutrophils, monocytes
  - Megakaryocytes can be large/hyperlobated, clustering, or mixed with small hypolobated forms
  - Blasts often <5%
  - Some dysplasia in other hematopoietic elements
  - Fibrosis present
  - Charcot-Leyden crystals may be present
  - Mast cell proliferations may be present, usually those with *PDGFRA* abnormalities

Ancillary Studies

Cytogenetic/molecular analysis

- del(4q12)/*PDGFRA* cannot be detected on karyotype (cryptic); FISH and or RT-PCR study is needed for suspected cases
- *PDGFRB* at 5q33 translocations have many partners; t(5;12)(q31-q33;p12)/*ETV6-PDGFRB* most common

8p11/*FGFR1* translocations have many partners; t(8;13)(p11;q12) most common

PCM1-JAK2 or t(8;9)(p22;p24.1); should be distinguished from t(8;9)(p11;q33)/*CEP110-FGFR1*.FISH for *JAK2* and *FGFR1* molecular abnormalities should be performed to confirm the rearrangement as suggested by karyotyping result

Differential Diagnosis

- Reactive eosinophilia owing to allergy, parasitic disease, other infections, hypersensitivity, autoimmune disorders, neoplastic diseases such as T-cell lymphoma
- Clonal eosinophilia associated with CML, AML, and other MPNs

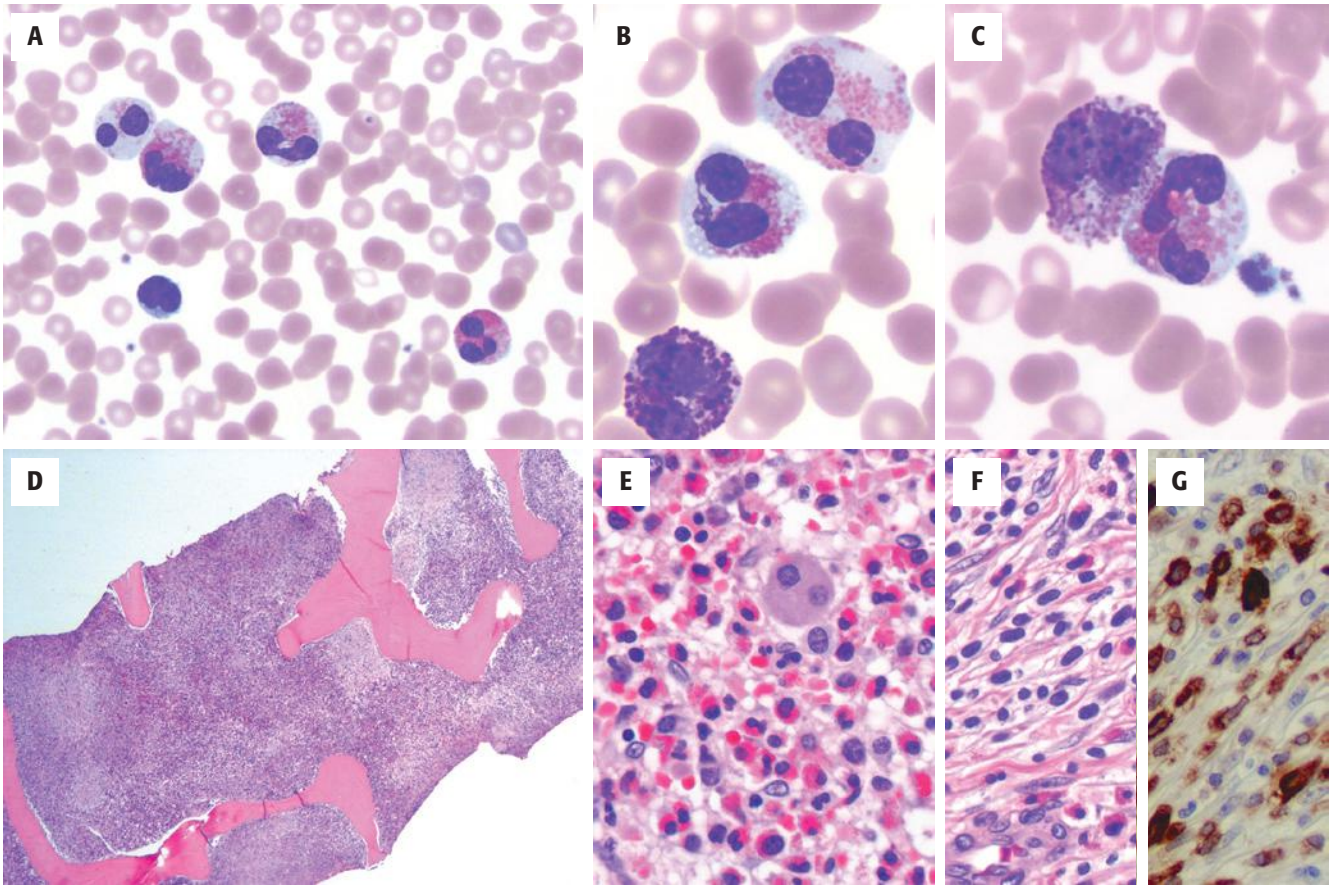


FIG. 17.30

Myeloid neoplasm with eosinophilia: This 32-year-old male patient had a sustained eosinophil count in the blood of greater than 15 × 10^9/L (A). Some of the eosinophils are hypogranular (B), but this is not sufficient to consider the eosinophils malignant, as this can be seen in reactive states. There was also a slight basophilia (B). Occasional eosinophils are immature with primary (blue) granules (C), but these are not the abnormal granules in the eosinophils of acute myeloid leukemia with inv(16). The biopsy was markedly hypercellular (D) with some areas of fibrosis. The increased cellularity was owing to a proliferation of mature eosinophils, sometimes associated with abnormal or dysplastic cells such as the small megakaryocytes (E). In this patient, there were spindled cells in the areas of fibrosis (F) that were positive for mast cell tryptase (G), indicating a mast cell component to the process. This case was associated with *FIP1L1-PDGFRA* identified by a *CHIC2* deletion by fluorescence in situ hybridization.

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[B-ALL] with t[5;14]), and mastocytosis. The lymphocytic variant of HES should also be considered. This is discussed in Chapter 13. Disorders that may have an eosinophilia in which the eosinophils are part of the clone include CML, AML, other MPN, and MDS. Obviously these too must be excluded.

Prognosis and Therapy

Patients with abnormalities of *PDGFRA* on 4q and *PDGFRB* on 5q31-3 have been shown to have excellent response to treatment with imatinib, because both the α and β subunits of *PDGFR* are TKs that are abnormally activated because of the respective fusions. This therapy can provide impressive resolution of the eosinophilia and of the harmful sequelae of the eosinophilic infiltration. Patients who are responsive to treatment may become resistant because of mutations that are analogous to the mutations in CML that result in a TK resistance.

Patients with a diagnosis of 8p11/*FGFR1* rearranged cases are treated with a variety of therapies, including those for ALL, AML, or MPN. Overall, these therapies have been inadequate, as there are only a few long-term survivors, with a median survival of only 15 months. The third-generation TK inhibitor ponatinib has shown treatment efficacy in individual cases. Recently, an *FGRF1* monoclonal antibody is in clinical trials and its efficacy is yet to be determined. Stem cell transplantation is the only option that has produced better results.

CHRONIC EOSINOPHILIC LEUKEMIA, NOS/ HYPEREOSINOPHILIC SYNDROME

Chronic eosinophilic leukemia, not otherwise specified (CEL, NOS) and the HES are disorders associated with hypereosinophilia (HE) in the blood ($\geq 1.5 \times 10^9$/L) and bone marrow that can infiltrate into other organs. By the current WHO definition, CEL, NOS is associated with an elevated blast count (>5% but <20%) in PB or BM or is clonal by the finding of an associated clonal cytogenetic abnormality or a mutation of a gene. Idiopathic HES is a disease diagnosed by exclusion when the eosinophilia is persistent ($\geq 1.5 \times 10^9$/L for at least 6 months), has no known underlying cause, and cannot be shown to be clonal or associated with increased blasts. Idiopathic HES has to show end-organ damage, either proven by biopsy, or radiographic suggestion or clinical manifestations; if absent, *idiopathic HE of uncertain significance* is the preferred terminology. For the diagnosis of CEL, NOS/HES, other diagnoses that need to be excluded include disease entities associated with HE in addition to well-described rearrangements involving *PDGFRA, PDGFRB, FGFR1*, and *PCM1-JAK2*. Though not well studied in literature, there are certain clinical features that can distinguish between CEL, NOS and idiopathic HES. CEL, NOS is seen more commonly in older individuals and presents more frequently with constitutional symptoms, anemia, thrombocytopenia, and symptoms related to cytopenias, frequent organomegaly, elevated LDH levels, and less frequently with skin manifestations such as allergy, urticarial/rash, edema, asthma, myalgia/arthralgia, or eosinophil-mediated organ injury. In contrast, patients with HES with no identifiable mutations present at a much younger age and have more frequent symptoms associated with eosinophil activation, such as dermatologic, pulmonary, gastrointestinal, and rheumatologic manifestations.

CHRONIC EOSINOPHILIC LEUKEMIA, NOS AND IDIOPATHIC HYPEREOSINOPHILIC SYNDROME—FACT SHEET

Definition

- CEL, NOS is a hematopoietic stem cell neoplasm characterized by hypereosinophilia ($\geq 1.5 \times 10^9$/L); idiopathic HES is a group of disorders featured by a proliferation of eosinophils ($\geq 1.5 \times 10^9$/L for at least 6 months) with associated organ/tissue damage, for which the underlying causes are unknown
- Due to overlapping features and difficulty in proving clonality, CEL, NOS and idiopathic HES are currently listed together under the WHO classification
- A diagnosis of CEL, NOS and idiopathic HES requires
  - Absence of Ph chromosome or *BCR-ABL1* fusion gene or other myeloproliferative neoplasms (PV, ET, PMF, systemic mastocytosis) or MDS/MPN or inv(16)(p13q22) or t(16;16)(p13;q22) or lymphocytic variant of hypereosinophilia
  - No rearrangement of *PDGFRA, PDGFRB; FGFR1* or *PCM-JAK2*

Incidence

- Rare, unknown

Clinical Features

- Mean age is fourth decade for HES and sixth decade for CEL, NOS
- Both more common in men
- CEL, NOS frequently presents with constitutional symptoms, anemia, thrombocytopenia, and symptoms related to cytopenias, frequent organomegaly, elevated lactate dehydrogenase levels, and less frequently with skin manifestations such as allergy, urticarial/rash, edema, asthma, myalgia/arthralgia, or eosinophil-mediated organ injury
- HES more frequently shows symptoms associated with eosinophil activation, such as dermatologic, pulmonary, gastrointestinal, and rheumatologic manifestation. Endomyocardial fibrosis is most serious complication

Prognosis and Therapy

- The goal of therapy is to reduce eosinophil-mediated organ damage
- Corticosteroids are first line of therapy for patients with idiopathic HES
- Hydroxyurea and interferon-α have demonstrated efficacy as initial treatment and for steroid-refractory cases of HES
- A trial of TKI may be appropriate
- Early data from clinical trials on the use of anti-IL-5 (mepolizumab) and anti-CD52 (alemtuzumab) antibodies in HES have shown some efficacy

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Pathologic Features

Although not clearly stated in the WHO classification, a number of PB/BM morphologic features can assist in the separation of CEL, NOS from true HES. In CEL, NOS, bone marrow is often markedly hypercellular because of a prominent proliferation of eosinophils and granulocytic cells. Blasts are usually less than 2% in the blood and less than 5% in the marrow but can be elevated, which is one of the criteria to separate CEL, NOS from idiopathic HES. Megakaryocytes often exhibit abnormal features, but they are not typical of myeloproliferative neoplasm-type megakaryocytes but more MDS-like or with a mixed MDS/MPN like megakaryocytic morphology. Dysgranulopoiesis and/or dyserythropoiesis may be present in some cases. In contrast, idiopathic HES often shows a normal or only slightly increased cellularity due to increased eosinophils in BM. Normal BM topography/architecture is maintained. Trilineage hematopoiesis is present, and megakaryocytes are normal appearing. Mild dyserythropoiesis may be seen after hydroxyurea treatment, but marked dyserythropoiesis or dysgranulopoiesis is uncommon. BM infiltration by eosinophils is often associated with mild fibrosis; however, MF2 and MF3 fibrosis are more frequently seen in CEL, NOS over idiopathic HES. Increased bone marrow and peripheral blood eosinophils are seen in both the groups; however, patients with CEL, NOS show significantly more abnormalities in eosinophil granulation and more nuclear lobation (multilobation, hypolobation, nuclear branching) abnormalities than that seen in idiopathic HES cases. Dense clusters of mast cells are absent in both groups, but scattered and loose clusters of mast cells may be seen, more commonly in idiopathic HES than CEL, NOS.

Ancillary Testing and Differential Diagnosis

When considering a patient with eosinophilia for a possible myeloid/lymphoid disorder, it is necessary first to rule out reactive causes of eosinophilia, clinically and through evaluation of blood and bone marrow. This is critical because reactive causes of eosinophilia owing to allergic processes, infectious agents, drugs, Hodgkin lymphoma, solid tumor (e.g., squamous cell carcinoma of head and neck) or T-cell lymphomas are far more common than any of CEL, NOS/idiopathic HES. It is noteworthy that many patients with idiopathic HES have symptoms similar to those of autoimmune diseases or allergy, but no underlying causes can be identified. In these patients, hypereosinophilia is persistent over years and responsible for tissue injury and clinical symptoms.

CEL, NOS and idiopathic HES are diagnoses of exclusion. Therefore, for anyone presenting with persistent hypereosinophilia, studies for *BCR/ABL1*/Ph-chromosome rearrangements of *PDGFRA, PDGFRB, FGFR1*, and *PCM-JAK2* should be performed to confirm their absence. Furthermore, other well-defined myeloid neoplasms associated with eosinophilia must be ruled out.

The distinction between CEL, NOS and idiopathic HES remains challenging. The presence of clonal karyotypic abnormality supports a diagnosis of CEL, NOS. However, karyotypic abnormalities only occur in a subset of cases in any given MPN or MDS/MPN (e.g., 5% to 10% in ET, 10% to 20% in PV and up to 30% in PMF). Mutations in genes frequently associated with MPNs, such as *JAK2, MPL, CALR, RAS,* and *KIT,* are very infrequent in CEL, NOS. Recently, using NGS technology, mutations, mostly in genes related to DNA methylation and histone modifications such as *ASXL1, TETS,* and *DNMT3A,* are detected in about 25% to 30% of idiopathic HES (by the WHO classification). Although mutation data may provide evidence of clonality and help to identify some idiopathic HES as a clonal hematopoietic neoplasm, an appropriate interpretation needs to consider the caveats of the high prevalence of clonal hematopoiesis of uncertain significance in the elderly population.

Prognosis and Therapy

The goal of therapy is to reduce eosinophil-mediated organ damage. Corticosteroids are first-line therapy for patients with idiopathic HES. Hydroxyurea and interferon-α have demonstrated efficacy as initial treatment and for steroid-refractory cases of HES. Some patients with CEL, NOS and idiopathic HES may benefit from a trial of TK inhibitor therapy, because there can be difficulty in detecting the molecular abnormalities, such as *PDGFRA* and *PDGFRB* fusions, and treatment with a TK inhibitor has little toxic effect. If patients respond, it must be assumed that they have clonal disease associated with an undescribed molecular change that is responsive to the TK inhibitor. Early data from clinical trials on the use of anti-IL-5 (mepolizumab) and anti-CD52 (alemtuzumab) antibodies in HES have shown some efficacy.

MYELOPROLIFERATIVE NEOPLASMS, UNCLASSIFIABLE (MPN-U)

The WHO committee writing on hematopoietic tumors included a category of MPN for cases that did not fit into the entities described previously. MPN-U was not intended for cases in which a better classification could not be made because of lack of information or clinical data. It is intended for cases that have clear clinical, laboratory, and morphologic features of an MPN but do not meet criteria for a specific type or for cases that have features that overlap two or more MPN categories. Most cases in the category are either early cases for which a more definitive diagnosis cannot be made or advanced cases in which a definitive diagnosis at an earlier stage was not made, and in which secondary changes have masked more typical features. The former situation commonly

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arises for patients with very early PV, ET, and PMF. Follow-up studies at 6-month intervals might be necessary before a better classification can be attempted. For the latter situation, when marked secondary changes such as fibrosis or osteosclerosis obscure the diagnosis, it is necessary to rule out CML with secondary changes as seen in the accelerated phase. This may be important because the correct diagnosis of the accelerated phase of CML should initiate a trial of imatinib with adjuvant chemotherapy or one of the newer more potent TK inhibitors. A third category is for cases that are obviously an MPN but in which the classification is complicated by a coexisting disease that obscures applying the diagnostic criteria. A typical example is an MPN coexisting with chronic lymphocytic leukemia, where the CLL infiltrate may obscure a full assessment of the underlying MPN.

MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS

Myelodysplastic/myeloproliferative neoplasm (MDS/MPN), a nosologic group of disorders, was first introduced in the 2008 WHO as a new disease category to include myeloid neoplasms with clinical, laboratory, and morphologic features overlapping between MDS and MPN. This category includes several well-defined entities—namely, chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), atypical (*BCL/ABL1*-negative) chronic myeloid leukemia (aCML), and MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T). The latter was a provisional entity in the 2008 WHO classification, and at that time, it was given a name of refractory anemia with ring sideroblasts with marked thrombocytosis (RARS-T). The discovery of frequent association with spliceosome gene *SF3B1* mutation (highly associated with MDS with ring sideroblasts) as well as mutations in *JAK2 V617F* or less frequently (<10%) with *CALR,* or *MPL* genes (mutations highly associated with MPN), provides sufficient evidence to support MDS/MPN-RS-T as a full entity in the 2016 WHO revision. There are still a handful of cases that cannot fit into any of the above entities and remain as "unclassifiable" MDS/MPN (MDS/MPN-U).

CHRONIC MYELOMONOCYTIC LEUKEMIA

Chronic myelomonocytic leukemia was considered as a form of MDS in the French-American-British (FAB) classification system. However, the clinicopathologic features were somewhat perplexing; in some cases, there was a prominent proliferative component. CMML was therefore further divided into two types—a dysplastic type (MD) with lower white counts and a proliferative type (MP) with high counts (13×10^9/L being the dividing line). However, it was questioned if such classifications had any clinical utility or biologic meaning and this subdivision was not recognized by the earlier editions of the WHO classification schemes. In recent years, studies have shown that CMML with a high WBC differs from CMML with a low WBC in clinical and molecular characteristics, particularly the aberrations in the RAS/MAPK signaling pathways. With the emerging evidence, a subclassification of CMML into MD and MP subtypes is warranted, and it is finally recognized by the 2016 WHO revision.

In addition, it has been shown that a more precise prognostication can be obtained with three rather than two blast-based groupings (CMML1 and CMML2). In the 2016 WHO revision, CMML has been further divided into CMML0, CMML1, and CMML2, based on BM and PB blast counts. The new subcategorization poses further challenges in counting blasts, since promonocytes (blast equivalent cells) are notoriously difficult to be separated reliably from dysplastic/abnormal monocytes. With recent advances in next-generation sequencing, mutations in epigenetic machinery have been found frequently in CMML, providing prognostic information for patients with CMML.

Clinical Features

Chronic myelomonocytic leukemia occurs most commonly in older adults, with a median age of 65 to 75 years, showing a male predominance. It is the most common type of MDS/MPN. CMML occurs primarily de novo, and a small subset can occur in patients who have prior exposure to cytotoxic agents, as therapy-related CMML. CMML also can transform from preexisting MDS, which is often accompanied by acquisition of additional molecular genetic events, such as *ETV6* and signaling gene mutations.

CMML is characterized by a proliferation of peripheral monocytes that is often accompanied by anemia or thrombocytopenia. Splenomegaly is present in up to 25% of patients and is often accompanied by hepatomegaly, lymphadenopathy, or nodular cutaneous leukemic infiltrates. Constitutional symptoms such as fevers, unexplained weight loss, and night sweats, similar to those associated with primary myelofibrosis, are observed in some patients with CMML.

Pathologic Features

Blood

White blood cell count in CMML varies widely from 2 to 500×10^9/L. The median counts are between 10 and 20 $\times 10^9$/L (Figs. 17.31 and 17.32). Some patients have thrombocytopenia, but some may actually have normal or even slightly increased platelet counts. Anemia is common but usually mild.

A characteristic feature in the blood, and a necessary diagnostic criterion, is an increase in monocytes, both proportionally (≥10%) and absolutely ($1 \times \geq 10^9$/L). This point is important, because it is easy to reach $1 \times \geq 10^9$/L

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CHRONIC MYELOMONOCYTIC LEUKEMIA—FACT SHEET

Definition

- A clonal hematopoietic neoplasm characterized by a proliferation of monocytes in blood and marrow (blood monocytosis ≥1 × 10^9/L and ≥10% of the WBCs)
- Ineffective hematopoiesis leading to anemia and or thrombocytopenia

Subclassification

By WBC

- Proliferative type (WBC ≥13 × 10^9/L)
- Dysplastic type (WBC <13 × 10^9/L)

By blast count

- CMML-0 (<2% blasts in PB and <5% blasts in BM)
- CMML-1 (2% to 4% blasts in PB and/or 5% to 9% blasts in BM)
- CMML-2 (5% to 19% blasts in PB and/or 10% to 19% blasts in BM and/or any Auer rods present)

Incidence, Gender, and Age Distribution

- Age-adjusted incidence of CMML in the United States is approximately 0.3 per 100,000
- Occurs in elderly, median age at diagnosis 65 to 75 years
- Male predominance

Clinical Features

- Symptoms: asymptomatic but with an abnormal blood count; or presenting with symptoms or complications due to unrecognized cytopenia(s); or splenomegaly
- Physical findings: splenomegaly in up to 25% of patients

Prognosis

- Survival varies widely; overall median survival 20 to 40 months

absolute monocyte count cutoff in other myeloid neoplasms with a high white blood cell count, even with a very low proportion of monocytes (e.g., when WBC count is 50 × ≥10^9/L, an absolute monocyte count only requires 2% monocytes to reach 1 × ≥10^9/L absolute monocyte count). In CMML, monocytosis can range from minimal (1 × ≥10^9/L) to rather dramatic with counts as high as 100 to 200 × 10^9/L; however, most patients have modest numbers (~5 × 10^9/L). Monocytes can show various dysplastic features and may appear slightly immature. Blasts and promonocytes usually account for less than 10% of the cells but are always less than 20%. Granulocytes also vary significantly in number but are usually not decreased. Neutrophil precursors (promyelocytes, myelocytes, and metamyelocytes) are usually not as prominent but, if present, have been shown to be adverse features in CMML. Granulocytes may or may not exhibit dysplastic features. Basophilia and eosinophilia are uncommon. If a prominent eosinophilia, it may be associated with a recurring cytogenetic abnormality, t(5;12)(q33;p13) involving *PDGFRB* or *PCM1-JAK2*. Such a case should be classified as myeloid neoplasm with *PDGFRB or PCM1-JAK2* rearrangement.

Bone Marrow

Bone marrow is hypercellular in most patients, and increased cellularity is often due to a granulocytic proliferation and, surprisingly, often not monocytes. Monocytic proliferation sometimes can be difficult to appreciate without the aid of a nonspecific esterase reaction, although sometimes it is obvious, even on the biopsy (Fig. 17.33). Dysgranulopoiesis may or may not be present. The myeloid cells may be left

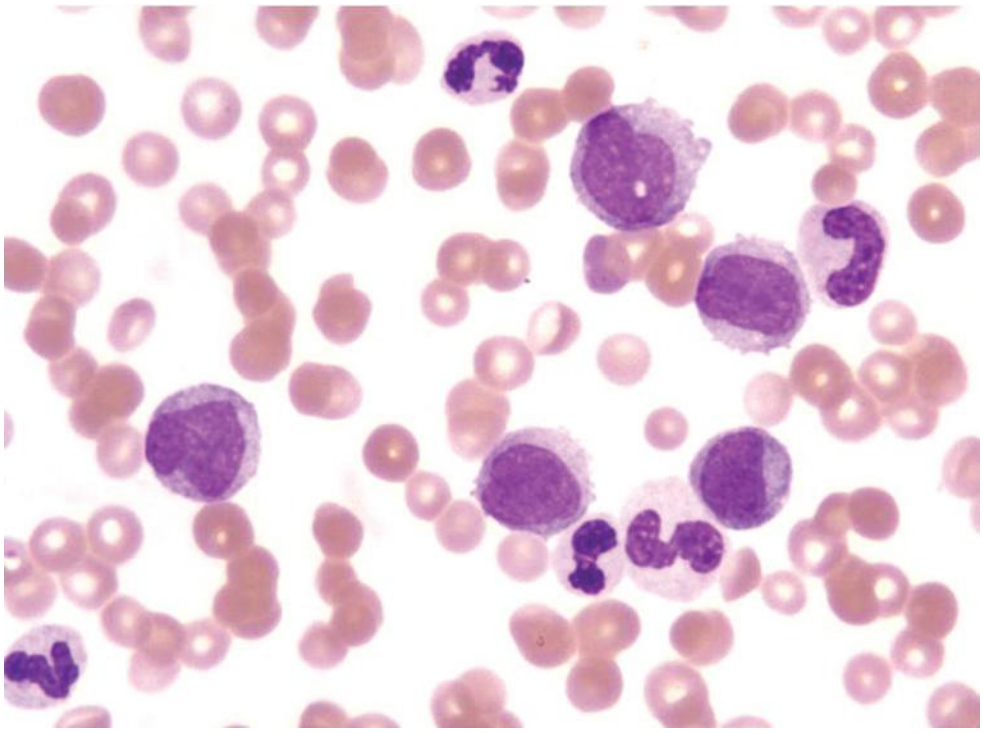


FIG. 17.31
Peripheral blood smear in chronic myelomonocytic leukemia. Note the monocytes, dysplastic granulocytes, and absent platelets.

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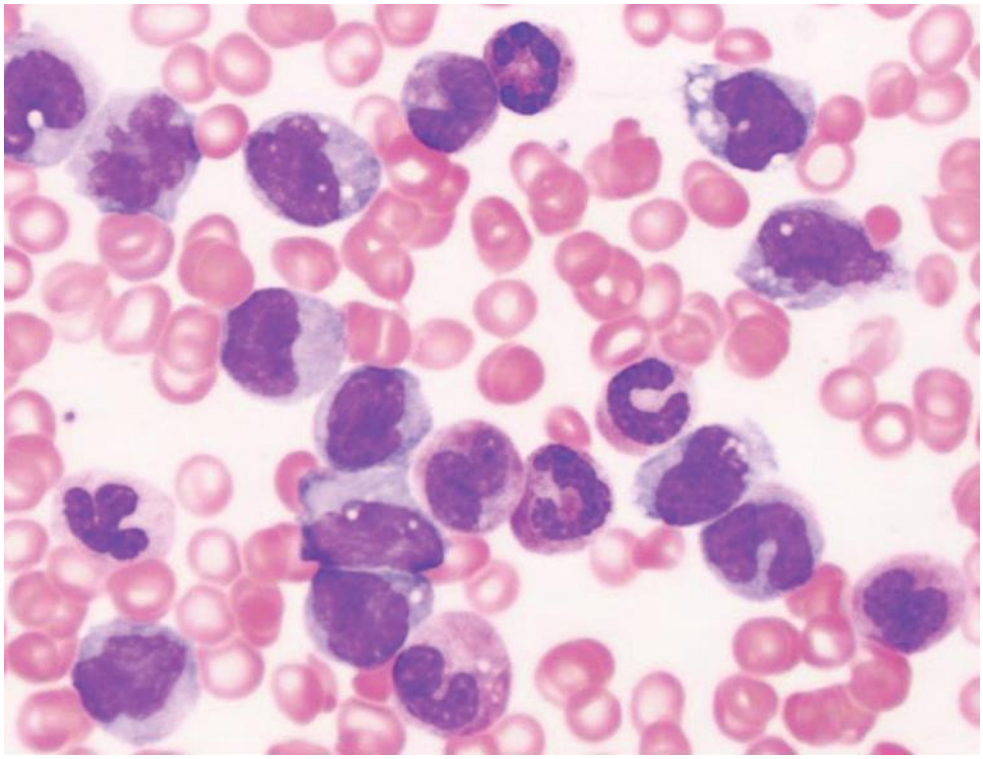


FIG. 17.32

Peripheral blood smear in a case of chronic myelomonocytic leukemia with high count. Note the minimal dysplasia in the granulocytes.

CHRONIC MYELOMONOCYTIC LEUKEMIA—PATHOLOGIC FEATURES

Diagnostic Criteria (WHO 2016)

- Persistent peripheral blood monocytosis ≥1 × 10^9/L and ≥10% of WBC
- No Philadelphia chromosome or *BCR-ABL1*
- No rearrangement of *PDGFRA, PDGFRB, FGFR1,* or *PCM1-JAK2*
- Fewer than 20% blasts (myeloblasts, monoblasts and promonocytes) in blood or marrow

Dysplasia in one or more myeloid cell lines or, if absent or minimal dysplasia, the diagnosis can be made if the other requirements are met and include

- An acquired clonal cytogenetic or molecular abnormality is present
  - The monocytosis has persisted for at least 3 months and other causes of monocytosis have been excluded

Microscopic Features

Blood

- WBC varies from 2 to 500 × 10^9/L; a WBC 13 × 10^9/L divides CMML into proliferative vs. dysplastic type
- Monocytosis, both relative and absolute, ≥10% monocytes, and ≥1 × 10^9/L
- Thrombocytopenia and mild anemia common
- Blasts and promonocytes may present in variable numbers but <20%
- Granulocytic dysplasia may be present

Bone marrow

- Hypercellular, myeloid-to-erythroid ratio is often increased
- Monocytes may or may not increase; sometimes difficult to appreciate
- Dysplasia in granulocytes, erythroid precursors, and megakaryocytes often variably present; in some cases, may be subtle
- MF-1 fibrosis may be present; MF-2 to -3 fibrosis is not common
- Plasmacytoid dendritic cell nodules or mast cell infiltrate may be seen in some cases

Ancillary Studies

- Nonspecific esterase reaction useful to help identify monocytes on smears
- Immunohistochemistry with CD11c, CD14, lysozyme, CD68 can be used to stain monocytes CD117 and tryptase to identify associated mast cell proliferation
- CD123 and TCL1 help to stain plasmacytoid dendritic cell nodules

Flow cytometry immunophenotype

- CD34+ myeloblasts almost always show aberrancy
- Monocytes exhibit numerous abnormalities, see description

Cytogenetic analysis

Karyotypic abnormalities are seen in ~30% of CMML, providing prognostic information. It is important to exclude t(9;22) and *BCR-ABL1* and recurrent genetic abnormalities associated with specific entities

Molecular studies

Mutations have been found very common in CMML, including *SRSF2, TET2,* and/or *ASXL1*, *SETBP1, NRAS/KRAS, RUNX1, CBL*, and *EZH2*.

Differential Diagnosis

- Reactive monocytosis
- Myelodysplastic syndromes
- AML, monocytic
- CML
  - Atypical CML (aCML)
- Myeloid neoplasms with t(5;12)(q33;p13) *ETV6/PDGFRB* or t(8;9)(p22;p24) *PCM1-JAK2*

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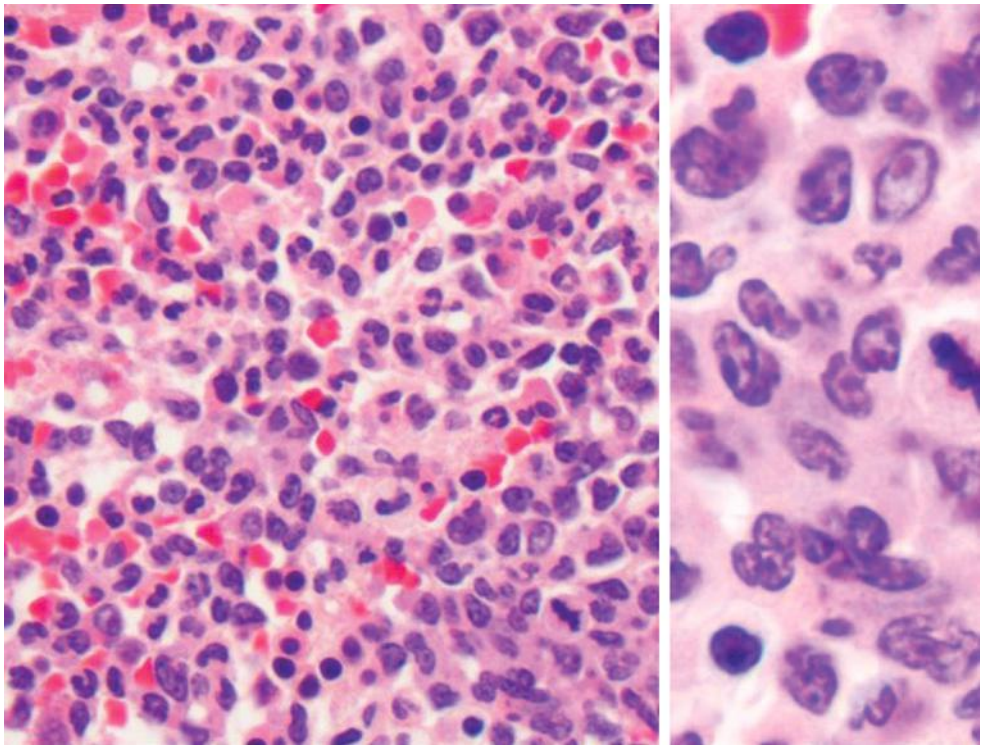


FIG. 17.33

Bone marrow biopsy in chronic myelomonocytic leukemia showing a proliferation of granulocytes and monocytic elements. The monocytes are prominent in this case and show clustering *(right),* but in some cases they are only slightly increased in the marrow.

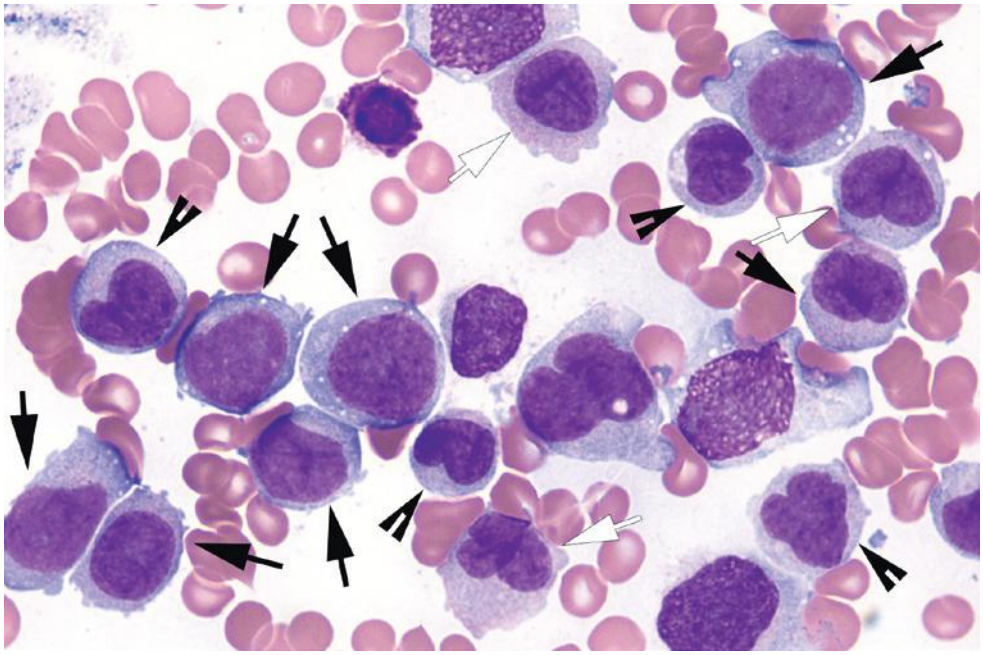


FIG. 17.34

Monoblasts, promonocytes, and abnormal monocytes. A case of acute monocytic leukemia transformed from chronic myelomonocytic leukemia. Monoblasts are large, with round nuclei, moderate to abundant cytoplasm, open chromatin, and nucleoli *(black arrows);* promonocytes: cells with delicate folds, open chromatin, and moderate cytoplasm *(white arrows);* abnormal monocytes: cells have mature chomatin and deeper folds *(arrowheads).*

shifted, but myeloblasts, monoblasts, and promonocytes are less than 20%. It is important to recognize promonocytes (blast equivalent) (Fig. 17.34), which have immature chromatin and delicate folds, in contrast to abnormal/dysplastic monocytes, which often have mature chromatin and convoluted/segmented nuclei. Erythroid precursors are often reduced and can show dysplasia; however, ring sideroblasts (RS) are not common (<10%). Cases with RS are frequently associated with spliceosome mutations involving *SRSF2, SF3B1,* and *U2AF35*, particularly *SF3B1.* Megakaryocytes are usually abnormal and can include large atypical forms, sometimes with widely spaced small nuclei or small micro-megakaryocytes. Reticulin fibrosis is commonly mildly increased, and reactive lymphoid aggregates may be seen. In some cases, CMML can be associated with a mast cell proliferation, and the latter fulfills the criteria of systemic mastocytosis.

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In such cases, mast cells are often a part of the neoplastic hematopoiesis, sharing same molecular genetic abnormalities as CMML.

Other Extramedullary Tissues

The spleen is frequently enlarged, with abnormal myelomonocytic cells infiltrating the red pulp. Such infiltrates can be seen elsewhere, but lymph node involvement is rare. Transformation to acute leukemia can occur in the extramedullary sites.

Ancillary Studies and Differential Diagnosis

Reactive Monocytosis

The diagnosis of CMML should be considered when persistent (≥3 months), unexplained peripheral monocytosis is present in an older adult (median age at diagnosis: 65 to 75 years). In a patient who seeks medical attention in less than 3 months, absolute ($\geq 1 \times 10^9$/L) and relative (≥10%) monocytosis should be shown by at least two sets of CBC. Although monocytosis is the hallmark of CMML, it also can be associated with a number of non-neoplastic conditions. Reactive monocytosis can be seen in infections, such as brucellosis, cat-scratch disease, HIV-1, infectious mononucleosis, malaria, Rocky Mountain spotted fever, trypanosomiasis, tuberculosis, and autoimmune diseases (e.g., vasculitis, systemic lupus erythematosus, rheumatoid arthritis, and inflammatory bowel disease; lymphoma; sarcoidosis and lipid storage disease or obesity; or bone marrow recovering from acute infection or chemotherapy). Therefore, it is critical to prove a clonal neoplastic process in CMML.

Karyotypic abnormalities are seen in 20% to 40% of CMML. Cytogenetic findings are not unique in CMML and include +8, –7 or -7q, del(20q), +21, or complex abnormalities. CMML occurring in patients who received chemoradiation therapy (t-CMML) tends to carry a significantly higher frequency of cytogenetic abnormalities. Of note, whereas -5/-5q which is common in MDS, it is very uncommon in CMML: when present, it is seen mostly in therapy-related CMML. In recent years with the application of next-generation sequencing technology, much has been learned about the common molecular abnormalities in patients with CMML. Mutations in myeloid neoplasm-associated genes have been found in more than 90% of CMML. Commonly mutated genes in CMML are *SRSF2, TET2,* and/or *ASXL1* in more than 80% of cases; other mutations occurring at lower frequency include *SETBP1, NRAS/KRAS, RUNX1, CBL*, and *EZH2*. Mutational analysis can be a helpful adjunct study in difficult cases but should not be used alone as proof of neoplasia because some of these mutations occur in healthy older patients as so-called clonal hematopoiesis of indeterminate potential (CHIP). *NPM1* mutation is seen in a rare subset of CMML (3% to 5%), often at a low allele burden (<10%); if a high allele burden is detected, it often heralds a more aggressive clinical course.

Flow cytometry studies have recently shown that there is always a small population of CD34+ myeloblasts in the BM of patients with CMML. Although CD34+ myeloblasts only constitute a small number of BM cells, not corresponding to the morphologic blast count, they are almost always aberrant, supporting a neoplastic hematopoietic process initiated from early precursors. The common aberrancies include overexpression of CD13, CD117, and CD123 and under expression of CD38. Monocytes also show a number of changes, including aberrant expression of CD2 and CD56 (>30% cells); and altered expression of CD45, CD16, CD15, and human leukocyte antigen-D related (HLA-DR).

Myeloid Neoplasms With Recurrent Genetic Abnormalities

A diagnosis of CMML also requires exclusion of CML with molecular or cytogenetic analysis for *BCR-ABL1* and t(9;22) because rare cases of CML with *BCR-ABL1* gene associated with the p190 protein may present with increased monocytes. t(5;12)(q33;p13) can be seen in cases with monocytosis and eosinophilia. Currently, such cases would be best classified as myeloid neoplasms with *PDGFRB* rearrangement.

Acute Monocytic Leukemia

In some cases of acute monocytic leukemia (AMoL), monocytes in peripheral blood can be extremely mature, mimicking CMML. BM examination is very critical in such cases. Bone marrow biopsy in AMoL is often packed with monocytic blasts, with markedly decreased granulocytes and precursors. In contrast, CMML often shows granulocytic hyperplasia. BM aspirate smears show increased monoblasts and promonocytes in AML with monocytic differentiation but less than 20% immature cells in CMML. The finding of inv(16), or t(16;16), would indicate AML with abnormal eosinophils, and the finding of translocations involving 11q23 (*MLL*) would also suggest AML; both can present with borderline blast counts. *NPM1* mutation is very rare in CMML (3% to 5%), often at a low allele burden; if a high allele burden, it often heralds a more aggressive clinical course, biologically closer to AMML than CMML.

Atypical CML, MDS/MPN-U, MDS, and MPN

In general, the presence of persistent monocytosis in a patient with no prior history of a hematopoietic stem cell neoplasm, a diagnosis of CMML takes precedence over MDS, MDS/MPN, or atypical CML. Borderline or relative elevations in the monocyte count are common in MDS, and monocytes may fluctuate over time but they are not a persistent feature. Although some of these cases may represent early CMML, many remain to be MDS and never reach the criteria for a

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diagnosis of CMML. Dysplastic subtype of CMML and MDS share many biological, clinical, and prognostic features. Of note, some MDS may acquire a proliferation of monocytes and evolve to CMML in the course of disease. Other features more in favor of CMML over MDS are splenomegaly, leukocytosis, and constitutional symptoms. Atypical CML often presents with a very high WBC with dysgranulopoiesis and 10% or more circulating immature neutrophil precursors (promyelocytes, myelocytes, and metamyelocytes). MDS/MPN-U also can present with a high WBC. Whenever there is a high WBC, the absolute monocyte count can easily reach more than 1×10^9/L; on the other hand, in some patients with MDS/MPN, CBC may show transient monocytosis. Therefore, it is critical to require not only absolute but also relative (≥10%) monocytosis that is persistent in the diagnosis of CMML. In other myeloid neoplasms other than CMML with a high WBC, the percent of monocytes is less than 10%, often less than 5%. Some CMML cases can have an i(17q) as a sole abnormality and can be associated with prominent pseudo–Pelger-Huët granulocytes with condensed chromatin. These cases were initially thought to be classified as aCML, but it seems that most have sufficient monocytes to categorize them as CMML.

The *JAK2* V617F can be seen in about 5% to 10% CMML. The presence of *JAK2* V617F should not be the reason to favor a diagnosis of MPN over CMML. Of note, *JAK2* V617F mutation could occur as a secondary event in some cases of CMML. Finally, primary myelofibrosis at the time of diagnosis or during disease course may develop monocytosis. These cases should not be classified as CMML but rather be considered within the spectrum of PMF, particularly if the bone marrow feature is diagnostic of the latter. This in combination with the presence of MF2 or more myelofibrosis and increased LDH levels, seen commonly in PMF, also help in achieving the correct diagnosis.

Prognosis

The survival in CMML varies widely, and accurate data are lacking because of difficulty in diagnosis and classification. Overall the median survival is 20 to 40 months after diagnosis. AML progression occurs in approximately 15% to 20% of patients. Independent prognostic factors include an older age, poor performance status, thrombocytopenia, anemia, increased BM blasts, leukocytosis, chromosome 7 or complex cytogenetic abnormalities, and a prior history of red blood cell transfusions. Therapy-related CMML has an inferior survival, with a median survival of fewer than 12 months. The discovery of gene mutations in CMML has resulted in the development of molecular prognostic models. *ASXL1* is found to be a predictor of aggressive disease behavior and has been incorporated into a proposed prognostic scoring system for CMML alongside karyotype and clinicopathologic parameters; however, mutation data have not been found to be significant in other studies.

JUVENILE CHRONIC MYELOMONOCYTIC LEUKEMIA

Juvenile chronic myelomonocytic leukemia (JMML) is a rare MDS/MPN occurring in infancy and early childhood that is characterized by hypersensitivity of the myeloid progenitor cells to granulocyte–macrophage colony-stimulating factor (GM-CSF). There is an association with neurofibromatosis type 1 (NF1) and with Noonan syndrome in a small percentage of cases. Although uncommon, these associations have led to an understanding of some of the underlying molecular pathology of the disease. In NF1 and Noonan syndrome, there are mutations in *NF1* and *PTPN11*, respectively. These genes are important in the controls of the RAS/MAPK pathway. This pathway regulates a cell's proliferative response to GM-CSF when GM-CSF is bound to the cell surface. In JMML, dysregulation of the RAS signaling pathway through the mutations associated with NF1 or Noonan syndrome, as well as recently described mutations in other regulators, is believed to be the underlying molecular mechanism leading to a marked hypersensitivity of the myeloid progenitors to GM-CSF and to the proliferation of the leukemic clone. The clinical and pathologic definition of JMML is not substantially changed in the 2016 WHO revision; however, molecular diagnostic parameters have been refined. The updated diagnostic findings are listed in the table under "Diagnostic Criteria."

Clinical Features

Juvenile myelomonocytic leukemia, which is uncommon, accounts for 1.2 per million children per year. It shows a male predominance, is more frequent in children younger than 2 years (median age 20 months, 1 to 85 months) and is more common in boys. The association with NF1 is seen in approximately 15% of cases, and children with NF1 have a 200- to 500-fold increased risk of this leukemia.

Symptoms of JMML are due to the infiltration of organs by malignant cells. Children with JMML usually have impressive hepatosplenomegaly, lymphadenopathy, and erythematous maculopapular rash on the face with a butterfly distribution. The rash can precede other symptoms by months. Fever associated with bronchitis or tonsillitis is seen in approximately half of the cases. Some patients present with hemorrhage. Café au lait spots are seen in patients with NF1.

Pathologic Features

Blood and Bone Marrow

Pediatric patients with JMML have features similar to those seen in adult patients with CMML. There is leukocytosis, absolute monocytosis, and thrombocytopenia with or without

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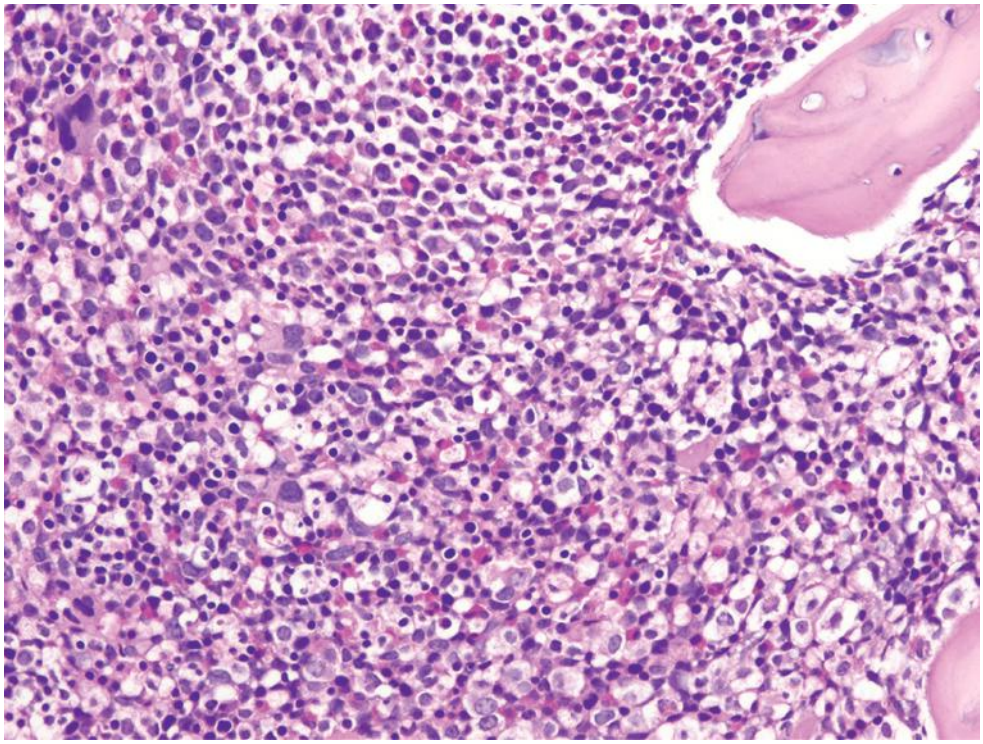


FIG. 17.35

A case of juvenile myelomonocytic leukemia (JMML) with monosomy 7 and *KRAS* mutation. Bone marrow biopsy shows a hypercellularity with left-shifted myeloid maturation, increased monocytes and histiocytes, occasional small megakaryocytes, and modestly increased eosinophils. *(Courtesy of Andrea Marcogliese, MD, Texas Children Hospital.)*

JUVENILE MYELOMONOCYTIC LEUKEMIA—FACT SHEET

Definition and Other Names

- Clonal hematopoietic disorder of infancy and early childhood characterized by an excessive proliferation of monocytic and granulocytic lineages with frequent somatic or germline mutations of *PTPN11, KRAS, NRAS, CBL,* or *NF1*

Incidence, Gender, and Age Distribution

- Uncommon, 1.2 per million children per year
- Male predominance
- More frequent in children <2 years
- *PTPN11, KRAS, NRAS, CBL*, or *NF1,* usually mutually exclusive mutations, activate the RAS/MAPK pathway in ~90% patients

Clinical Features

- Impressive hepatosplenomegaly, lymphadenopathy, erythematous maculopapular rash on face
- Fever with bronchitis or tonsillitis is common
- Café au lait spots in patients with NF1

Prognosis

- Variable course with disparate survival
- Older age, high Hgb F level, *PTPN11* mutation, and high-risk cytogenetics may predict worse course
- Transformation to AML in approximately 10% to 15%

anemia. The median WBC count is between 25 and 35 × 10^9/L but may range from 10 to 100 × 10^9/L. Monocytes may range from 1 to 60 × 10^9/L. Granulocytic elements can include some immature forms, but blasts plus promonocytes are usually less than 5% of the cells, and by requirement less than 20%. Dysplasia is not prominent. The bone marrow is hypercellular, but this is generally normal in children younger than 2 years. The myeloid-to-erythroid ratio can vary but is usually increased (Fig. 17.35). Dysplasia, as in blood, is only minimal, with increased monocytes (Fig. 17.36). Erythroid elements may be megaloblastoid. Megakaryocytes do not show prominent dysplasia. Granulocytes with hypogranulation and pseudo–Pelger-Huet forms may present. Monocytes may be difficult to appreciate.

Ancillary Studies

Mutation studies become essential in establishing a diagnosis of JMML. Approximately 90% of patients with JMML present with somatic and/or germline mutations in the genes of the RAS pathway, including *PTPN11, NRAS, KRAS, NF1,* and *CBL*. *PTPN11, NRAS,* and *KRAS* are somatic, and *NF1* and *CBL* are germline mutations. *CBL* alterations also include loss of heterozygosity or heterozygous splice site mutations. Overall in JMML, *CBL* mutation frequency is about 10% to 15%, *PTPN11* 35%, *KRAS/NRAS* 20%, and *NF1* 15%. *SETBP1* and *JAK3* mutations are reported in 15% JMML. Other genes reported to be mutated in JMML include *EZH2, DNMT3A, RUNX1*, and *ASXL1*. Hypermethylation of *RASA4* isoform 2, BMP4, CALCA, CDKN2B, and RARB have been found in patients with JMML, associated with a significantly poor prognosis.

Karyotypic abnormalities are seen in about one third of the cases. Of those, monosomy 7 comprises about two thirds of the cases, and other abnormalities, including trisomy 8 and

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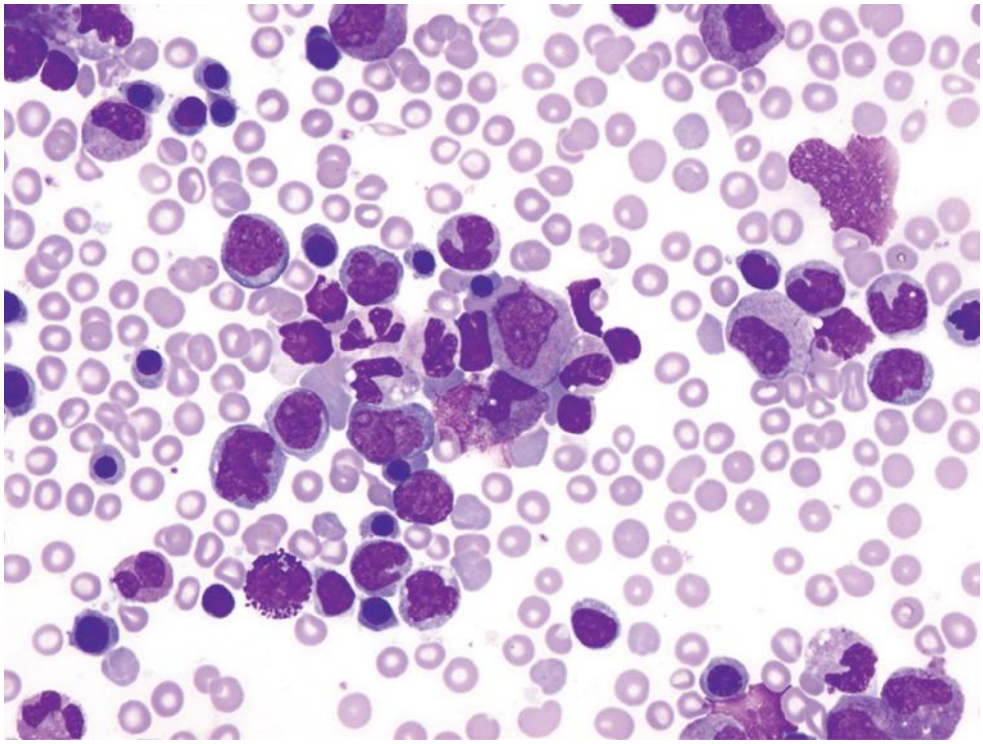


FIG. 17.36

The bone marrow aspirate smear of the case of juvenile myelomonocytic leukemia (JMML) with monosomy 7 and *KRAS* mutation. There are increased monocytes with atypical features, minimal dysplasia in erythroid and granulocytic cells. *(Courtesy of Andrea Marcogliese, MD, Texas Children Hospital.)*

JUVENILE MYELOMONOCYTIC LEUKEMIA—PATHOLOGIC FEATURES

Diagnostic Criteria (WHO 2016)

Category I: Clinical and hematologic features (all 4 features mandatory)

- Peripheral blood monocytosis >1 × 10^9/L
- Blasts (including promonocytes) <20% in blood and bone marrow
- Splenomegaly
- No Philadelphia chromosome/*BCR-ABL1*

Category II: Genetic studies (1 finding sufficient)

- Somatic mutation in *PTPN11* or *KRAS* or *NRAS*
- Clinical diagnosis of *NF1 or NF1* mutation
- Germline *CBL* mutation or loss of heterozygosity of *CBL*

Category III: For patients without genetic features, besides the clinical and hematologic features listed under category I, the following criteria must be fulfilled:

- Monosomy 7 or any other chromosomal abnormality or at least 2 of the following criteria:
  - Hemoglobin F increased for age
  - Circulating myeloid or erythroid precursors
  - GM-CSF hypersensitivity in colony assay
  - Hyperphosphorylation of STAT5

Note: The diagnosis of JMML is made if a patient meets all of the category I criteria and one of the category II criteria without needing to meet the category III criteria. If there are no category II criteria met, then category III must be met. *PTPN11 or KRAS or NRAS* germline mutations indicate Noonan syndrome and need to be excluded. *CBL* in occasional cases can be heterozygous splice site mutations

Microscopic Features

Blood

- Median WBC count, 25 to 35 × 10^9/L
- Absolute monocytosis >1 × 10^9/L
- Blasts usually <5%, have to be <20%
- Dysplasia not common

Marrow

- Hypercellular
- Increased M:E ratio
- Increased monocytes
- Minimal dysplasia

Ancillary Studies

- Mutation studies of *PTPN11, KRAS, NRAS, NF1, CBL*
  - Cytogenetic analysis shows abnormal clones in about one third of cases
- Hgb F levels typically increased
- Hypersensitivity of myeloid precursors to GM-CSF in vitro

Differential Diagnosis

- Reactive monocytosis
  - AML (acute myelomonocytic or acute monocytic type)
  - Wiskott-Aldrich syndrome
  - Osteopetrosis

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21, are not uncommon. Less frequent abnormalities include t(13;14)(q12.2;q32.3) and defect of 3q. The cases associated with NF1 more frequently have monosomy 7, which also may be acquired during the disease course.

Hemoglobin F levels are typically increased for the age of the patient, although this may not be the case in some patients. More than half of the patients have a polyclonal hypergammaglobulinemia, and 25% of patients have a positive direct Coombs test result. Hypersensitivity of myeloid progenitors to GM-CSF in vitro studies is a characteristic finding related to the underlying molecular pathogenesis. This test is not a routine offering in most laboratories, although it is one alternative criterion among others.

Differential Diagnosis

The diagnosis of JMML can be difficult, and differential diagnoses must include leukemoid reactions and other myeloid diseases. Children with Epstein-Barr virus (EBV), cytomegalovirus (CMV), and human herpesvirus-6 (HHV-6) can present with similar clinical and morphologic features. The finding of mutation in genes in the *RAS* pathway or a cytogenetic clone can be helpful in ruling out a reactive process. Cytogenetic analysis is also important in ruling out CML, which can develop occasionally in young children. The distinction of JMML from acute myelomonocytic leukemia must be made based on accurate blast and promonocyte counts.

Wiskott-Aldrich syndrome (WAS) is a rare X-linked recessive disorder, characterized by micro-thrombocytopenia, eczematous skin disease, and recurrent infections. JMML-like clinical and pathologic features may develop before the full clinical picture of WAS becomes apparent. Patients with WAS do not carry the molecular markers of JMML and often have no splenomegaly. Infantile malignant osteopetrosis (IMO) is a rare genetic disorder of bone resorption due to mutations leading to a defect of osteoclasts. Patients with IMO can present with leukocytosis, monocytosis, anemia, thrombocytopenia, a leukoerythroblastic blood count, and organomegaly, resembling JMML. Radiologic imaging displaying increased bone density is the key diagnostic method for an unequivocal distinction of these two diseases. Children with Noonan syndrome may develop a myeloproliferative disorder resembling JMML but have spontaneous remission without treatment.

Prognosis and Therapy

A case of JMML has a variable course with widely disparate survivals. One third of patients have rapidly progressive disease, whereas two thirds of patients have a relatively indolent course. The transformation to AML occurs in only 10% to 15% of patients. Older age (>2 years), higher hemoglobin F (>10%), and lower platelet counts may predict a worse prognosis. *SETBP1* mutation, aberrant methylation, and somatic *PTPN11* mutation are reported to be associated with an inferior prognosis. However, these have not been proved in large clinical studies.

ATYPICAL CHRONIC MYELOID LEUKEMIA, *BCR-ABL1*-NEGATIVE

Atypical CML (aCML) is an uncommon hematopoietic neoplasm characterized by leukocytosis that is *BCR-ABL1* negative and associated anemia and/or thrombocytopenia. The process suffers from a confusing name, which always must be clarified to the clinician and be explained as being distinct from what might sound like an unusual case of CML that is merely atypical. It is always helpful to refer to it as atypical CML, *BCR-ABL1* negative for clarity. The WHO 2008 classification gave aCML a formal designation, and recently molecular discoveries further helped in better defining this entity. The absence of monocytosis separates it from CMML. It becomes easier to be separated from chronic neutrophilic leukemia, knowing that *CSF3R* mutation is strongly associated with CNL but very rare in aCML (<10%). The so-called MPN-associated driver mutations (*JAK2, CALR, MPL*) are typically absent in aCML, assisting in a differential diagnosis from MPNs. aCML can occur in the therapy-related setting. Patients affected by aCML have a dismal prognosis.

Clinical Features

The incidence of aCML is not known, but it likely represents only a very small fraction of all MPNs and MDS/MPNs. In fact, fewer than two cases of aCML are found for every 100 cases of *BCR-ABL1*–positive CML. Patients are usually older, with a median age between 65 and 74 years, and there is a male predominance (2 : 1). Patients usually exhibit symptoms related to anemia and thrombocytopenia. About 40% to 50% patients have splenomegaly and hepatomegaly and may have symptoms related to organomegaly. LDH is elevated in majority of the patients.

Pathologic Features

Blood

Patients usually have leukocytosis with a median WBC count around 35 to 45 $\times$ 10^9/L, although in some cases the counts can exceed 200 to 300 $\times$ 10^9/L. Most of the leukocytes in the blood are granulocytes with 10% or more immature forms (promyelocytes, myelocytes, and metamyelocytes). Circulating blasts may be present, but these are generally

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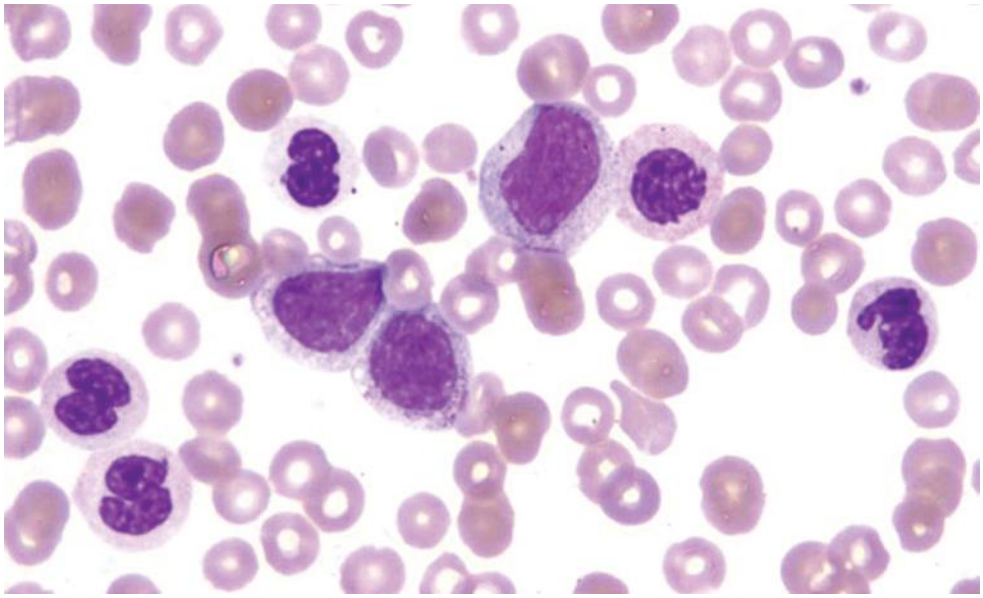


FIG. 17.37

Peripheral blood smear in a case of atypical chronic myeloid leukemia. Note the severe dysplasia in the mature granulocytes and the absence of platelets. The immature cells are dysplastic immature granulocytic elements. These could be distinguished from monocytes by a negative nonspecific esterase reaction.

ATYPICAL CHRONIC MYELOID LEUKEMIA—FACT SHEET

Definition

- A Ph-chromosome/*BCR-ABL1*-negative chronic leukemia that has a proliferative component (leukocytosis ≥13 × 10^9/L) and a dysplastic component with ineffective hematopoiesis
- Proliferation of granulocytic elements, which are frequently dysplastic, with left-shifted granulocytic components ≥10% in peripheral blood, and ineffective hematopoiesis usually manifest as anemia and thrombocytopenia

Incidence, Gender, and Age Distribution

- Incidence unknown, rare
- Male to female = 2–3 : 1
- Median age, 65 to 74 years

Clinical Features

- Symptoms related to anemia and thrombocytopenia most common
- Splenomegaly and hepatomegaly in some patients
- Molecular genetic features
- Karyotypic abnormalities in about half of the patients, +8 most common
- *CSF3R* mutation very uncommon; *SETBP1* and/or *ETNK1* mutations in up to one third of cases

Prognosis

- High leukocyte count, severe thrombocytopenia at diagnosis associated with a poor prognosis
- Some transform to AML (30% to 40%); more die of marrow failure (60% to 70%)
- Median survival, 12 months

less than 5%. Granulocytes are dysplastic with hypogranular cytoplasm, nuclear atypia, and hypolobation (Fig. 17.37). It is also observed in some cases that neutrophils may show abnormal chromatin clumping with multiple nuclear lobes (Fig. 17.38). Basophils are typically not elevated and account for less than 2% of the cells in most cases. Hypogranular myelocytes can be difficult to distinguish from monocytes, and nonspecific esterase may be helpful in the evaluation. Due to a high WBC count, it is not uncommon to have more than 1 × 10^9/L monocytes, but the percentage of monocytes is low and less than 10% of the leukocytes. There is commonly anemia, and the red blood cells frequently show anisopoikilocytosis, in keeping with the dysplasia that can be seen in the red blood cell precursors in the marrow. Platelets are decreased in approximately one third to one half of the patients. Thrombocytosis is not common but can be observed in a few patients. Hypogranulated and giant forms of platelets might be seen.

Bone Marrow

The bone marrow is usually hypercellular, mainly owing to a granulocytic proliferation. The granulocytes may be left shifted with increased blasts, but these must account for less than 20% of the cells. As in the peripheral blood, dysgranulopoiesis is prominent. Dysgranulopoiesis, hypogranulation, and nuclear hypolobation, including pseudo–Pelger-Huët anomaly, is very similar to those observed in MDS. In some cases, neutrophils may show abnormal chromatin clumping with multiple nuclear lobes (see Fig. 17.38). This form of dysplasia is not common in MDS and should not be interpreted as hypersegmentation, a benign change seen in megaloblastoid anemia or after hydroxyurea treatment. Monocytes are not increased. Dysmegakaryopoiesis is common, many resembling dysplastic megakaryocytes in MDS (Fig. 17.39), whereas

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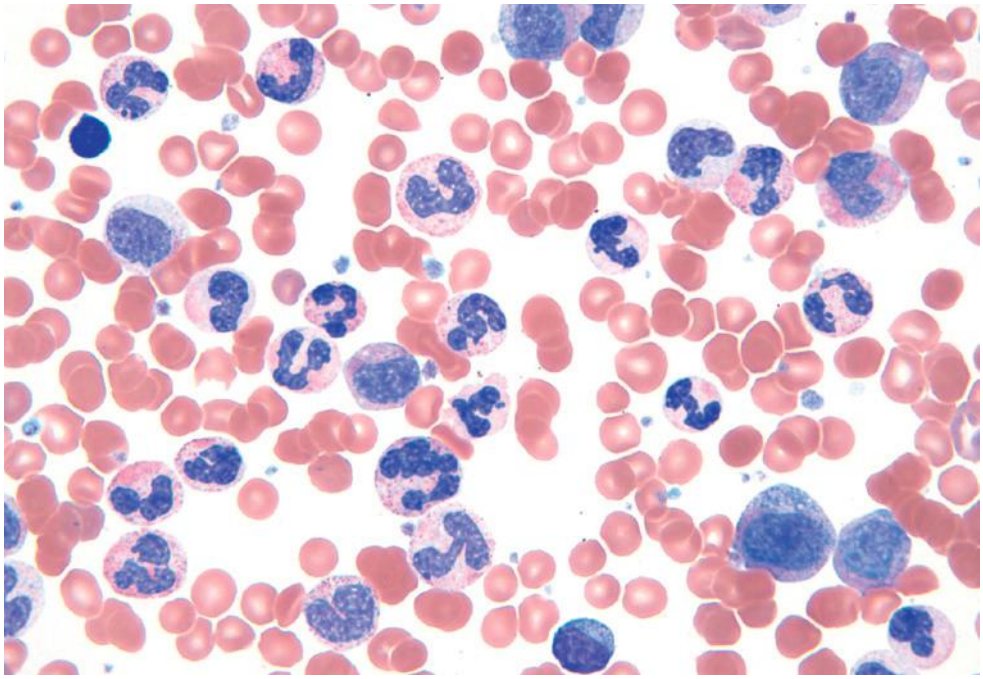


FIG. 17.38

In some atypical chronic myeloid leukemia, granulocytes show multiple segmentations with abnormal chromatin clumping. This type of change in granulocytes differs from the dysgranulopoiesis commonly seen in myelodysplastic syndromes and should not be interpreted as hypersegmentation, a normal change in megaloblastoid anemia or after hydroxyurea treatment.

ATYPICAL CHRONIC MYELOID LEUKEMIA—PATHOLOGIC FEATURES

Microscopic Features

Blood

- Leukocytosis ≥13 × 10^9/L, median 35 to 45 × 10^9/L, can go over 200 × 10^9/L
- Presence of ≥10% left-shift neutrophil precursors, including promyelocytes, myelocytes, and metamyelocytes, but blasts are usually <5%
- Dysplasia is usually marked, some with abnormal chromatin clumping
- Basophilia is uncommon, often ≤2%
- May have absolute monocytes ≥1 × 10^9/L due to a high WBC count, but monocytes are <10%
- Anemia with anisopoikilocytosis is common
- Thrombocytopenia is common

Bone marrow

- Hypercellular, granulocytic proliferation with a markedly increased M:E ratio
- Blasts <20%
- Multilineage dysplasia frequently present; granulocytic dysplasia is prominent
- Reticulin fibrosis may be present

Ancillary studies

- Cytogenetic and molecular analysis: must be t(9;22)/*BCR-ABL1*-negative; negative for *PDGFRA, PDGFRB,* or *FGF1R* or *PCM1-JAK2* rearrangement
- About half of the cases show karyotypic abnormality, with trisomy 8 being the most common, followed by -7/-7q, i17q, and a complex karyotype. However, none of these is specific for aCML
- CSF3R is very frequent in chronic neutrophilic leukemia but very infrequent in aCML
- SETBP1 and/or ETNK1 mutations in up to one third of cases, KRAS/NRAS are seen in about 20% to 30% cases
- The so-called MPN-associated driver mutations (JAK2V617F, CALR, MPL) are typically absent in aCML.

Differential Diagnosis

- Chronic myeloid leukemia
- Chronic neutrophilic leukemia
- Chronic myelomonocytic leukemia
- MDS/MPN-unclassifiable
- MDS

some show a mixture of small and large megakaryocytes. Erythroid dysplasia is also common but may be difficult to assess owing to a marked increased M:E ratio. Reticulin fibrosis may be increased.

Ancillary Studies and Differential Diagnosis

In CMML absolute monocytosis as defined by 1 × 10^9/L or greater can be easily reached in a patient with a high WBC count. For example, with a WBC count of 50 × 10^9/L, the presence of 3% monocytes will make an absolute monocyte count of 1.5 × 10^9/L. Therefore, it is very important to require the proportion of monocytes 10% or greater in addition to absolute monocytosis to consider a differential diagnosis of CMML. Some patients with aCML may have transient borderline monocytosis, both in monocyte percentage and absolute count, but monocytosis is not a persistent feature, indicating the importance of having CBC data from multiple time points to determine peripheral blood features. On the other hand, some patients with CMML may not have relative

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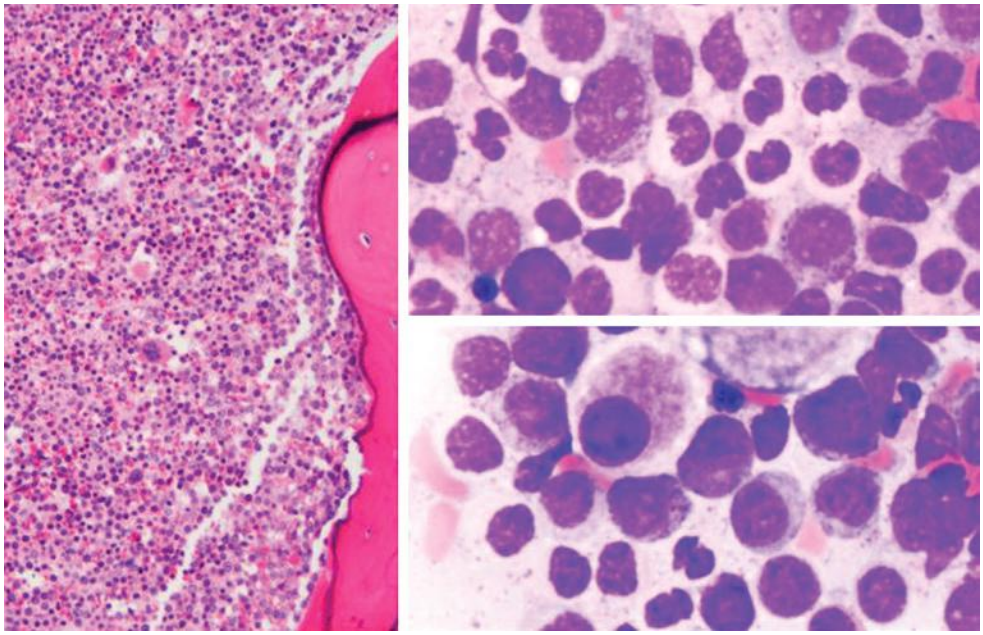


FIG. 17.39
Bone marrow biopsy and aspirate in a case of atypical chronic myeloid leukemia. Note the hypercellularity and marked dysplasia in mature granulocytes and megakaryocytes.

or absolute monocytosis at presentation but later show persistent monocytosis, and a diagnosis or reclassification of such cases as CMML is appropriate.

In CML the PB and BM of patients usually do not show dysgranulopoiesis. Circulating neutrophil precursors are present in both CML and aCML. However, CML is a genetic-defined disease, and cytogenetic or molecular studies must be performed to rule out t(9;22)/*BCR-ABL1*.

In CNL, patients often present with marked leukocytosis ($\geq 25 \times 10^9$/L) with neutrophilia (≥80% neutrophils). Neutrophilic precursors are less than 10%. There is no dysgranulopoiesis. *CSF3R T618I* or other activating *CSF3R* mutation is detected in more than 90% of CNL cases. In contrast, in aCML, *CSF3R* mutation is very uncommon; instead, *SETBP1* and/or *ETNK1* mutations are seen in up to one third of cases.

MDS/MPN-U comprises a heterogeneous group of cases with hybrid MDS and MPN features, but these cases cannot fit into any specific category of MDS/MPN. There is some overlap between aCML and MDS/MPN-U. For example, if a patient has leukocytosis, with 10% or greater neutrophilic precursors in PB, but no significant dysgranulopoiesis, or a patient has leukocytosis with dysgranulopoiesis but no increase in PB neutrophilic precursors, both patients will be diagnosed with MDS/MPN-U rather than aCML. It is debatable whether such a division is clinical or biologically meaningful. There is no molecular genetic marker to differentiate one from another.

Prognosis and Therapy

Patients with aCML have a poor prognosis, with a median survival of approximately 12 months. Some patients (~40%) show disease progression to AML, whereas many more (60%) die of marrow failure. Thrombocytopenia and severe anemia, as well as marked leukocytosis at diagnosis, are poor prognostic indicators.

MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASM WITH RING SIDEROBLASTS AND THROMBOCYTOSIS (MDS/MPN-RS-T)

Initially named refractory anemia with ring sideroblasts and marked thrombocytosis (RARS-T), MDS/MN-RS-T was first introduced as a provisional entity under MDS/MPN-unclassifiable (MDS/MPN-U) in the 2001 WHO classification of hematopoietic neoplasms. The initial definition required a minimal platelet count of 600×10^9/L and the presence of 15% or more ring sideroblasts (RS). In the 2008 WHO classification, the platelet count criterion was lowered from 600×10^9/L to 450×10^9/L to be consistent with the decision to lower the platelet count threshold for essential thrombocythemia (ET). The definition also required no circulating blasts and no increase in myeloblasts in bone marrow (<5%).

Whether the process is a distinct entity has been debated over the years. Some believed that it was a heterogeneous mix of three things: (1) cases of ET or (2) cases of early or cellular phase of primary myelofibrosis (PMF) with ringed sideroblasts and (3) cases of MDS with increased platelet count. More than a decade ago, *JAK2* V617F mutation was discovered in classic MPN; subsequent studies found *JAK2*V617F mutation in a substantial subset of cases of MDS/MPN-RS-T (50% to 60%), providing molecular support for the proliferative nature of this neoplasm. In several recent years, *SF3B1* mutation that is found in MDS with ring sideroblasts was also detected in high frequency (80% to 90%) in MDS/MPN-RS-T. Furthermore, somatic mutations in epigenetic regulators characteristic of MDS, such as *TET2,*

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ASXL1, DNMT3A, and *IDH2,* are also frequent in MDS/MPN-RS-T. These molecular findings are in keeping with clinicopathologic hybrid features, supporting the inclusion of RARS-T as an MDS/MPN overlap syndrome. In the 2016 WHO update, MDS/MPN-RS-T is considered a formal subtype of MDS/MPN.

Clinical Features

Patients are older, with a median age of 72 to 75 years and a male-to-female ratio approximately 1 : 1. Patients present with macrocytic anemia, but anemia is often less severe than in patients with RARS. By definition, all patients have a platelet count 450×10^9/L or greater, and the median platelet count is around 600×10^9/L. Some patients may have splenomegaly. Rates of thrombotic events are similar in MDS/MPN-RS-T and ET cases and higher than in cases of RARS.

Ancillary Studies and Differential Diagnosis

In a patient with macrocytic anemia, thrombocytosis (≥450 $\times 10^9$/L), and bone marrow 15% or greater RS, the list of differential diagnoses is relatively short. Similar to MDS with RS, benign conditions causing RS include alcohol, drugs, medications, pregnancy, nutritional deficiency, or congenital/hereditary disorders. Of the neoplastic conditions, if a patient has a known history of MPN, the presence of secondary RS should not reclassify such cases as MDS/MPN-RS-T. An exception may apply to MDS with RS, a reclassification to MDS/MPN-RS-T may be appropriate. When struggling with a differential diagnosis of MPN versus MDS/MPN-RS-T, presence of an *SF3B1* mutation with or without *JAK2 V617F* mutation would favor a diagnosis of MDS/MPN-RS-T, whereas lack of *SF3B1* but positive *MPL* or *CALR* would put a diagnosis of MDS/MPN-RS-T in question. MDS cases with inv(3)(q21q26.2) may present with RS and thrombocytosis, and in such patients, the disease often has an aggressive clinical course even in the absence of increased blasts. Approximately 5% of patients with isolated del(5q) may harbor *JAK2*V617F mutation and some may present with thrombocytosis; fortunately, RS are not common in these cases. Nonetheless, the current recommendation is to classify these cases as MDS with isolated del(5q) and to note the presence of *JAK2* V617F in the diagnosis.

MDS/MPN-RS-T—Fact Sheet

Definition (WHO 2016)

A form of MDS/MPN with anemia associated with erythroid dysplasia with or without multilineage dysplasia, ≥15% ring sideroblasts as well as persistent thrombocytosis with platelet count ≥450 × 10^9/L. There are <1% blasts in peripheral blood and <5% blasts in the bone marrow. No *BCR-ABL1* fusion gene, no rearrangement of *PDGFRA, PDGFRBI,* or *FGFR1;* or *PCM1-JAK2;* no (3;3)(q21;q26), inv(3)(q21q26) or del(5q). No preceding history of MPN, MDS (except MDS-RS), or other types of MDS/MPN.

Clinical Features

- Older patients present with macrocytic anemia and persistent thrombocytosis. Some patients may experience thrombotic events (2% to 20%)

Molecular Genetic Features

- High frequency of *SF3B1* mutation (80% to 90%) and *JAK2V617F* mutation (50%)
- Cytogenetic abnormalities in about 10% to 30% of cases

Prognosis

- Survival is variable, with a reported median survival around 70 months
- AML transformation is low

MDS/MPN-RS-T—Pathologic Features

Microscopic Features

Blood

Platelets are increased, including large and giant and hypogranulated forms. Anemia is often macrocytic, and red cells often show anisocytosis with a dimorphic pattern, due to the presence of ring sideroblasts. Neutrophils, though often normal, may show mild dysgranulopoiesis or left-shifted maturation in some cases.

Bone Marrow

Bone marrow often shows a hypercellularity with a decreased/reversed M:E ratio owing to ineffective erythropoiesis. Ring sideroblasts are present, minimal 15%, and median 30% to 50%. Megakaryocyte proliferation is invariably present in all cases, frequently with focal clustering. Megakaryocytes are often composed of predominantly large hypersegmented megakaryocytes. Of note, MDS/MPN-RS-T used to be described as resembling ET but with RS. Recent studies have described more heterogeneous findings in megakaryocytes in MDS/MPN-RS-T that can mimic megakaryocytes in ET, PV, PMF, or MDS (Fig. 17.40). Various grades of reticulin fibrosis (ranging from MF1 to MF3) can be seen in some patients at the time of initial diagnosis or develop in the course of disease. The morphologic spectrum of MDS/MPN-RS-T has been expanded with recent studies. Although some cases have morphologic features resembling MPN, lacking dysplasia other than the presence of RS (Fig. 17.41), some patients show mixed MDS and MPN features, and some cases resemble MDS with no MPN features. *JAK2V617* mutations have been described as the highest in cases resembling MPN and lowest in cases resembling MDS.

Prognosis and Therapy

The prognosis is considered to be inferior to ET but better than MDS with RS. The reported median survival is about 76 months in one large cohort study. The presence of *SF3B1*

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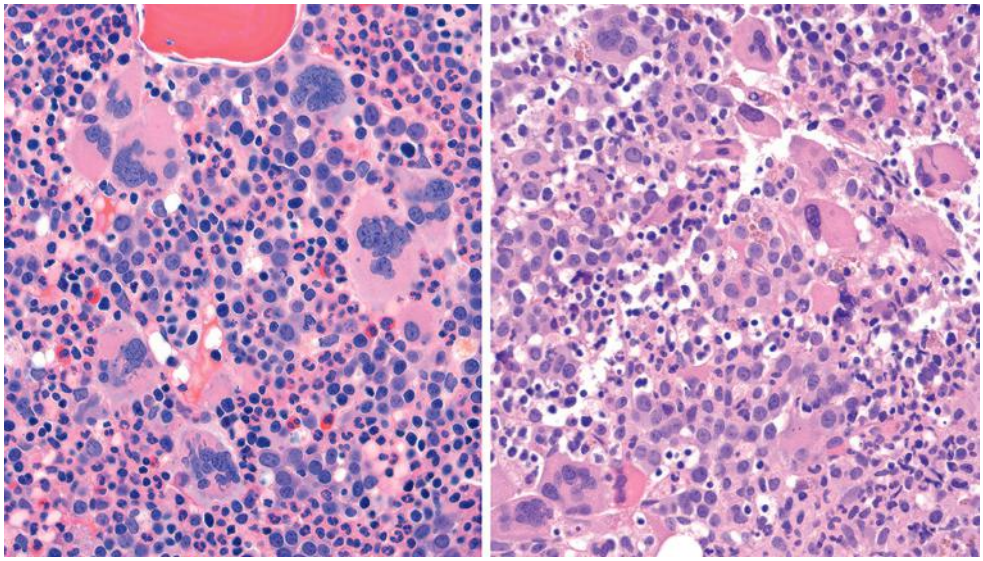


FIG. 17.40
Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T). Bone marrow biopsies show a hypercellularity with erythroid hyperplasia. Megakaryocytes *(left)* are often large, hypersegmented, and clustering. However, in some cases, megakaryocytes may show a mixture of large hypersegmented forms and small hypolobated forms *(right)*.

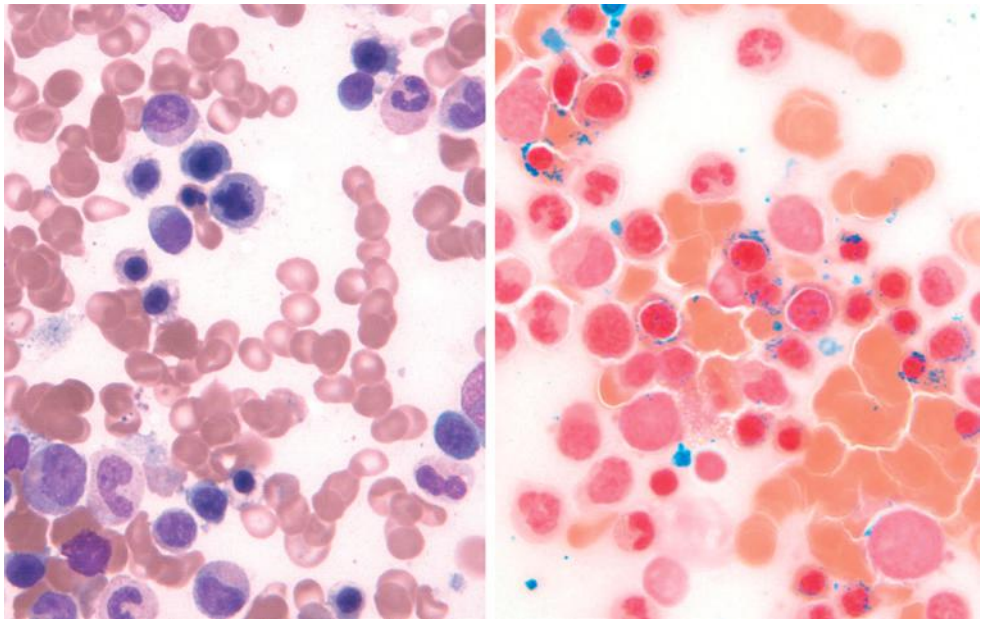


FIG. 17.41
Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T). Bone marrow aspirate shows dyserythropoiesis *(left)* with numerous ring sideroblasts (*right*, Perl stain). Of note, in some cases, erythroid precursors may only show ring sideroblasts without obvious dyspoiesis.

and *JAK2* V617F mutations are found to be independent favorable prognostic factors for improved survival in MDS/MPN-RS-T. The presence of *SETBP1* or *ASXL1* mutations is reported to be associated with an inferior prognosis. An abnormal karyotype and old age are also considered to be negative prognostic indicators.

MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS, UNCLASSIFIABLE

Similar to that for MPNs, the WHO classification allows for an unclassifiable category for MDS/MPN. The designation is not meant to be used for cases in which diagnostic data are lacking but instead for when the entity, once fully characterized, does not fit the known types of diseases. Cases usually have features of MDS but also have a myeloproliferative component with no history of a preceding MPN. After excluding molecular genetic defining entities, as well as cases fitting in any specific subtype of MDS/MPN, cases with hybrid MDS and MPN features will be placed under this category.

Clinical Features

Patients with MDS/MPN-U are generally older, with a median age 70 years, and show male predominance. Anemia is common. About one third of patients present with thrombocytopenia and another one third with thrombocytosis. Leukocytosis ($\geq 13 \times 10^9$/L) can be seen in around two thirds of

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patients. Of patients, 20% to 30% may present with splenomegaly. For patients with a known history of MPN who develop dysplasia, disease progression should be considered instead of a diagnosis of MDS/MPN-U. However, if a chronic phase of MPN has not previously diagnosed, a diagnosis of MDS/MPN would be appropriate.

Morphologic Features

The PB and BM findings are more heterogeneous than other well-defined MDS/MPN entities. PB may show dysplastic neutrophils, left-shifted neutrophil precursors, nucleated red cells, and circulating blasts (but <20%). Mild basophilia or eosinophilia may be seen in some patients. Bone marrow shows a hypercellularity, and moderate to marked fibrosis can be observed in 20% to 30% cases. M:E ratio may vary from case to case, ranging from markedly increased to markedly decreased/reversed. Although many of the cases show dysplastic megakaryocytes, about 20% to 30% of cases show large megakaryocytes with clustering, mimicking MPN megakaryocytes, and some cases show mixed MDS-like and MPN-like megakaryocytes. The heterogeneity is in part owing to an inclusion of cases that are not perfectly fitting for other entities. For example, if a patient presents with marked leukocytosis with dysgranulopoiesis but PB neutrophil precursors are less than 10%, the case will be best classified as MDS/MPN-U rather than aCML. If a patient with thrombocytosis and more than 15% RS in bone marrow, but also has more than 5% BM blasts, the case would fit MDS/MPN-U rather than MDS/MPN-RS-T.

Ancillary Studies and Differential Diagnosis

A diagnosis of MDS/MPN-U is one of exclusion, including cases not fitting perfectly into other well-defined entities. It has been questioned whether cases with marked leukocytosis, 10% or more neutrophil precursors in PB, and dysplasia in lineages other than granulocytes should be considered as aCML instead of MDS/MPN-U. For the time being, these cases are placed under MDS/MPN-U. If patients have a monocytosis, both in absolute count and percentage, a diagnosis of CMML should trump a diagnosis of MDS/MPN-U. MDS/MPN-U, however, should not be used for cases for which a complete assessment is not possible owing to poor quality specimen, incomplete clinical information, or lack of molecular genetic data.

MDS/MPN-U—FACT SHEET

Definition

Have hybrid MDS and MPN clinical features but do not fit into any specific type of well-defined MDS/MPN, including CMML, aCML, JMML, and MDS/MPN-RS-T. Negative for *BCL-ABL1, PDGFRA, PDGFRB, FGFR1* rearrangement, or *PCM1-JAK2*

Clinical Features

Patients present with both cytosis (leukocytosis as defined by ≥13 × 10^9/L and or thrombocytosis ≥450 × 10^9/L) and cytopenia (anemia and or thrombocytopenia, occasionally leukopenia). Some patients may have splenomegaly.

Morphologic Features

Dysplasia has to be present in at least one lineage. But PB and BM findings are more heterogeneous than other well-defined MDS/MPN owing to the inclusion of a heterogeneous group of cases that do not fit perfectly into other MDS/MPN entities

Molecular Genetic Features

Approximately 50% of patients show clonal cytogenetic abnormality, with +8 to be the most common abnormality, followed by -7/-7q and a complex karyotype. *JAK2V617F* can be seen in 20% to 30% of patients and *RAS* mutation in 10% to 15% of patients. Epigenetic regulator *ASXL1, TET2i,* and *EZH2* mutations can be seen in about 30% of patients.

Prognosis and Therapy

The median overall survival is around 12 to 18 months, and AML transformation is approximately 20% to 30%. Thrombocytopenia has a negative impact and thrombocytosis is a favorable predictor for an improved survival. Other factors that have a negative impact on survival include old age, the presence of circulating blasts, 5% or greater BM blasts, and the presence of more than 10% PB neutrophil precursors.


The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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18

Myelodysplastic Syndromes

■ Phuong L. Nguyen, MD ■ Robert Paul Hasserjian, MD

■ OVERVIEW

The myelodysplastic syndromes (MDSs) are a heterogeneous group of clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis. Clinically, this ineffective hematopoiesis manifests as progressive bone marrow failure with cytopenias and a variable probability of progression to acute myeloid leukemia (AML). Pathologically, MDSs are usually characterized by dysplastic morphologic features in one or more of the three hematopoietic lineages, with or without an accompanying increase in myeloblasts in the peripheral blood (PB) and bone marrow (BM) but at a percentage lower than the 20% threshold defining AML. MDSs can arise de novo, or they can be therapy related following exposure to cytotoxic chemotherapy, ionizing radiation, or both. Therapy-related MDSs are discussed with other therapy-related myeloid neoplasms in Chapter 14.

Clinical Features

Epidemiology

Myelodysplastic syndrome is a disease of the elderly, with a median age at diagnosis of 76 years and approximately 86% of patients aged 60 years or older at diagnosis; most MDS subtypes are more common in men than in women. The incidence of MDS increases sharply with age, from 9.7 per 100,000 individuals between the ages of 60 and 69 years to 59.1 per 100,000 among those older than 80 years according to data between 2008 and 2012 from the Surveillance, Epidemiology, and End Results (SEER) program. MDS is extremely rare in children younger than 14 years, among whom the annual incidence rate is 1.8 per 1,000,000. Patients with therapy-related MDS tend to be younger than those with de novo MDS. Based on the average annual age-adjusted incidence rate from 2003 to 2008 SEER data, it is estimated that approximately 9000 to 10,000 MDS cases are diagnosed annually in the United States. However, because many cancer registries rely on in-patient surveillance, these numbers are likely underestimates; when a claims-based algorithm is applied, the annual incidence of MDS has been estimated to be as high as 45,000 new cases. Moreover, because many patients with unexplained cytopenia never undergo a diagnostic BM evaluation, the true prevalence of MDS (the total number of people living with MDS) in the United States is unknown, but it has been estimated to range from 60,000 to 90,000.

Whereas the association between cytotoxic chemotherapy and ionizing radiation and therapy-related MDS is well established, risk factors for de novo MDS, which comprises the majority of cases, have not been fully elucidated. A variety of risk factors, such as cigarette smoking, exposure to solvents (benzene and derivatives) and agrochemicals (e.g., insecticides, pesticides, fertilizers), and increased body mass index have been reported, but most cases remain unexplained. Among children and young adults with MDS, 30% to 50% have a predisposing condition such as prior cytotoxic therapy, a congenital or acquired BM failure syndrome, or an underlying germline predisposition. Childhood MDS in the setting of Down syndrome is currently classified with Down syndrome–associated myeloid leukemia and is excluded from population-based studies of MDS.

Pathogenesis

The rate of cell proliferation in the BM of patients with MDS is increased compared to that in healthy individuals, but the maturation of such hematopoietic precursors is impaired, with increased apoptosis owing in part to overexpression of the apoptosis receptor CD95/Fas on hematopoietic precursors. This pro-apoptotic milieu appears to be driven also in part by increased production of the proinflammatory cytokine tumor necrosis factor alpha (TNF-α), with reports of elevated BM and plasma levels of TNF-α and elevations of the TNF-related apoptosis-inducing ligand and interferon γ on peripheral blood lymphocytes in patients with MDS. This apparently pro-apoptotic BM environment could explain the paradox of ineffective hematopoiesis in MDSs, in which there is increased marrow cellularity because of increased cell proliferation, but in which increased apoptosis of BM hematopoietic progenitors results ultimately in peripheral cytopenias (Fig. 18.1).

Autoimmunity plays a role in a subset of patients with MDS, particularly those with a trisomy 8 cytogenetic

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MYELODYSPLASTIC SYNDROMES—FACT SHEET

Definition

- MDS encompasses a heterogeneous group of clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis with a variable probability of progression to acute myeloid leukemia

Incidence, Age Distribution, and Predisposition

- It has been conservatively estimated that 9000 to 10,000 new MDS cases are diagnosed per year in the United States; the true annual incidence is like ly to be higher
- MDS is a disease of the elderly: 86% of MDS diagnoses are in individuals older than 60 years; conversely, de novo MDS is very rare in children under 14 years
- Predisposing risks for MDS include inherited or acquired bone marrow failure syndromes or other germline predisposition syndromes, previous exposure to cytotoxic chemotherapy, ionizing radiation, and certain toxins

Morbidity and Mortality

- Morbidity and mortality are related to the MDS subtype, extent of cytopenia(s), specific cytogenetic abnormality, as well as patient-related factors such as age and comorbid conditions

Clinical Features

- Symptoms are usually related to the type and extent of cytopenias; some patients may be asymptomatic

Prognosis and Therapy

- Patients without increased blood or bone marrow blasts, with normal cytogenetics, or with del(5q) as the sole chromosomal abnormality have the best prognosis
- Patients with increased blood or marrow blasts, complex chromosomal abnormalities, or therapy-related MDS have the worst prognosis
- The current standard curative treatment is allogeneic hematopoietic stem cell transplantation

abnormality. Overexpression of Wilms tumor protein (WT1) in these patients has been associated with increased programmed cell death that appears to be immunologically mediated, in that T cells from certain expanded Vβ subfamilies and specific for WT1 selectively kill trisomy 8 cells in vitro, whereas depletion of cytotoxic T cells enables growth of trisomy 8 colonies. Moreover, WT1-specific $CD4^+$ and $CD8^+$ T-cell responses appear to be associated with clinical responsiveness to immunosuppressive therapy.

Somatic mutations have emerged as a common mechanism in all MDS subtypes, as evidenced by the long list of single-gene mutations reported in patients with MDS within the past several years. Mutations in genes affecting RNA splicing (*SF3B1, SRSF2, U2AF1, ZRSR2*), chromatin remodeling and epigenetic regulation (*ASXL1, DNMT3A, TET2, IDH1/IDH2, EZH2*), as well as transcription factors (*RUNX1, ETV6, TP53, GATA2*), growth factor signaling (*NRAS/KRAS* and *CBL*), and cohesins, among others, have been found in MDS. Multiple diverse mutations are often present, suggesting that the acquisition of genetic aberrations in MDS likely represents a multistage process. Gene expression profiling of hematopoietic stem cells from patients with MDS identifies multiple frequently deregulated pathways. Among MDS patients with del(5q), the most frequently deregulated pathways include those involved in cell cycle regulation, integrin signaling, and chromatin assembly, whereas pathways involving cell survival appear to be most deregulated among patients with MDS and −7 or del(7q). In MDS with trisomy 8, the most significantly deregulated pathways appear to be those associated with the immune response, which correlates with the responsiveness of some of these patients to immunosuppressive therapy (see above).

Of great relevance are the reports from several groups that as many as 10% to 20% of individuals without known hematologic abnormalities harbor some of the gene mutations otherwise associated with MDS in their circulating hematopoietic cells, with *DNMT3A, TET2*, and *ASXL1* being the most common followed by *JAK2, TP53, SF3B1*, *SRSF2,* and *CBL*. This observation has given rise to the term *clonal hematopoiesis of indeterminate potential* (CHIP). With age, both the frequency and the number of such gene mutations increase, with approximately 10% of individuals over the age of 65 years harboring these mutations versus 1% of individuals under the age of 50 years. There is a 10- to 15-fold increased risk of a hematologic malignancy (not necessarily MDS) developed among individuals with a detectable mutation; this risk rises to 50-fold if the variant allele fraction (VAF) of the mutation(s) exceeds 10% to 20%. However, the absolute risk is small, and the majority of these patients do not develop a hematologic malignancy even after follow-up of several years. These observations, taken together, suggest that MDS likely arises as a result of progressive and selective accumulation over time of multiple somatic point mutations in hematopoietic stem cells (HSC). One hypothesis is that a so-called driver or initiating mutation primes a pool of long-lived HSC to acquire additional further somatic mutations, some of which in turn propel certain individuals along this multistep process toward the eventual development of MDS.

Clinical and Laboratory Features

Patients with MDS usually exhibit symptoms related to cytopenia, most frequently fatigue, as well as signs and symptoms related to bleeding, infections, or both. Other patients may have a diagnosis of MDS made while asymptomatic after detection of an abnormal complete blood cell count during health screening or as part of a medical evaluation for other unrelated reasons. With increased awareness of inherited predispositions to AML and MDS, the diagnosis may also be made in asymptomatic patients during the course of a family evaluation following the identification of the index case. Laboratory evaluation commonly reveals anemia (often macrocytic) with reticulocytopenia, neutropenia, and/or

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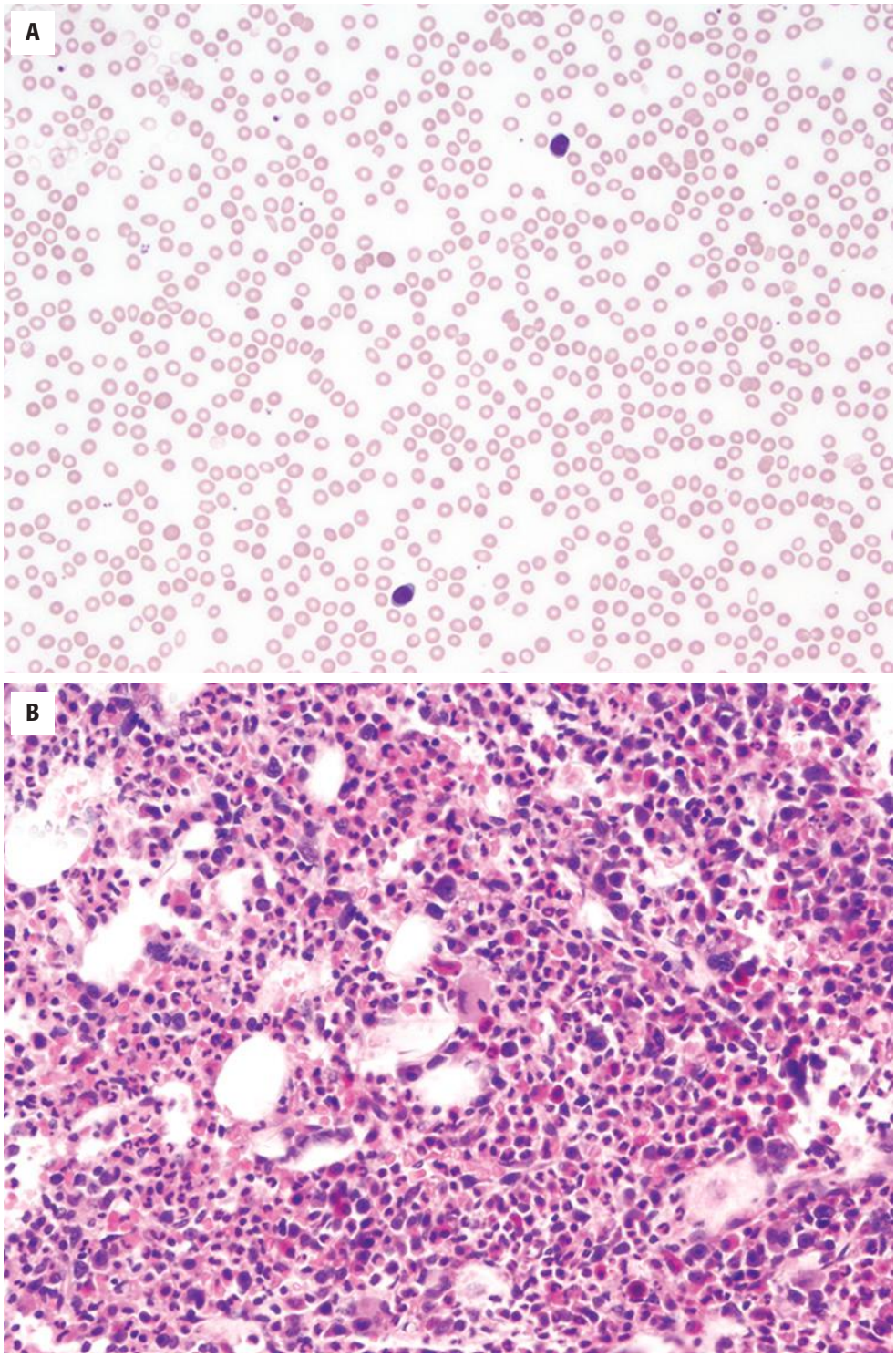


FIG. 18.1

Ineffective hematopoiesis in myelodysplastic syndromes. A, The peripheral blood of this patient with myelodysplastic syndrome shows pancytopenia (Wright-Giemsa stain). B, In contrast, the marrow is hypercellular, with increased hematopoietic precursors of all lineages (hematoxylin and eosin).

thrombocytopenia. Organomegaly and lymphadenopathy are usually absent.

For patients clinically suspected of having MDS, the National Comprehensive Cancer Network (NCCN) recommends that the initial evaluation includes, at the minimum, a comprehensive medical history (including transfusion history) and physical examination, complete blood count with leukocyte differential, reticulocyte count, measurement of erythropoietin, thyroid-stimulating hormone, serum iron/ferritin and vitamin B_{12}, red blood cell folate, a peripheral blood smear review, BM aspiration with iron stain, BM biopsy, and complete conventional karyotyping. Assessment of copper levels, interrogation for MDS-specific cytogenetic aberrations by fluorescence in situ hybridization (FISH), flow cytometry immunophenotyping, and molecular genetic testing may also be considered as part of the workup.

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Pathologic Features

Morphology

Because currently no single biological or genetic marker reliably identifies all cases of MDS, morphologic and cytogenetic evaluation remains the cornerstone of diagnosis. For optimal morphologic evaluation of cellular details, it is essential to obtain fresh and well-prepared blood and BM aspirate smears stained with Wright-Giemsa or May-Grunwald-Giemsa stains. Poor technical quality of the blood or BM smears is a common obstacle in reaching the accurate diagnosis of MDS, because informative differences in the degree of neutrophil granulation and in other nuclear and cytoplasmic details may be obscured or distorted. A good quality BM biopsy (at least 1.5 cm in length and at a right angle to the cortical bone) is invaluable for an accurate histologic assessment of cellularity, cell distribution, and megakaryocyte morphology and for immunohistochemical studies such as CD34 staining to identify blasts in cases with a non-representative aspirate smear.

Peripheral Blood

In addition to the quantitative abnormalities mentioned previously, microscopic examination of the blood can reveal

MYELODYSPLASTIC SYNDROMES—PATHOLOGIC FEATURES

Laboratory Findings
- One or more cytopenias, most often anemia with reticulocytopenia, neutropenia, and/or thrombocytopenia
- Macrocytosis, a dimorphic red blood cell pattern, or circulating blasts (<20%) may be present

Microscopic Findings
- Accurate morphologic evaluation of well-prepared and well-stained blood and bone marrow slides is essential in the diagnosis of MDS
- An accurate and exact count of circulating blasts is critical to classify and properly risk-stratify MDS: thresholds for circulating blasts are <1%, 1% (confirmed on at least 2 separate occasions), 2% to 4%, and ≥5%
- The marrow is typically hypercellular, with morphologic dysplasia in at least 10% of cells from at least one lineage (preferably at least 30% or 40% when applied to megakaryocytes); increased blasts (but <20%) and/or Auer rods may be present

Cytochemistry
- Iron stains of BM slides should be performed to identify ring sideroblasts. Bone marrow aspirate slides with spicules are optimal for iron staining; un-decalcified BM clot/particle preparations also can be used, but ring sideroblasts are difficult to detect in the decalcified core biopsy
- Globular or punctate periodic acid–Schiff (PAS) reactivity in dysplastic vacuolated erythroblasts may be seen
- Increased dual butyrate esterase and chloroacetate esterase reactivity may be seen in dysplastic myeloid elements

Immunohistochemistry
- Increased numbers (at least 5%) and/or compact, non-paratrabecular clusters of $CD34^+$ blasts may be present
- CD61 immunohistochemistry can highlight dysplastic megakaryocytes, especially micromegakaryocytes or abnormally small forms with hypolobated nuclei

Cytogenetics
- Conventional karyotyping is preferable to targeted FISH or sequencing technologies
- Cytogenetic analysis is essential in the diagnosis, subclassification, and prognostication of MDS
- According to the World Health Organization (WHO) classification of MDS, certain MDS-related cytogenetic abnormalities can form the basis for a diagnosis of MDS in the setting of persistent unexplained cytopenias even when there are insufficient morphologic features of myelodysplasia

Molecular Genetics
- As clinical studies continue to accumulate, molecular genetic data are likely to impact the diagnosis, classification, and prognostication of MDS. At present, however, even in the setting of cytopenia(s), the mere identification of somatic mutations may indicate clonal hematopoiesis but is not viewed as sufficient in itself to establish a diagnosis of MDS

Differential Diagnosis
- Cytopenia with hypercellular marrow: hemolytic anemia, immune-mediated or hypersplenism-related neutropenia or thrombocytopenia, megaloblastic anemia, viral infections (e.g., human immunodeficiency virus [HIV], chronic parvovirus B19, cytomegalovirus, Epstein-Barr virus), hemophagocytosis, acute leukemias, marrow replacement by lymphoma, or solid tumor
- Cytopenia with normocellular or hypocellular marrow: inherited or acquired BM failure syndromes, myelosuppressive infections, medication effect, toxic exposure, graft-versus-host disease affecting the marrow, T-cell large granular lymphocytic leukemia, hypocellular acute myeloid leukemia
- Dyserythropoiesis: severe hemolysis, marrow regeneration, megaloblastic anemia, effects of antimetabolite or antineoplastic therapy or other medications, congenital dyserythropoietic anemia, thalassemias
- Ring sideroblasts: alcohol, mitochondrial cytopathy, arsenic poisoning, zinc toxicity, pyridoxine deficiency, copper deficiency, anti-tuberculosis therapy
- Dysgranulopoiesis: granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF) therapy, trimethoprim-sulfamethoxazole, valproate, mycophenolate mofetil, or tacrolimus therapy
- Dysmegakaryopoiesis: sectioning artifact, HIV infection, valproate or thrombopoietin analogue therapy, certain inherited predisposition syndromes, such as those with germline mutations in *RUNX1*, *ANKRD26, ETV6*, or *GATA2*, idiopathic thrombocytopenic purpura (ITP), hematopoietic recovery following cytotoxic chemotherapy
- Increased blood or marrow blasts: marrow regeneration, G-CSF or GM-CSF therapy, leukemoid reaction, acute leukemia

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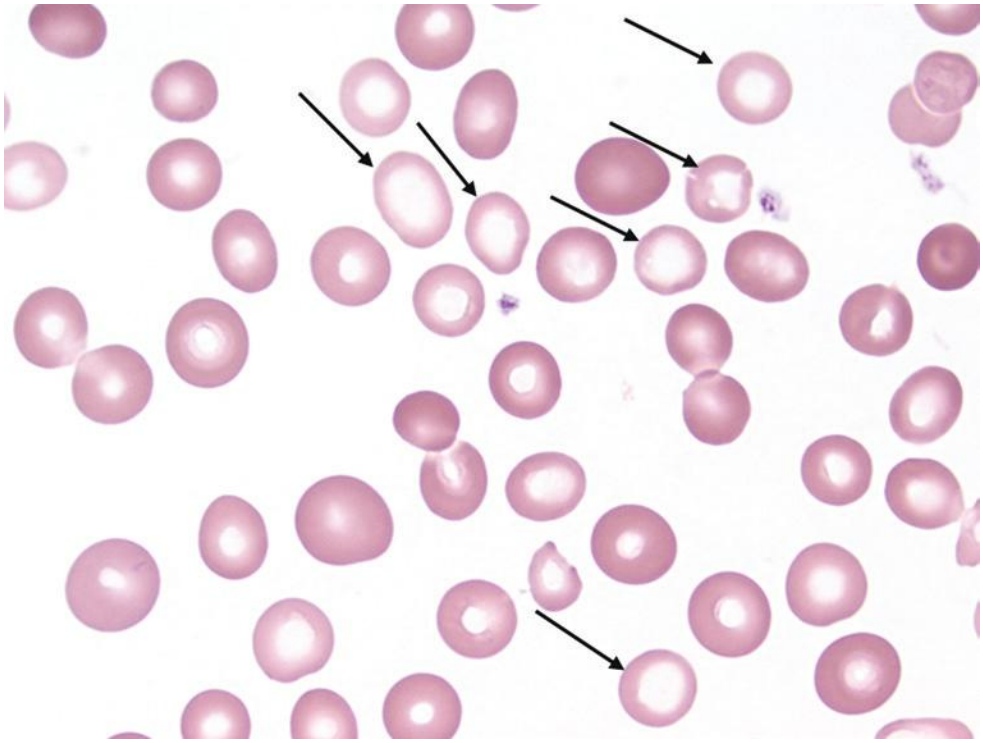


FIG. 18.2

Dimorphic red blood cells in myelodysplastic syndrome. Peripheral blood smear from a patient with myelodysplastic syndrome (MDS with ring sideroblasts and single lineage dysplasia), showing two populations of red blood cells—one hypochromic *(arrows)* and one normochromic (Wright-Giemsa stain).

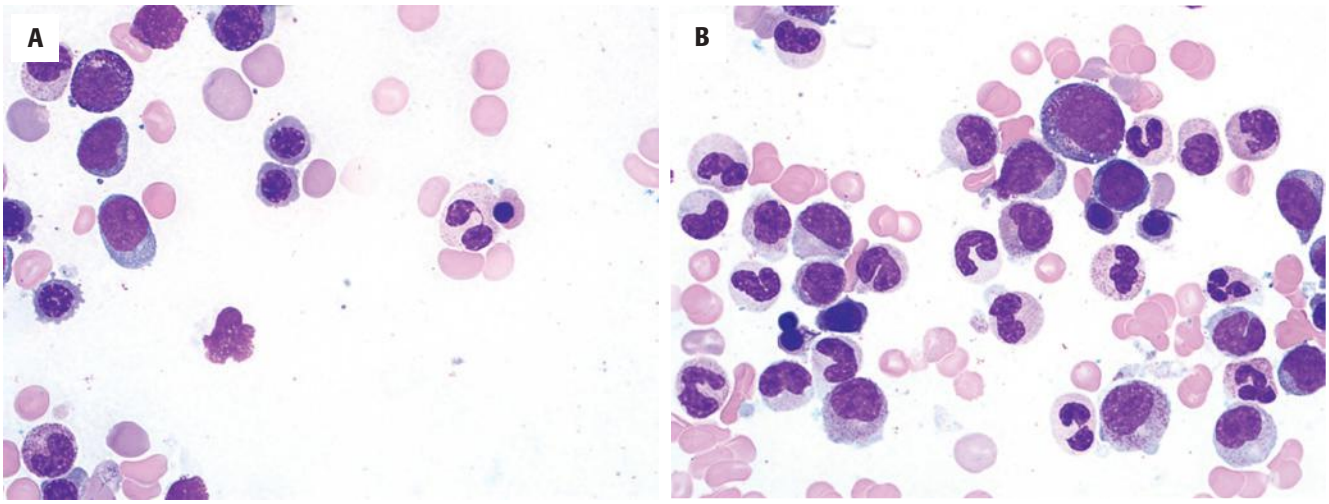


FIG. 18.3

Examples of dysgranulopoiesis. A, Dysplastic neutrophil exhibiting pseudo–Pelger-Huët anomaly with nuclear hyposegmentation in bone marrow aspirate. Note the thin, threadlike chromatin strand between the two nuclear lobes (Wright-Giemsa stain). B, A dysplastic granulocytic cell in the center exhibiting cytoplasmic hypogranulation in bone marrow aspirate (Wright-Giemsa stain).

abnormalities in one or more of the hematopoietic lineages. Dysplastic changes in the red blood cells can manifest as macrocytosis (unrelated to nutritional deficiencies), anisopoikilocytosis, or a dimorphic pattern with a mixture of normochromic and hypochromic erythrocytes (Fig. 18.2). Atypically large and hypogranular platelets may be present. Neutrophilic dysplasia may be observed in the cytoplasm as deficient or absent secondary granulation, aberrantly fine or coalescent secondary granules, or pseudo–Chédiak-Higashi granules. Dysplastic neutrophils may show nuclear hyposegmentation (pseudo–Pelger-Huët anomaly) or, less commonly, bizarre nuclear hypersegmentation (Fig. 18.3). Most cases of MDS will retain a certain proportion of normal neutrophils with an adequate complement of secondary granulation; these should be contrasted with the hypogranular or agranular neutrophils to rule out the possibility of poor or pale staining as an explanation for the apparent abnormal granulation of neutrophils. Atypical hypogranulated eosinophils and basophils may also be seen in MDSs, although such findings are infrequent. Auer ods (thin red cytoplasmic crystalline structures) may be seen in circulating blasts or immature myeloid cells in high-grade MDS cases.

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Leukocyte Differential Count and the Enumeration of Circulating Blasts

The blood smear should be carefully examined for the presence of circulating blasts. If blasts are present in the peripheral blood, an accurate determination of their exact percentage is essential both for subclassification and for prognostication. In a study of patients with low-grade MDS, Knipp and colleagues observed that, compared to patients without PB blasts, those with 1% circulating blasts had both a shorter median survival and a higher cumulative risk of AML evolution. Both the International Working Group on Morphology of Myelodysplastic Syndrome and the WHO recommend that a count of at least 200 cells be performed, with some investigators recommending a differential count of as many as 500 cells. When the presence of 1% circulating blasts forms the basis for the diagnosis of MDS-Unclassifiable (see below), this finding should be observed on at least two separate occasions.

Bone Marrow

The BM in MDS is often hypercellular. This paradox of marrow hypercellularity despite peripheral blood cytopenias constitutes the ineffective hematopoiesis that is characteristic of MDS. In some MDS cases, the BM may be normocellular or hypocellular, in which case the differential diagnosis of an evolving aplastic anemia, hypoplastic AML, or a sampling artifact should also be considered, particularly when there are no definitive morphologic features of dysplasia.

Dyserythropoiesis

Dysplastic erythroid precursors may exhibit asynchronous nuclear-cytoplasmic development (so-called megaloblastoid features), nuclear abnormalities such as nuclear lobulation, irregular nuclear contours, karyorrhexis, and atypical multinucleation (Fig. 18.4A–C). Dyserythropoiesis may also manifest as ring sideroblasts, which are defined as erythroid precursors with five or more iron granules encircling at least one third of the nuclear circumference (Fig. 18.4D). Dysplastic erythroblasts may show cytoplasmic vacuoles that stain positively for periodic acid-Schiff in a globular or coarsely punctate fashion (Fig. 18.4B and E). Of the three hematopoietic lineages, dysplasia of the erythroid lineage is the least specific for MDS.

Dysgranulopoiesis

Dysplastic neutrophils and precursors with cytoplasmic hypogranulation or nuclear hyposegmentation, including pseudo–Pelger-Huet forms, can be seen both in the blood and in the BM aspirate smears. Dysplastic neutrophils with non-segmented nuclei can be distinguished from neutrophil myelocytes by the clumped, more condensed and more mature-appearing nuclear chromatin in the former. Some authors have emphasized the finding of "agranular" neutrophils as being more reliable than hypogranular neutrophils in establishing the diagnosis of MDS.

Dysmegakaryopoiesis

Dysplastic megakaryocytes may be abnormally small with non-lobated or hypolobated nuclei, or they may be normal to large in size but with abnormally disconnected nuclear lobes (so-called osteoclast-like megakaryocytes; Fig. 18.5A–C). Care must be taken when evaluating megakaryocyte morphology in the bone marrow biopsy sections, because sectioning artifact may create the illusion of hypolobated nuclei or widely separated nuclear lobes. Clustering of small megakaryocytes is best visualized in the core biopsy sections and can be a helpful feature in support of a myeloid neoplasm, although myeloproliferative neoplasms or marrow regeneration can also be associated with this finding. Whereas the WHO guidelines advise the finding of dysplastic morphologic features in at least 10% of erythroid and granulocytic cells, a higher threshold at 30% to 40% has been suggested as being more specific for dysmegakaryopoiesis; moreover, some consider the finding of micromegakaryocytes to be more specific for MDS compared to other morphologic manifestations of megakaryocyte dysplasia.

Increased Blasts

Accurate enumeration of bone marrow blasts is essential in the diagnosis, classification, and prognostication of MDSs. At the minimum, a 500-cell differential count should be performed on the aspirate smear. By morphologic evaluation, normal BM smears usually contain fewer than 3% blasts, although the cut-off level defining excess blasts as first proposed by the French-American-British (FAB) group has remained at 5%. Substitution of a morphologic blast count with one obtained by flow cytometry immunophenotyping is not recommended; dilution of the marrow aspirate by peripheral blood, specimen manipulation during processing for flow cytometry, and, in some cases, the presence of blasts lacking in CD34 expression may distort the marrow blast percentage derived from flow cytometric immunophenotyping.

Auer Rods

Based on the observation that Auer rods are associated with more aggressive disease, their detection in MDS, regardless of the PB or BM blast percentages, is considered sufficient to categorize an MDS case as "MDS with excess blasts-2" (MDS-EB-2). Rarely, Auer rods may be identified in cases with morphologic features that otherwise might be viewed as lower grade MDS. In one retrospective study involving three institutions, cases of MDS with less than 5% marrow blasts, but where Auer rods were present, had a worse prognosis than would have been expected according to their International Prognostic Scoring System (IPSS) scores, with rapid progression to AML, shortened survival, or both.

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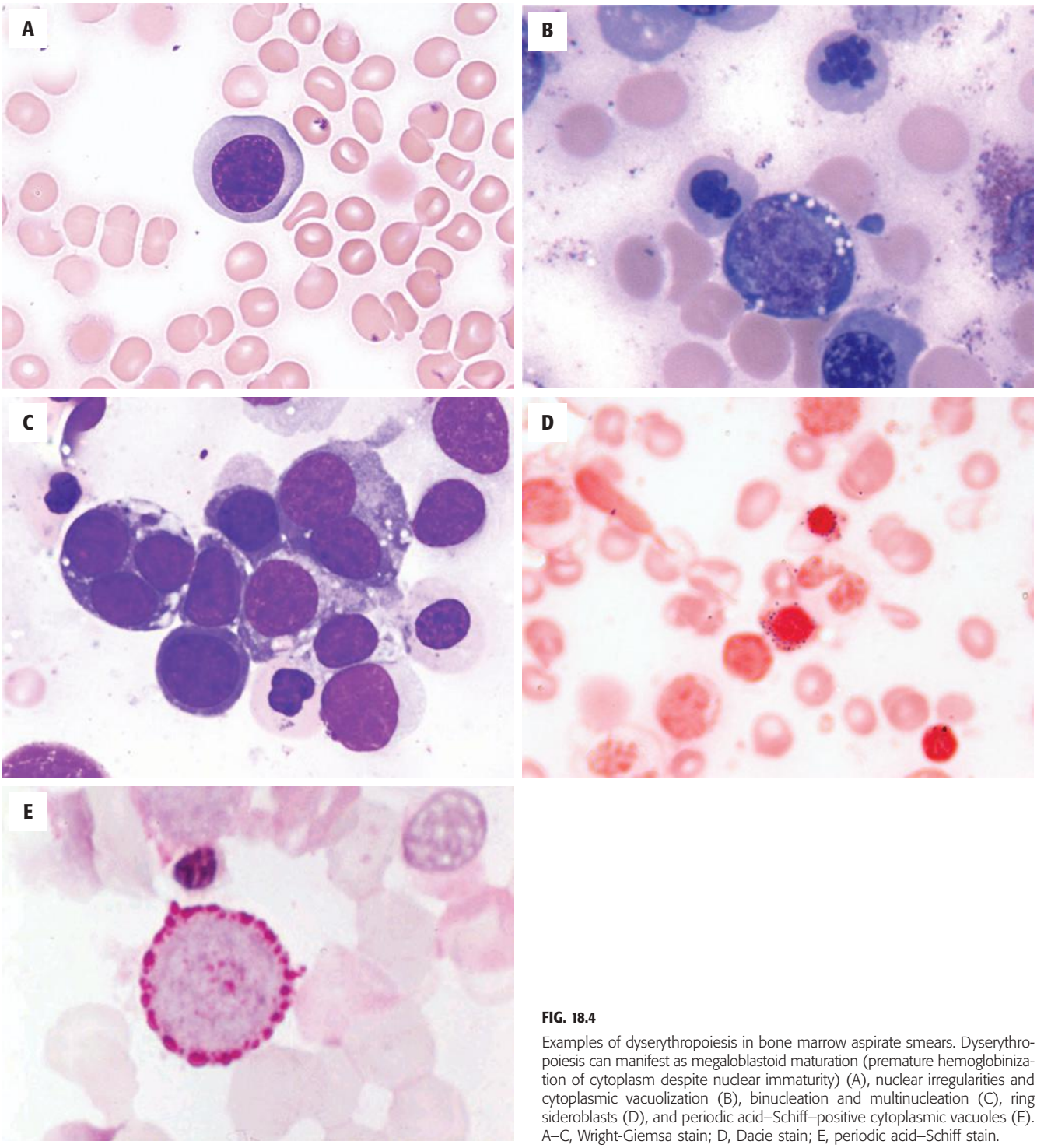


FIG. 18.4

Examples of dyserythropoiesis in bone marrow aspirate smears. Dyserythropoiesis can manifest as megaloblastoid maturation (premature hemoglobinization of cytoplasm despite nuclear immaturity) (A), nuclear irregularities and cytoplasmic vacuolization (B), binucleation and multinucleation (C), ring sideroblasts (D), and periodic acid–Schiff–positive cytoplasmic vacuoles (E). A–C, Wright-Giemsa stain; D, Dacie stain; E, periodic acid–Schiff stain.

Ancillary Studies

Cytochemistry

A periodic acid–Schiff stain of the marrow aspirate may highlight globular or coarsely punctate positive reactivity in erythroblasts with cytoplasmic vacuoles (see Fig. 18.4E). An iron stain of the marrow aspirate smear is necessary to look for ring sideroblasts (see Fig. 18.4D). Regarding the granulocytes, a combined butyrate esterase and chloroacetate esterase stain can reveal an abnormally increased population of dual-esterase–positive cells in some cases of MDS, thus uncovering an additional feature of abnormal myeloid maturation. However, this feature is not pathognomonic of MDS and can also be seen in acute myeloid leukemia with monocytic

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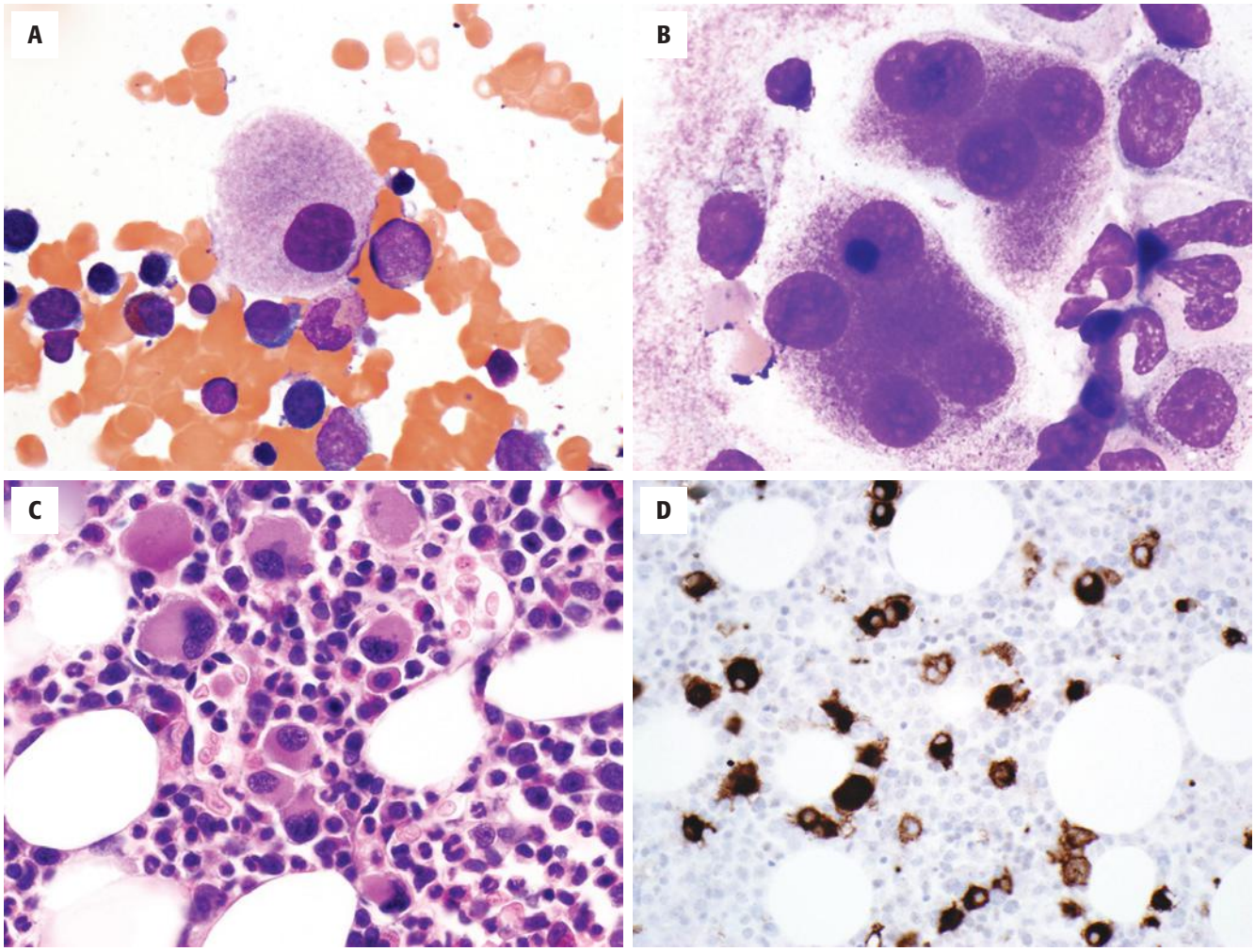


FIG. 18.5

Examples of dysmegakaryopoiesis. A, Dysplastic small megakaryocyte with a nonlobated nucleus. The cell is mature, as evident by the abundant platelet granules present in the cytoplasm (Wright-Giemsa stain). B, Dysplastic megakaryocytes with widely separate nuclear lobes (Wright-Giemsa stain). C, Clusters of dysplastic small and hypolobated megakaryocytes in this bone marrow biopsy section (hematoxylin and eosin). D, An immunohistochemical stain for CD61 highlights numerous small megakaryocytes with nonlobated nuclei and micromegakaryoctes.

differentiation or chronic myelomonocytic leukemia and in hematopoietic regeneration.

Immunohistochemistry

A basic panel of antibodies that can be useful in suspected cases of MDS includes the blast marker CD34 and a megakaryocytic marker such as CD31, CD42, or CD61. CD34 stains the blast cells in most cases of MDS and can be of great value in assessing the number and distribution of these cells in the bone marrow biopsy and/or clot section. This is particularly important in MDS with fibrosis and in hypocellular MDS, in which the aspirate smear is often hemodilute and may underrepresent medullary blasts. CD34 immunohistochemistry is also useful for assessing small, compact blast aggregates (clusters of at least three $CD34^+$ cells located away from bone trabeculae) that are highly suggestive of a neoplastic process. When these blast aggregates are detected in MDS, they are usually associated with the MDS subtypes with excess blasts and have been variously referred to as "multifocal accumulations of $CD34^+$ progenitor cells" or "abnormal localization of immature precursors" (ALIP). In the few cases of MDS in which the blast cells are CD34-negative, CD117 may be used as an alternate marker, although caution should be used when interpreting this stain, because promyelocytes and early erythroid precursors can stain positively for this marker. Megakaryocyte markers (CD31, CD42, or CD61) are used in the detection of both atypical accumulations (groups or clusters) and cytomorphologic atypia of megakaryocytes, particularly micromegakaryocytes that may be overlooked on the routine histologic sections (see Fig. 18.5D).

In rare cases in which mastocytosis is associated with the MDS, staining for tryptase and CD117 helps highlight abnormal, dense infiltrates of spindle-shaped mast cells. Demonstration of co-expression of CD2 or CD25 on the abnormal mast cell population can lend further support to a concurrent mast cell disease.

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Immunophenotype

The role of flow cytometric immunophenotyping in the diagnosis or management of patients with MDS continues to remain unsettled. "Immunophenotypic dysplasia" represents abnormal antigen expression patterns in maturing granulocytic, monocytic, and erythroid cells that are often seen in MDS cases subjected to flow cytometry. Reproducible abnormalities in blast cells in MDS include abnormal expression patterns of CD117, CD38, CD33, or aberrant expression (so-called lineage infidelity) of CD2, CD5, CD7, CD19, or CD56. A marked decrease in hematogones has also been described in patients with MDS compared to non-cytopenic and non-MDS cytopenic control subjects.

The presence of paroxysmal nocturnal hemoglobinuria (PNH) phenotype cells, with loss of the glycosylphosphatidylinositol (GPI)-anchored proteins CD55 or CD59, has been associated with a more indolent course and better hematologic responsiveness to immunosuppressive therapy in MDS. Westers and colleagues reported that among MDS patients with IPSS low- and intermediate-1 risk, there was a poorer response to erythropoietin-stimulating agents, granulocyte colony-stimulating factor (G-CSF) therapy, or both, in patients with immunophenotypically aberrant blasts (characterized as exhibiting aberrant expression of CD5, CD7, or CD56; expression of CD7 with loss of CD45; or loss of the myeloid antigen CD33) compared to those with immunophenotypically normal blasts. However, despite the increasing number of studies documenting immunophenotypic abnormalities in MDS, the specificity of such observations remains unclear, owing largely to persistent uncertainties regarding the potential overlap with normal, reactive non-neoplastic disorders as well as uncertainties regarding changes in blast phenotypes during hematopoietic regeneration and following growth factor stimulation. Moreover, there continues to be a lack of standardization among the various flow cytometry immunophenotyping laboratories on the minimum required panel of antibodies to be employed. Thus, while acknowledging that abnormal immunophenotypic patterns can help support a diagnosis of MDS, the 2016 revised WHO classification of MDS also recommends that such abnormalities be interpreted in the larger context of the clinical, morphologic, and genetic findings and not be used in isolation to establish a primary diagnosis of MDS.

Cytogenetics

Cytogenetic analysis of the BM is essential in the evaluation of patients with suspected MDS, for both diagnostic and prognostic purposes. The overall estimated frequency of chromosomal abnormalities among all patients with MDS is about 50%, with a higher proportion of abnormal karyotypes among MDS subtypes with excess blasts. Whereas a normal cytogenetic result does not exclude the possibility of MDS, the presence of specific clonal cytogenetic abnormalities in patients with unexplained cytopenias and borderline or no appreciable morphologic abnormalities can be of great help in establishing the diagnosis of MDS. Table 18.1 lists those specific cytogenetic abnormalities that, according to the revised 2016 WHO guidelines for classifying the MDS, may be considered as presumptive evidence of MDS in the setting of unexplained persistent cytopenia, even in the absence of diagnostic morphologic features of dysplasia.

When the diagnosis of MDS is clear, detection of certain types of cytogenetic anomalies can provide valuable prognostic information. For example, the presence of three or more chromosomal anomalies or of abnormalities involving chromosome 7 is considered to portend a poor prognosis. In contrast, a normal karyotype or a del(5q) (either as a single finding or with one other low-risk abnormality), is generally associated with a relatively favorable prognosis. Reports from large MDS registries, including merged multinational databases, those including both de novo and therapy-related patients with MDS, and those including patients receiving a variety of treatments

TABLE 18.1

Myelodysplastic Syndrome–Defining Cytogenetic Abnormalities and Their Frequencies

Abnormality	Primary MDS (%)	Therapy-Related MDS (%)
Unbalanced		
+8[a]	10	
–7 or del(7q)	10	50
–5 or del(5q)	10	40
del(20q)[a]	5–8	
–Y[a]	5	
i(17q) or t(17p)	3–5	
–13 or del(13q)	3	
del(11q)	3	
del(12p) or t(12p)	3	
del(9q)	1–2	
idic(X)(q13)	1–2	
Balanced		
t(11;16)(q23;p13.3)		3
t(3;21)(q26.2;q22.1)		2
t(1;3)(p36.3;q21.2)	1	
t(2;11)(p21;q23)	1	
inv(3)(q21q26.2)/ t(3;3)(q21.3;q26.2)	1	
t(6;9)(p23;q34)	1	

[a]In the 2008 and 2016 World Health Organization classification, the presence of these abnormalities as the sole cytogenetic abnormality in the absence of morphologic dysplasia is not considered as definitive evidence for MDS. In contrast, the other abnormalities listed are considered to be "presumptive" evidence of MDS in the setting of persistent cytopenias even when there are no definitive morphologic features.

MDS, myelodysplastic syndrome.

Adapted from Swerdlow SH, Campo E, Harris NL, et al, editors: *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue*, ed 4, Lyon, 2008, IARC.

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have over the years repeatedly confirmed the prognostic value of cytogenetics in MDS.

Molecular Genetics to Estalbish Clonality

Although clonal cytogenetic abnormalities are demonstrated in only approximately 50% of patients with MDS, targeted next-generation genetic sequencing has identified recurrent mutations in 80% to 90% of those patients (Table 18.2). Although the presence of such mutations has shed light on the molecular pathogenesis of MDS, their use in MDS diagnostics has been tempered by several important observations. First, as mentioned above, in the CHIP phenomenon, similar MDS-type somatic mutations have been reported in as many as 10% to 20% of apparently healthy individuals over the age of 70 years—the very same age group in which MDS becomes a frequent diagnostic consideration! Moreover, when more sensitive techniques capable of detecting clonal mutations at a frequency as low as 0.03% VAF, clonal hematopoiesis may affect as many as 95% of healthy individuals between the age of 50 and 60 years, as recently evaluated in the Nurses' Health Study. Because of the high frequency of CHIP in older individuals (much higher than the age-matched incidence of MDS), it is generally not recommended to use the presence of MDS-type somatic mutations to diagnose MDS, even in patients with cytopenias, lest non-neoplastic etiologies for cytopenias be overlooked and missed in these elderly patients among whom there may be comorbid conditions. Second, with the exception of *SF3B1*, no single mutation is seen in more than a third of MDS cases; in fact, the majority of mutations occurs at a frequency below 5%. Third, many of these MDS-associated mutations have also been reported in a variety of other non-MDS diseases. For example, *SF3B1* mutation occurs in chronic lymphocytic leukemia and in myelodysplastic/myeloproliferative neoplasm (MDS/MPN) overlap entities, whereas *TET2* and *DNMT3A* mutations are seen in lymphomas. The contribution of molecular genetics to our understanding of MDS undoubtedly will be immense, but its application to the diagnosis of MDS requires further investigation and may rest on a combination of the numbers and types of mutations and their allele fraction, as well as other as-yet-undefined parameters. At the time of this writing, there is no single simple genetic test for MDS.

TABLE 18.2

Common Recurrent Gene Mutations in Myelodysplastic Syndromes

Pathway and Genes	Approximate Frequency in MDS	Prognosis
Splicing factors		
SF3B1[a]	20%–35%	Favorable
SRSF2[a]	10%–15%	Adverse
U2AF1[a]	5%–10%	Adverse
ZRSR2	5%–10%	Uncertain
Epigenetic regulators		
Chromatin modifiers		
ASXL1[a]	15%–20%	Adverse
EZH2	5%–10%	Adverse
DNA methylation		
TET2[a]	20%–30%	Uncertain
DNMT3A[a]	10%–15%	Adverse
IDH1/2	~5%	Uncertain
Transcription factors/ Tumor suppressors		
RUNX1	10%–15%	Adverse
TP53[a]	5%–10%	Adverse
BCOR[a]	~5%	Adverse
ETV6	<5%	Adverse
Growth factor/signaling		
NRAS/KRAS	5%–10%	Adverse
CBL[a]	~5%	Adverse
Cohesin complex		
STAG2	~5%	Adverse

[a]Also reported in hematologically normal individuals with "clonal hematopoiesis of indeterminate potential" (CHIP).

Molecular Genetics to Elucidate the Pathogenesis and Biology of MDSs

The application of high-throughput DNA sequencing to longitudinally collected clinical MDS and AML samples has contributed to the paradigm of MDS as a multistage malignancy, whose ultimate clinical phenotype is a result of highly dynamic clonal selection, with evidence of clonal evolution, increasing clonal complexity, and even disappearance of clonal sub-populations as the disease progresses.

In addition to the numerous gene mutations identified in patients with MDS as described above, gene expression profiling has implicated certain pathways in the pathogenesis of MDS. For example, Pellagatti and colleagues found that the interferon and thrombopoietin signaling pathways are the most frequently dysregulated pathways in MDS, with upregulation of *STAT1, IRF9, IFIT1,* and *IFITM1* genes and of *TPOR, STAT1,* and *SOS1* genes, respectively. In this same study, immunodeficiency, B-cell receptor, IL4, and chemokine signaling pathways were the most frequently deregulated pathways in lower grade as compared to higher grade MDS; this observation is intriguing in view of the known clinical responsiveness to immunosuppressive therapy in some patients with low-grade MDS.

Although not specific to MDS, the concept of epigenetic changes exerting another level of genetic control provides a

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new and exciting intellectual framework for the purpose of therapeutic development in MDSs. In this paradigm, expression or silencing of a gene does not require actual alterations at the level of the primary nucleotide sequence. Instead, it may occur by enzymatic reorganization of the three-dimensional structure of chromatin, thereby modulating the accessibility of that genetic region to transcription factors and other proteins involved in gene expression. Hypermethylation of *CDKN2b,* encoding the cell cycle regulator $p15^{INK4B}$, the pro-apoptotic *DAPK1* gene, the ribosomal-processing gene *RPS14*, and the *FHIT* gene, among others, has been identified in approximately 50% of patients with MDS, which may explain the success of the hypomethylating agent 5′ azacytidine in the treatment of patients with MDS (see Epigenetic Therapies later in this chapter). Histone acetylation at specific lysine residues on histones H3 and H4 has also been shown to represent another means of reorganizing the three-dimensional structure of chromatin that may allow for either repressive or permissive gene transcription.

Diagnostic Classification

French-American-British Classification

In 1975 the FAB cooperative group published the first major international MDS classification. This classification was based entirely on morphologic features identified in the PB and BM aspirate smears. In the initial proposal, two subtypes of "dysmyelopoietic syndromes" were defined: refractory anemia with excess blasts (RAEB) and chronic myelomonocytic leukemia (CMML). In 1982, refractory anemia (RA), refractory anemia with ring sideroblasts (RARS), and RAEB in transformation (RAEB-T) were added. This subclassification considered the percentage of peripheral blood and marrow blasts, the percentage of ring sideroblasts, and the presence of a blood monocytosis. However, the number and extent of cytopenias and the extent of dysplasia were not explicitly addressed in the FAB classification system. Furthermore, among patients in those FAB MDS categories without increased blasts (RA, RARS, and CMML), the wide range of median survivals (from <1 year to 5 to 6 years) caused some to question the clinical reliability of this classification system.

World Health Organization (WHO) Classification

Compared to the FAB classification, the 2001 WHO guidelines differed in several substantial ways. First, the proportion of blasts required for a diagnosis of AML was lowered from 30% to 20%, which effectively eliminated the FAB category of RAEB-T. Second, the high-grade MDS subcategory of RAEB was subdivided into RAEB-1 and RAEB-2. Third, to reflect the distinct impact of multilineage dysplasia (two or three of the hematopoietic lineages manifesting significant dysplasia) on overall survival and on the likelihood of transformation to acute leukemia, the low-grade FAB subtypes of RA and RARS were more strictly and narrowly defined, and a new category of "refractory cytopenia with multilineage dysplasia" (RCMD), with ring sideroblasts (RCMD-RS) or without, was added. Fourth, the 2001 WHO guidelines recognized a distinct low-grade MDS entity, defined by the presence of del(5q) as the sole chromosomal abnormality with certain associated morphologic and clinical features. Finally, the subcategory of CMML was removed from the MDS category and assigned to the overlap group of MDS/MPN.

2008 WHO Classification

The 2008 WHO classification of MDS introduced refinements of the 2001 WHO guidelines. An emphasis was placed on the exact percentages of circulating blasts, even when less than 5%, in the definition of the various MDS subtypes. Two new subcategories were added. The first, "refractory cytopenia with unilineage dysplasia" (RCUD), included patients with RA as well as those rare patients with refractory neutropenia (RN) or refractory thrombocytopenia (RT), thus accounting for all three individual cytopenias. A second new and important category was that of childhood MDS, including the provisional entity "refractory cytopenia of childhood" (RCC).

2016 Revision to the WHO Classification

In recognition of the fact that MDS among adults is defined mainly by the degree of myelodysplasia and proportions of blasts, and because the specific cytopenia(s) often does/do not correlate with the type or number of dysplastic lineages (see MDS-SLD section below), the 2016 revision introduces nomenclature changes such that the preface "myelodysplastic syndrome" replaces previous references to "refractory anemia" and "refractory cytopenia" in the names of the various MDS entities, followed by the modifiers specific to the different subtypes according to the number of dysplastic lineages, presence of ring sideroblasts, excess blasts, or the del(5q) cytogenetic abnormality.

Based in part on a multiinstitutional study showing that in both AML subtypes (acute erythroleukemia) and MDS with over 50% bone marrow erythroid precursors, patient overall survival depended on the cytogenetic risk group but not on the blast count. In the 2016 revision, the percentage of marrow blasts is now calculated as a percentage of all nucleated cells, not just of the "nonerythroid" cells. This altered definition of acute erythroid leukemia (see Chapter 14) results in the re-classification of most cases of acute erythroid leukemia (erythroid/myeloid subtype) as "MDS with excess blasts." One recent study from the Grupo Espanol de Sindromes Mielodisplasicos suggested that quantifying marrow blasts as a percentage of total non-erythroid cells in all MDS might provide better IPSS prognostic stratification, but it is not clear if this holds true also in the new IPSS-R scheme with its finer subdivisions of marrow blasts.

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When the presence of 1% circulating blasts and less than 5% bone marrow blasts forms the basis for the diagnosis of "MDS, unclassifiable" (MDS-U, see below), the reproducibility of the 1% circulating blasts must now be confirmed on at least two separate occasions.

Since the 2008 classification, multiple studies have demonstrated the close association between mutations of the spliceosome gene *SF3B1* and the presence of ring sideroblasts. In the 2016 WHO revision, the category of MDS-RS was expanded to include also cases with ring sideroblasts and multi-lineage dysplasia (previously encompassed within "RCMD" in the 2008 classification). In addition, given the lack of any prognostic relevance related to the actual percentage of ring sideroblasts in the context of an *SF3B1* mutation, the category of MDS-RS was further broadened to include cases with as few as 5% RS as long as an *SF3B1* gene mutation is present, or with at least 15% ring sideroblasts if there is no *SF3B1* mutation.

Finally, in a large multicenter study of de novo MDS with del(5q), Mallo and colleagues showed no statistically significant differences in overall survival between those with only an isolated del(5q) abnormality and those with one additional clonal cytogenetic abnormality, as long as the additional abnormality did not involve chromosome 7. Based on these data, the 2016 WHO revision now includes within the category "MDS with isolated del(5q)" also those cases with one additional cytogenetic abnormality, as long as the additional abnormality is not monosomy 7 or del(7q). Moreover, since *TP53* mutation appears to predict poorer response to lenalidomide therapy, it is recommended that patients with MDS with isolated del(5q) be evaluated also for *TP53* mutation, either by immunohistochemistry or by molecular genetic analysis.

The following section discusses in detail the diagnostic features of the different categories of MDS recognized in the 2016 WHO classification. The key diagnostic criteria are presented in Table 18.3.

MYELODYSPLASTIC SYNDROME WITH SINGLE LINEAGE DYSPLASIA (MDS-SLD)

The MDS-SLD designation encompasses 10% to 20% of MDS cases and includes patients with refractory cytopenia or bicytopenia associated with significant dysplasia limited to one cell line. Refractory pancytopenia that is associated with single lineage dysplasia is classified as "MDS, unclassifiable" (MDS-U, see below). There are fewer than 1% blasts in the peripheral blood and fewer than 5% in the marrow. No Auer rods are present. Morphologic features of dysplasia must be present in at least 10% of the cells in the affected hematopoietic lineage. Ring sideroblasts account for fewer than 15% of erythroid precursors in the absence of an *SF3B1* gene mutation, or less than 5% in the presence of such a mutation. Clonal cytogenetic abnormalities may be present, but there cannot be isolated del(5q).

In most cases of MDS-SLD, dysplasia is limited to the erythroid lineage (i.e., the FAB "refractory anemia" category), which is considered as a low-grade MDS with median survival in the range of 6 to 7 years and with less than 5% of cases progressing to AML. The diagnosis of MDS-SLD is one of exclusion and requires that the cytopenia(s) is/are of at least 6 months' duration and that potential reactive or inherited causes of cytopenia and dysplasia be rigorously excluded (see Differential Diagnosis, Qualitative Defects, Dyserythropoiesis). MDS-SLD with dysplasia limited only to the neutrophilic lineage or megakaryocytic lineage is uncommon, and cases presenting with isolated neutropenia or thrombocytopenia (formerly "refractory neutropenia" and "refractory thrombocytopenia," respectively) account for less than 2% of all MDS cases. Non-neoplastic causes for isolated neutropenia or thrombocytopenia are much more common than clonal diseases. Morphologic features of dysmegakaryopoiesis can be seen in certain germline predisposition syndromes such as those associated with *RUNX1, ANKRD26, ETV6*, or *GATA2* mutations, among others. Moreover, morphologic features of dysplasia have been described in both apparently healthy individuals as well as those with non-neoplastic cytopenias, and inter-observer variability remains a concern in the microscopic identification of dysmyelopoietic features. (See Differential Diagnosis, Quantitative Defects and Qualitative Defects, Dysgranulopoiesis and Dysmegakaryopoiesis.) Importantly, in MDS-SLD, the cytopenia(s) observed in the blood does/do not necessarily correspond to the lineage in which dysplasia is observed. For example, a patient with MDS-SLD and isolated anemia may entirely lack dysplasia in the erythroid lineage and instead shows dysplasia in the granulocytic lineage or in megakaryocytes, despite normal neutrophil and platelet counts.

MYELODYSPLASTIC SYNDROME WITH RING SIDEROBLASTS (MDS-RS)

In MDS with ring sideroblasts (MDS-RS), abnormal mitochondrial iron metabolism manifests as ring sideroblasts, which are defined as erythroblasts in which five or more iron granules encircle at least one third of the cells' nuclear circumference identified on an iron stain. MDS-RS is characterized by anemia, dyserythropoiesis, and 5% or more ring sideroblasts with *SF3B1* mutation (or ≥15% ring sideroblasts in the absence of an *SF3B1* mutation). In MDS-RS with single lineage dysplasia (MDS-RS-SLD), there is no significant granulocytic or megakaryocytic dysplasia. The red blood cells often exhibit a dimorphic pattern with a mixture of normochromic and hypochromic cells, but a normochromic and macrocytic anemia may also be observed. There are no circulating blasts, and marrow blasts number fewer than 5%. It should be noted that ring sideroblasts and *SF3B1* mutation can also be found in other subsets of MDS (including MDS with excess blasts), in AML, and ring sideroblasts without SF3B1 mutations may be seen in some non-neoplastic diseases (see Differential Diagnosis, Qualitative Defects, Ring Sideroblasts). Patients with MDS-RS-SLD have a good prognosis, with

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TABLE 18.3

Key Diagnostic Features of Myelodysplastic Syndrome (MDS) Subtypes According to the 2016 World Health Organization

Name	Peripheral Blood	Bone Marrow	Cytogenetics
MDS with single lineage dysplasia (MDS-SLD)	1 or 2 cytopenia(s) <1% blasts; no Auer rods	<5% blasts; no Auer rods <15% ring sideroblasts and no *SF3B1* mutation, or <5% ring sideroblasts with *SF3B1* mutation Unilineage erythroid, granulocytic, or megakaryocytic dysplasia in ≥10% of cells	Any, except those associated with MDS with isolated del(5q)
MDS with multilineage dysplasia (MDS-MLD)	1–3 cytopenias <1% blasts; no Auer rods	<5% blasts; no Auer rods Dysplasia in ≥10% cells in ≥2 lineages	Any, except those associated with MDS with isolated del(5q)
MDS with ring sideroblasts with single lineage dysplasia (MDS-RS-SLD)	1 or 2 cytopenias <1% blasts; no Auer rods	Unilineage dysplasia, <5% blasts; no Auer rods ≥5% ring sideroblasts and *SF3B1* mutation (or ≥15% ring sideroblasts if no *SF3B1* mutation)	Any, except those associated with MDS with isolated del(5q)
MDS with ring sideroblasts with multilineage dysplasia (MDS-RS-MLD)	1–3 cytopenias <1% blasts, no Auer rods	<5% blasts; no Auer rods ≥5% ring sideroblasts and *SF3B1* mutation (or ≥15% ring sideroblasts if no *SF3B1* mutation) Dysplasia in at least 10% cells in ≥2 lineages	Any, except those associated with MDS with isolated del(5q)
MDS with excess blasts-1 (MDS-EB-1)	1–3 cytopenias 2%–4% blasts, no Auer rods	5%–9% blasts; no Auer rods Dysplasia in 0–3 lineages Ring sideroblasts may or may not be present	Any clonal abnormalities
MDS with excess blasts-2 (MDS-EB-2)	1–3 cytopenias 5%–19% blasts, ± Auer rods[a]	10%–19% blasts, ± Auer rods[a] Dysplasia in 0–3 lineages, Ring sideroblasts may or may not be present	Any clonal abnormalities
MDS with isolated del(5q)	1–2 cytopenias; the platelet count may be normal or high; <1% blasts; no Auer rods	<5% blasts; no Auer rods Dysplasia in ≥10% cells in 1–3 lineages Ring sideroblasts may or may not be present	Del(5q) only, or with 1 additional clonal abnormality excluding del(7q), –7
Childhood MDS, including refractory cytopenia of childhood (RCC) (provisional)	1–3 cytopenias <2% blasts	<5% blasts Dysplasia in 1–3 lineages No ring sideroblasts	Any clonal abnormalities
MDS, unclassifiable (MDS-U)	1–3 cytopenias	<5% blasts; no Auer rods	Any clonal abnormalities
With 1% peripheral blood blasts	1% circulating blasts demonstrated on 2 separate occasions; no Auer rods	Dysplasia in 1–3 lineages	
With single lineage dysplasia and pancytopenia	3 cytopenias <1% blasts; no Auer rods	<5% blasts; no Auer rods Unilineage dysplasia;	Any clonal abnormalities
With defining cytogenetic abnormalities	1–3 cytopenias <1% blasts; no Auer rods	<5% blasts; no Auer rods No dysplasia	MDS-defining clonal abnormalities (see Table 18.1)

[a]Presence of Auer rods in MDS cases with <20% blasts in blood or bone marrow qualifies as MDS-EB-2, irrespective of the blast percentage. ±, With or without.
Arber DA, Orazi, A, Hasserjian R, et al: The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia, *Blood* 127(20):2391–2405, 2016.

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a median survival of 7 to 9 years or longer and with a low rate of conversion to AML (<5%). In MDS-RS-MLD, there is dysplasia involving the granulocytic and/or megakaryocytic lineages in addition to the erythroid lineage; these patients have a shorter survival. There is also a suggestion that patients with MDS-RS but without an *SF3B1* mutation may have a worse outcome compared to those with the mutation. Further study is needed to determine the relative contribution of multilineage dysplasia versus *SF3B1* mutation to the prognosis of MDS-RS.

When patients have both ring sideroblasts and a concurrent elevated platelet count (≥450 × 10^9/L) with megakaryocytic features resembling those seen in myeloproliferative neoplasms, consideration should be given to the entity "myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis*"* (MDS/MPN-RS-T). Whereas the *JAK2* V617F mutation is uncommon in MDS, it has been reported in 30% to 90% of patients with MDS/MPN-RS-T, with mutation of *SF3B1* occurring in approximately 72% of the cases.

MYELODYSPLASTIC SYNDROME WITH MULTILINEAGE DYSPLASIA (MDS-MLD)

Accounting for approximately 30% to 40% of MDS cases, MDS-MLD is characterized by one to three cytopenia(s) and dysplastic changes affecting at least 10% of cells in two or more of the hematopoietic lineages. There are fewer than 1% blasts in the blood, fewer than 5% blasts in the BM, and no Auer rods. Cases with multilineage dysplasia, 15% or greater ring sideroblasts, or 5% or greater ring sideroblasts and an *SF3B1* mutation are classified separately within the MDS-RS group (MDS-RS-MLD, see above). Cases meeting the criteria for MDS-MLD but with persistently 1% circulating blasts should be diagnosed as MDS-U. With a reported median survival of approximately 30 months and with approximately 10% of patients progressing to AML, the diagnosis of MDS-MLD carries a more unfavorable outcome compared to that of MDS-SLD.

MYELODYSPLASTIC SYNDROME WITH EXCESS BLASTS (MDS-EB)

As a group MDS-EB accounts for approximately 40% of cases of MDS and is subdivided into two subgroups, MDS-EB1 and MDS-EB2. Most cases of MDS-EB1 have 5% to 9% blasts in the BM, fewer than 5% blasts in the blood, and no Auer rods; a diagnosis of MDS-EB1 can be also made with fewer than 5% BM blasts if there are 2% to 4% circulating blasts. The category MDS-EB2 encompasses cases in which there are 10% to 19% blasts in the marrow and/or 5% to 19% blasts in the blood but fewer than 20% blasts in the BM and blood. The presence of Auer rods also qualifies an MDS case as MDS-EB2 irrespective of blast percentage. Changes in how bone marrow blasts are quantified in the new 2016 WHO classification will result in an influx of cases to the MDS-EB category: In the new guidelines, regardless of the number of BM erythroblasts, all nucleated cells will be included in the denominator when calculating the percentages of bone marrow blasts, not only the non-erythroid cells. As such, most of the AML cases previously diagnosed as "acute erythroid leukemia, erythroid/myeloid" subtype (because of the presence of >50% BM erythroblasts and BM blasts comprising at least 20% of the non-erythroid cells, but <20% of all cells) will be re-classified as MDS-EB1 or MDS-EB2.

Although not part of the WHO MDS classification scheme, there is a category "MDS-EB-T" ("MDS with excess blasts in transformation") with 20% to 30% BM blasts in the current NCCN guidelines, with the recommendation to the treating physician that discretion be employed in choosing either MDS- or AML-type therapy according to the specific clinical context.

Patients with MDS-EB have an unfavorable prognosis overall, regardless of whether they progress to AML. The median survival for patients with MDS-EB1 is approximately 18 months; for those with MDS-EB2, it is 10 months. Approximately 25% of patients with MDS-EB1 and 33% of patients with MDS-EB2 progress to AML, with the highest rate (nearly 50%) observed among patients whose MDS-EB2 diagnosis is based on a peripheral blast count exceeding 5% or on the presence of Auer rods.

MYELODYSPLASTIC SYNDROME WITH ISOLATED DEL(5q)

This type of MDS is characterized by one or two cytopenias, fewer than 5% BM blasts, fewer than 1% PB blasts, no Auer rods, single or multilineage dysplasia, and a cytogenetic abnormality of del(5q). In a retrospective, multicenter study of over 500 patients with de novo MDS and del(5q) recruited in the pre-lenalidomide era, Mallo and colleagues did not observe a significant difference in overall survival between patients with del(5q) as a sole abnormality and those with one additional clonal abnormality. Based on this observation, the revised 2016 WHO guidelines allow for the finding of one additional chromosomal abnormality besides the del(5q), as long as the additional abnormality is not del(7q) or monosomy 7. Patients with MDS and increased BM blasts or other types of additional cytogenetic abnormalities should not be placed in this category. Several regions on chromosome 5 may be deleted in patients with other types of MDS and AML, but an interstitial deletion affecting bands q31-q33 is characteristic of MDS with isolated del(5q) subtype. Mapping of the commonly deleted region suggests that haploinsufficiency for the ribosomal gene *RPS14* is central to the pathogenesis of this syndrome, with activation of TP53 and upregulation of the TP53 pathway resulting in defective erythropoiesis and anemia as observed both in a 5q– mouse model and in human MDS with isolated del(5q). Haploinsufficiency of the microRNA genes *miR-145* and *miR-146a* may

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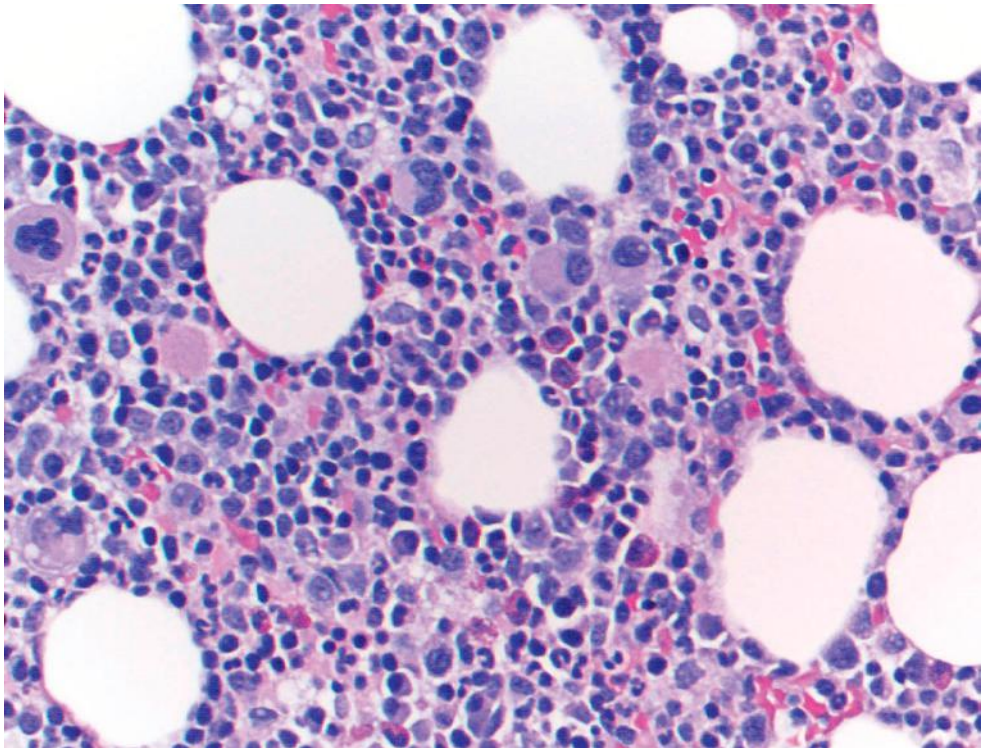


FIG. 18.6
Myelodysplastic syndrome with isolated del(5q). The dysplastic megakaryocytes in this bone marrow section are normal to small in size, but the nuclei are hypolobated and nonlobated (hematoxylin and eosin).

be other cooperating events that account for abnormalities of the megakaryocytic lineage, including the thrombocytosis seen in some patients with this MDS subtype. Although a spectrum of clinical presentations is associated with this MDS subtype, the typical profile (so-called 5q- syndrome) is that of an older woman with macrocytic anemia, normal or elevated platelet count, erythroid hypoplasia, and megakaryocytes with non-lobated or hypolobated nuclei (Fig. 18.6). MDS with isolated del(5q) is generally an indolent disease, and many cases are effectively treated with the thalidomide derivative lenalidomide (see section on treatment below). By next-generation sequencing, *TP53* mutation can be detected in nearly 20% of patients with low-risk MDS del(5q), and this finding is associated with both a lower probability of a complete cytogenetic response to lenalidomide and an increased risk of leukemic evolution.

MYELODYSPLASTIC SYNDROME, UNCLASSIFIABLE

The MDS, unclassifiable (MDS-U) category can be used in three defined situations:

1. Unequivocal single lineage dysplasia in greater than 10% cells of the affected lineage but with pancytopenia (hemoglobin <10 g/dL, absolute neutrophil count <1.8 $\times 10^9$/L, and platelets <100 $\times 10^9$/L)
2. Cases with features of MDS-SLD, MDS-MLD, or MDS with isolated del(5q) otherwise, but with 1% blasts in PB demonstrated on at least two separate occasions
3. Cases with fewer than 1% PB blasts, fewer than 5% BM blasts, dysplasia in fewer than 10% of the cells in any hematopoietic lineage but with a cytogenetic abnormality otherwise considered as presumptive evidence of MDS (see Table 18.1)

Note that this last scenario maintains an important change brought forth by the 2008 WHO classification of MDS, in which a diagnosis of MDS can be based on the strength of cytogenetic results and the clinical history of refractory unexplained cytopenias, despite the lack of definitive morphologic findings. The incidence, rate of transformation to AML, and survival in patients with MDS-U are not known.

CHILDHOOD MYELODYSPLASTIC SYNDROMES

Myelodysplastic syndromes represent only 4% of all pediatric hematologic cancers. In particular, de novo or primary MDS in children is exceedingly uncommon. Therefore, the diagnosis of de novo MDS in a child should be made with considerable caution; morphologic features mimicking or resembling dysplasia may be encountered in the BM of children with a wide variety of non-neoplastic conditions such as infections, metabolic disorders, vitamin deficiencies, and inherited or acquired BM failure syndromes.

Several differences contrast childhood MDS from adult MDS. Unlike MDS in adults, thrombocytopenia and neutropenia (rather than anemia) and BM hypocellularity are more frequently observed in children with MDS; MDS-RS

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and MDS with isolated del(5q) are exceedingly rare in children; the importance of multilineage versus single lineage dysplasia in childhood MDS is unknown; and it is not known at present whether a blast threshold of 20% is superior to a threshold of 30% in distinguishing MDS from AML in this pediatric patient population, nor is it clear whether the MDS-EB1 versus MDS-EB2 distinction is of clinical value in this age group. Currently, without data indicating the contrary, the WHO recommends that the same definitions for MDS-EB be used in the pediatric population as for adults with MDS.

REFRACTORY CYTOPENIA OF CHILDHOOD (RCC)

Refractory cytopenia of childhood (RCC) was introduced by the WHO in 2008 as a provisional MDS entity to include patients with persistent cytopenia, generally hypocellular BM, dysplasia in two different hematopoietic cell lineages or exceeding 10% in one single lineage, and fewer blasts than the thresholds established for MDS-EB (Fig. 18.7). RCC remains a provisional category in the 2016 WHO classification, with the recognition that this group of disorders should be examined closely to identify distinct genetic, histopathologic, and clinical subgroups. Morphologic features that are considered especially helpful in the diagnosis of hypocellular RCC as opposed to aplastic anemia are the presence of pseudo Pelger-Huët neutrophils, hypogranular or agranular neutrophils, micromegakaryocytes, and islands of more than 20 left-shifted or mitotically active erythroid precursors. More than half of patients with RCC have a normal karyotype; among the remaining patients, monosomy 7 is the most frequently detected clonal chromosomal abnormality. The diagnosis of RCC can be difficult to establish in the absence of a cytogenetic abnormality, because the hypocellular BM may yield too few cells for an adequate assessment for morphologic features of dysplasia and may also bring to the differential diagnosis an acquired or inherited BM failure syndrome. In difficult cases, repeated BM evaluations with cytogenetic analysis may be necessary.

CONSIDERATIONS IN THE DIFFERENTIAL DIAGNOSIS OF CHILDHOOD MYELODYSPLASTIC SYNDROMES

Acquired aplastic anemia (AA), at least in some patients, is thought to be an autoimmune disorder characterized by increased apoptosis of hematopoietic stem cells and progenitor cells mediated by aberrantly activated T-cell populations. Approximately 10% to 20% of patients with acquired AA develop a clonal stem cell disease, including MDS, within the decade following their diagnosis. This is thought to occur as a result of the selective pressure applied on the stem cell pool by the cytotoxic T-cell population, which favors the evolution of somatically mutated stem cells. It is unclear whether the development of MDS is part of the extended natural history of AA or whether it may be related to the use of immunosuppressive therapies or growth factors. In general, very slight dyserythropoiesis may be accepted in the BM of children with severe AA (SAA), but there should be no significant dysplasia in the granulocytic or megakaryocytic lineages. The finding of erythroid islands with increased numbers of immature or mitotically active erythroid precursors should prompt one to consider RCC as a diagnosis instead of SAA, according to the European Working Group of MDS in Childhood (EWOG-MDS) criteria. Although earlier studies have described the presence of clonal, transient cytogenetic abnormalities in some patients with AA, the detection of a cytogenetic abnormality in a patient with AA should be considered as strong evidence of potential or impending evolution to MDS and should prompt close clinicopathologic and genetic monitoring. In children and adults with AA, the most commonly found cytogenetic abnormalities are aberrations of chromosome 7 and trisomy 8. Because patients with AA who develop monosomy 7 or a complex karyotype have an unfavorable prognosis with poor response to immunosuppressive therapy and a high rate of conversion to AML, such developments should be viewed as disease progression to MDS, the precise subclassification of which depends on the combined morphologic and cytogenetic findings and on the recognition that the prognosis in these settings may differ from that seen with de novo MDS. In contrast, patients with AA with trisomy 8 frequently show a good hematologic response to immunosuppressive therapy. Although their roles remain to be defined, genomic studies are likely to add further complexity to our understanding of AA and of MDS in young patients, for examples, with respect to the contribution of germline or inherited predisposition syndromes. In a study of pediatric and young (aged ≤45 years) adult patients with AA and MDS who had been referred for hematopoietic stem cell transplantation and among whom the family history had been unrevealing, targeted gene capture revealed germline mutations in known inherited bone marrow failure or MDS-associated genes in 5% of patients with AA and 14% of patients with MDS.

Fanconi anemia (FA) is an inherited BM failure syndrome associated with a high risk of progression to MDS and AML. In data from the International Fanconi Anemia Registry, MDS was reported to develop in 7% of patients, with a cumulative incidence of 33% for developing some hematologic malignancy (AML or MDS) by 40 years of age. MDS is a particularly challenging diagnosis in FA because of the frequent presence of dysplastic features, especially dyserythropoiesis, in the marrow of patients with FA. Therefore, dyserythropoiesis as a sole abnormality should not be construed as evidence for MDS in these patients. A diagnosis of MDS should be considered in patients with FA when, in addition to dyserythropoiesis, dysplastic features are noted in one or both of the other two lineages. Most often, ring sideroblasts are seen, BM blasts are increased, or a clonal cytogenetic abnormality is detected. The most frequent clonal chromosomal abnormalities in patients with FA who develop MDS are gains of material

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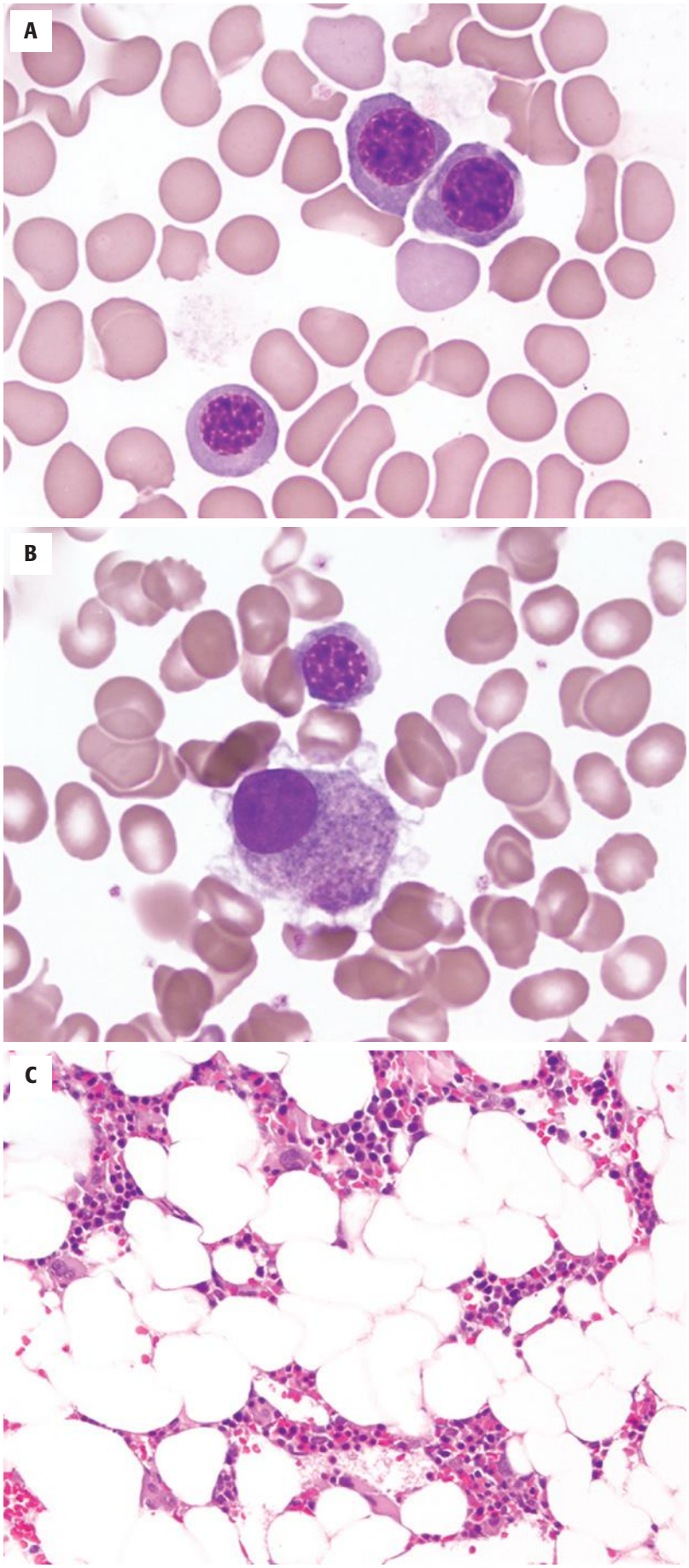


FIG. 18.7

Refractory cytopenia of childhood. The bone marrow aspirate of a child with anemia and neutropenia showing megaloblastoid erythroid precursors (A) and a dysplastic megakaryocyte with a nonlobated nucleus (B) (Wright-Giemsa stain). The marrow blast percentage was not increased and no circulating blasts were identified. C, The bone marrow is hypocellular for age (hematoxylin and eosin). Chromosome analysis showed a derivative chromosome resulting in monosomy 7q and trisomy 1q.

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on chromosomes 1 and 3, loss of material on chromosome 7, and monosomy 7.

SPECIAL CONSIDERATIONS

HYPOCELLULAR MYELODYSPLASTIC SYNDROME

Up to 20% of patients with MDS have hypocellular BM, defined as less than 30% cellularity in patients younger than 60 years and less than 20% in those older than 60 years. Marrow hypocellularity is more common in MDS arising from a pre-existing bone marrow failure or predisposition syndrome than in de novo disease. Patients with hypocellular MDS (h-MDS) tend to have lower white blood cell (WBC) counts and marrow blast percentages, but have otherwise similar IPSS scores and frequencies of karyotypic abnormalities compared with their normocellular or hypercellular MDS counterparts. According to the few published reports on this entity, hypocellularity in MDS appears to represent a favorable prognostic factor that is independent of other risk factors, with a survival benefit demonstrated in all IPSS risk groups and across different cytogenetic risk groups. The lower prevalence of *RAS* and *AML1* mutations and *SOCS1* hypermethylation in h-MDS—all of which are associated with poor prognosis—might in part explain the better prognosis of this group of patients.

The distinction between h-MDS and aplastic anemia (AA) may be difficult or virtually impossible in some cases because of a number of overlapping features, such as megaloblastoid changes and macrocytosis, the presence of PNH-type cells, a good response to immunosuppressive therapy, and a relatively indolent clinical course. Although some features, such as dysplastic granulocytes and megakaryocytes, increased CD34+ blasts in the bone marrow, increased reticulin fibrosis, and the identification of a clonal chromosomal abnormality, may favor a diagnosis of h-MDS over AA, the possibility of a preexisting AA, inherited bone marrow failure, or predisposition syndrome progressing to MDS should also be considered. In addition to AA, the differential diagnosis of h-MDS includes hypocellular AML, hairy cell leukemia, and T-cell large granular lymphocytic leukemia. Even when a diagnosis of h-MDS is made, subclassification may prove difficult, especially in terms of separating between MDS-SLD, MDS-MLD, and MDS-U. Some investigators have proposed that for cases of h-MDS without increased blasts and without an isolated del(5q) cytogenetic abnormality, a diagnosis of hypocellular MDS may suffice.

MYELODYSPLASTIC SYNDROME WITH FIBROSIS

Significant reticulin fibrosis has been reported in approximately 10% to 15% of patients with MDS. It is not clear whether MDS with fibrosis (MDS-f) should be considered as a distinct clinicobiological entity, largely because criteria for its recognition have not been defined clearly. Patients with MDS-f usually exhibit significant pancytopenia requiring red blood cell and platelet transfusions, multilineage dysplasia, and increased blasts. In this setting, most authors suggest that the diagnosis of "MDS-f" be considered if there is a diffuse and dense increase in reticulin fibers with extensive intersections (i.e., grade 2 or 3 reticulin fibrosis, in the 0 to 3 grading scale used in the European consensus guidelines and WHO classification). Recent studies have demonstrated that increased fibrosis in MDS is associated with shorter overall and leukemia-free survival and represents a risk factor that is independent of other MDS risk factors such as IPSS or WHO classification–based prognostic scoring system (WPSS) score.

One of the most important differential diagnostic considerations with MDS-f is acute panmyelosis with myelofibrosis, a rare subtype of acute myeloid leukemia that is characterized by the presence of at least 20% blasts, bone marrow fibrosis, and multilineage dysplasia, especially dysmegakaryopoiesis. Careful enumeration of blasts on bone marrow touch imprints and by CD34 immunohistochemical studies on the marrow core biopsy sections is necessary in this differential diagnosis.

Other considerations in the broad differential diagnosis of MDS-f include acute leukemias with fibrosis, myeloproliferative neoplasms, systemic mastocytosis, hairy cell leukemia and other non-Hodgkin lymphomas, metastatic tumors, and autoimmune myelofibrosis.

MINIMAL DIAGNOSTIC CRITERIA FOR MYELODYSPLASTIC SYNDROME

What is required for the diagnosis of MDS? Given the broad differential diagnosis for anemia, neutropenia, and thrombocytopenia, the somewhat subjective nature of assessing morphologic features of dysplasia, the variable degrees of dysplasia from case to case, and the absence of cytogenetic proof of clonality in approximately half of MDS cases, this question of minimal diagnostic criteria for MDS arises frequently in clinical practice.

Following the Working Conference on MDS in 2006, Valent and colleagues proposed that features already used in the various MDS classification schemes and in practice are organized into prerequisite criteria and MDS-related or decisive criteria, with the presence of both prerequisite criteria and one or more decisive criteria fulfilling the minimal requirements for a diagnosis of MDS. In this proposal, the two prerequisite criteria that must be fulfilled are (1) marked and constant (at least 6 months in duration) peripheral cytopenia and (2) the absence of all other possible hematopoietic and non-hematopoietic disorders as reasons for dysplasia or cytopenia. MDS-related or decisive criteria include the following: dysplasia in at least 10% of cells in one of the hematopoietic lineages, greater than 15% ring sideroblasts,

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5% to 19% marrow myeloblasts, or an MDS-related karyotype. The thresholds of cytopenia used to define MDS have varied from hemoglobin between 10 to 12 g/dL, absolute neutrophil count 1.5 to 1.8 $\times$ 10^9/L, and generally platelets less than 100 $\times$ 10^9/L. The 2016 WHO MDS guidelines suggest thresholds of cytopenia(s) at hemoglobin less than 10 g/dL, absolute neutrophil count less than 1.8 $\times$ 10^9/L, and platelets less than 100 $\times$ 10^9/L, but it recognizes as well that a diagnosis of MDS may be made in patients with a milder cytopenia if the cytopenia is persistent and unexplained and if other diagnostic criteria are present.

In the Valent proposal, the authors suggest the use of "co-criteria" if no decisive criteria are met. Although some of the co-criteria listed in this proposal, such as flow cytometric studies for immunophenotypic features of myelodysplasia or molecular genetic testing for clonal abnormalities, have gained wide use recently, at present there is no standardized acceptance of diagnostic criteria by these modalities in the diagnosis of MDS exists. Therefore, a positive result by means of one of these "co-criteria" may not be sufficient for a definitive diagnosis of MDS in the absence of other definitive and clinically accepted features, and the current recommended standard of care is to continue with follow-up and repeated assessment as necessary.

IDIOPATHIC CYTOPENIA OF UNCERTAIN SIGNIFICANCE (ICUS)

The term *idiopathic cytopenia of uncertain significance* (ICUS) has been proposed for patients whose blood and bone marrow findings do not meet the minimal diagnostic criteria for MDS, but whose cytopenia is of at least 6 months' duration and cannot be explained by any other hematologic or non-hematologic disorders. ICUS is not a diagnosis; rather it is an acknowledgment of diagnostic uncertainty. The designation indicates the necessity of continued hematologic follow-up to document or exclude evolution to MDS. Recommended follow-up investigations include complete blood count with differential at least at 6-month intervals, with a repeat BM examination in patients with a high level of suspicion for MDS (e.g., patients with macrocytic anemia and transfusion dependency).

Approximately 20% to 30% of individuals with ICUS are shown to harbor at least one MDS-related somatic mutation, with the rate rising to 55% among those with some morphologic features of MDS. *TET2, DNMT3A*, and *ASXL1* are the most commonly mutated genes. Some have suggested using the term *clonal cytopenias of undetermined significance* (CCUS) to describe these patients with ICUS and clonal hematopoiesis. In a retrospective analysis of patients with MDS or AML who had had a prior bone marrow sample that was not diagnostic of a myeloid neoplasm, Cargo and colleagues identified a somatic mutation and/or structural genetic abnormality in 91% of the pre-diagnostic samples, most commonly involving *TET2, SRSF2*, or *ASXL1*, with a median of two mutations per patient (range 1–5). Moreover, 64% of the pre-diagnostic specimens had two or more mutations, compared to only 8% reported in healthy individuals with CHIP, and there was a significantly higher median VAF (40%) among these pre-diagnostic samples versus the 9% to 10% VAF reported in healthy individuals with CHIP. These findings suggest that the presence of multiple mutations and a higher VAF may indicate a higher risk of progression from CCUS to MDS or another myeloid neoplasm, but prospective studies are needed to validate this.

DIFFERENTIAL DIAGNOSIS

The diagnosis of MDS represents a malignant condition, with a variable but finite probability of progressing to acute leukemia. Therefore, it is imperative that all other diagnostic possibilities be thoroughly evaluated and excluded before rendering a diagnosis of MDS. Following are discussions of such alternate diagnoses that should be carefully considered in the differential diagnosis of MDS.

QUANTITATIVE DEFECTS

Cytopenia With Hypercellular Marrow

Cytopenia with hypercellular marrow is a scenario that may be encountered in immune-mediated destruction of erythrocytes, neutrophils, or platelets, or it may be seen with sequestration owing to hypersplenism. However, without other confounding factors, immune-mediated destruction or hypersplenism typically does not result in a pancytopenia. Conversely, caution should be exercised when considering a diagnosis of MDS based on an isolated neutropenia or thrombocytopenia, which would be a rare presentation of MDS. For example, as rare as WHIM syndrome (an acronym for **w**arts, **h**ypogammaglobulinemia, **i**nfections, and **m**yelokathexis) may be, it is perhaps no less likely than MDS as an explanation for an isolated neutropenia! A high-quality core biopsy specimen of adequate size is also important to rule out the possibility of a sampling bias, in which the hypercellular area may be only focal whereas the remainder of the marrow is in fact normocellular or hypocellular. Viral disorders, particularly HIV infection, can cause cytopenias with a hypercellular marrow. However, whereas the presence of cellular debris in association with an increased number of plasma cells and lymphocytes is frequently seen in the BM of patients with HIV infection, these findings are usually not prominent in patients with MDS. Cytopenias and pancytopenia have been reported in patients with chronic parvovirus B19, Epstein-Barr virus, and cytomegalovirus infections, especially among those who are immunocompromised. Correlation with the clinical history and with serum serologies or viral DNA studies would be necessary, and a primary diagnosis of MDS should not be made in the face of active viral infections. Patients with hemophagocytic lymphohistiocytosis (HLH) may also exhibit pancytopenia and a hypercellular marrow, in which the presence of increased

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macrophages exhibiting varying degrees of active hemophagocytosis should discourage the diagnosis of MDS as a primary explanation for the cytopenias. Laboratory evaluation of ferritin and triglyceride levels, coagulation parameters, and other diagnostic features of HLH should be of help when considering this differential diagnosis. Last, although acute leukemia can at times present with cytopenia or pancytopenia despite a hypercellular marrow, the presence of 20% blasts or more in the blood or marrow should readily separate this consideration from that of MDS.

Cytopenia With Normocellular or Hypocellular Marrow

Other causes of cytopenia with a normocellular or hypocellular marrow include aplastic anemia, paroxysmal nocturnal hemoglobinuria, myelosuppressive infections, medication effect, toxic exposure, graft-versus-host disease affecting the marrow, T-cell large granular lymphocytic leukemia, and acute leukemia. Documentation of a normal complete blood count within the preceding 3 to 6 months would suggest a more acute or subacute onset and would make MDS unlikely; instead, such a time course should suggest exposure to medication, toxin, or infection as a cause for the cytopenia. The clinical history should help to determine whether graft-versus-host disease is a likely explanation for the cytopenia. In T-cell large granular lymphocytic leukemia, laboratory evaluation typically reveals neutropenia with or without anemia, whereas thrombocytopenia as an isolated finding at presentation is uncommon. These patients often have increased circulating large granular lymphocytes, and flow cytometric immunophenotyping of the blood with immunohistochemical studies of the BM biopsy sections can help to detect phenotypically distinct T-cell populations. In the rare cases of hypocellular acute myeloid leukemia, identification of 20% or more myeloblasts in the bone marrow aspirate or by CD34 immunohistochemistry, or both, should confirm the diagnosis of acute myeloid leukemia.

Although some cytologic atypia may be seen in granulocytes and megakaryocytes, definitive dysmyelopoietic features that are characteristic of MDS are rarely found in viral infections or at first presentation of a BM failure syndrome. Therefore, a careful microscopic examination of well-prepared and well-stained blood and BM slides is essential to evaluate accurately for such features. However, certain medications may cause morphologic changes reminiscent of dysgranulopoiesis (see Differential Diagnosis and Qualitative Defects). In the absence of an increased blast population, definitive dysplastic morphologic features, or a characteristic clonal cytogenetic abnormality, continued monitoring of the patient's clinical, and hematologic parameters with repeated BM examinations may be necessary.

Qualitative Defects

Dyserythropoiesis

Varying degrees of dyserythropoiesis may be seen in megaloblastic anemia, thalassemia (Fig. 18.8A and B), with antimetabolite therapy or with certain medications such as azathioprine and valproate, brisk hemolysis (so-called stress dyserythropoiesis), marrow regeneration, congenital dyserythropoietic anemia, and mitochondrial cytopathy. Patients with megaloblastic anemia owing to severe vitamin B_{12} or folate deficiency may develop pancytopenia with marked dyserythropoiesis. However, there should be no increase in blasts, and the morphologic features of megaloblastosis with "giant" neutrophilic bands and metamyelocytes as well as hypersegmented neutrophils are readily distinguishable from the cytoplasmic hypogranulation and nuclear hyposegmentation typical of MDSs. Laboratory evaluation for serum level of vitamin B_{12}, serum and red blood cell folate levels, and other related metabolites is crucial in confirming the diagnosis of a nutritional deficiency. A careful review of the patient's clinical and medication history with appropriate laboratory evaluation, including a workup for hemolysis, is always useful to rule out the possibility of dyserythropoiesis caused by marrow regeneration, medication effect, or severe hemolysis. In congenital dyserythropoietic anemia, despite considerable dyserythropoiesis, the granulocyte and megakaryocyte lineages are morphologically normal.

Ring Sideroblasts

Ring sideroblasts can be seen with arsenic poisoning, anti-tuberculosis therapy, pyridoxine deficiency, zinc toxicity, copper deficiency, and heavy alcohol use. A careful history of possible exposure or prior gastric bypass surgery is therefore essential to rule out these possibilities before embarking on a diagnosis of MDS-RS. In the pediatric patient, the presence of ring sideroblasts in the BM should prompt an evaluation for a mitochondrial cytopathy such as Pearson syndrome (a congenital multisystem disorder characterized by marked anemia, ring sideroblasts in the BM, neutropenia, thrombocytopenia, and pancreatic exocrine insufficiency), because MDS-RS in this age group is extremely uncommon.

Dysgranulopoiesis

The possibility of poor staining should first be excluded as an explanation for hypogranular-appearing neutrophils and precursors, and a diagnosis of MDS should never be based solely on the presence of pale neutrophil cytoplasm. G-CSF or granulocyte-macrophage colony-stimulating factor (GM-CSF) therapy may result in hypogranular-appearing neutrophils, although increased azurophilic granulation is more frequently seen. G-CSF or GM-CSF therapy and trimethoprim-sulfamethoxazole therapy can sometimes result in pseudo–Pelger-Huët neutrophils. Moreover, G-CSF or GM-CSF therapy can also cause left-shifted granulocytic maturation with circulating myeloblasts. Morphologic features of dysgranulopoiesis have also been described with valproate, mycophenolate mofetil, and tacrolimus therapy (see Fig. 18.8C and D). With mycophenolate mofetil, dysgranulopoiesis is characterized by loss of normal nuclear chromatin condensation with neutrophil maturation, resulting in mature neutrophils showing more reticulated and immature-appearing chromatin features resembling those seen in mature monocytes; atypical nuclear hypersegmentation may also be seen in some neutrophils.

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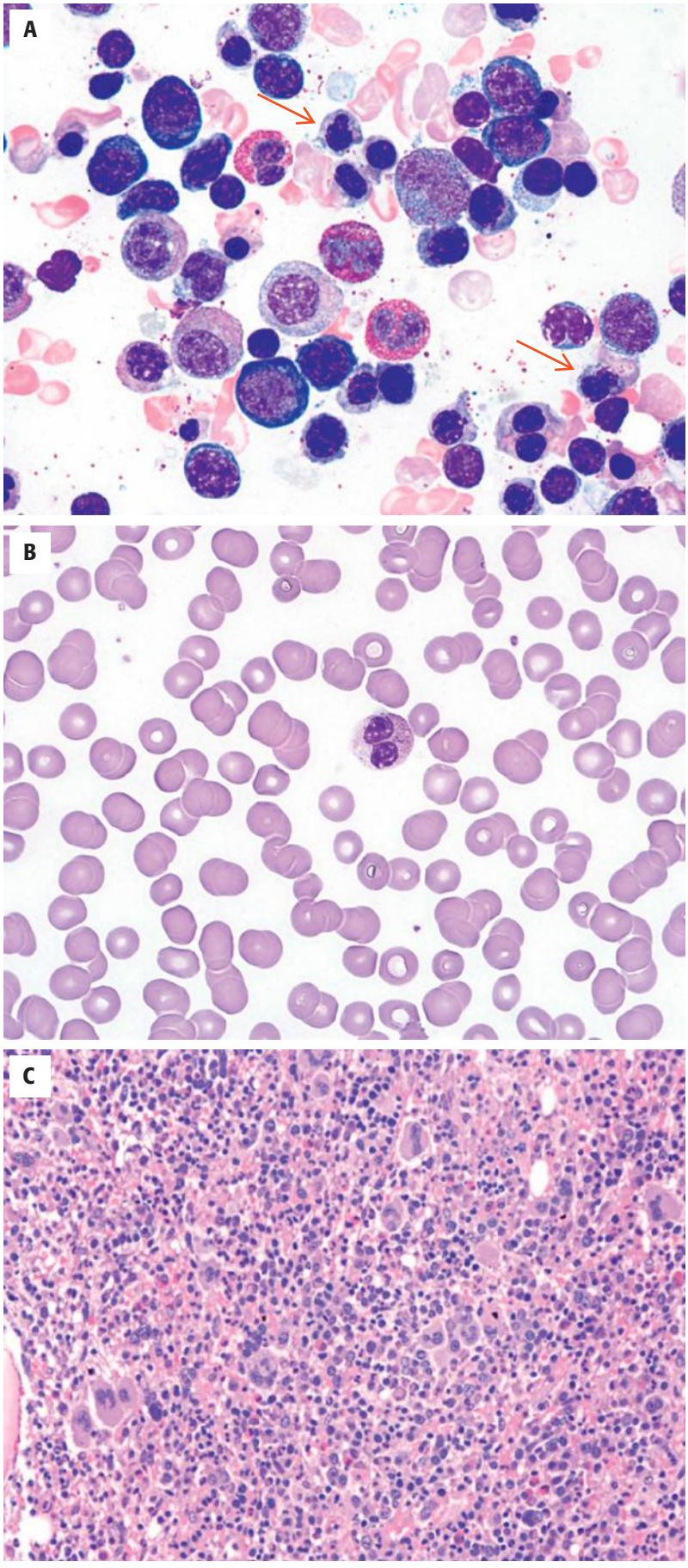


FIG. 18.8

Dysplastic morphologic features in non-MDS settings. Erythroid precursors with irregular nuclear lobulations in the bone marrow aspirate of a patient with thalassemia (A, Wright-Giemsa stain). Neutrophil with pseudo–Pelger-Hüet anomaly in the peripheral blood of a patient on tacrolimus therapy (B, Wright-Giemsa stain). Megakaryocytes with nuclear hypolobation in the bone marrow biopsy of a patient with hematopoietic regeneration following cytotoxic chemotherapy for acute lymphoblastic leukemia (C, hematoxylin and eosin).

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Nuclear fragments (akin to Howell-Jolly bodies in red blood cells) may also be seen in this setting as well as in patients with HIV infection and in some patients undergoing chemotherapy. It is therefore advisable to repeat the blood and marrow examination after discontinuation of growth factor, trimethoprim-sulfamethoxazole, or similar culprits before rendering the diagnosis of MDS.

Dysmegakaryopoiesis

Sectioning artifact on trephine sections can render the appearance of a megakaryocyte as small and the nucleus as hypolobated or even multinucleated. However, the more such abnormal-appearing megakaryocytes are seen in the BM trephine sections, the less likely is the possibility of artifact. Evaluation of the BM aspirate smears obviates this sectioning artifact and is helpful to confirm an impression of dysplastic megakaryocytes from the bone marrow biopsy. To improve both the diagnostic specificity and interobserver variability, some authors have proposed setting the threshold for dysmegakaryopoiesis at identifying at least 10% micromegakaryocytes (defined as a mononulcear megakaryocyte with a size comparable to that of a promyelocyte and lacking features of a blast cell), or at least 40% megakaryocytes showing any features of dysplasia. Other non-neoplastic causes of small and hypolobated megakaryocytes include HIV infection and certain medications, such as valproate. In addition to MDS, megakaryocyte clustering or even atypia can be seen in myeloproliferative neoplasms and hematopoietic regeneration (see Fig. 18.8E–F). With the recent introduction of thrombopoietin analogue therapy, the effect of such agents on the morphologic features and distribution of megakaryocytes needs to be considered, and repeated marrow evaluation after discontinuation of such therapies is recommended before a diagnosis of MDS may be considered.

Increased Blasts

Myeloblasts may be observed in the blood as part of a left-shifted granulocytic maturation resulting from G-CSF or GM-CSF therapy, a leukemoid reaction, or leukoerythroblastic reaction. Although occasionally hypogranular or hyposegmented neutrophils can be seen with G-CSF or GM-CSF therapy, the blood should exhibit a continuum of all intermediate stages of granulocytic precursors in addition to the blasts; the blast count rarely approaches 10% in the marrow, and there often is in parallel a notable left shift in granulocytic maturation with increased promyelocytes and myelocytes; megakaryocytic dysplasia should not be seen. If in doubt and if cytogenetic analysis reveals no characteristic abnormalities, one should exercise caution, and close clinical monitoring with follow-up hemograms and with a repeated BM examination may be necessary. When the percentages of circulating and/or BM blasts approach the 20% threshold, an accurate and well-sampled differential count of well-prepared and representative blood and marrow slides is essential to determine whether the blast proportions meet the definition of AML. If necessary, several fields, slides, and preparations may have to be examined to obtain a representative blast percentage. Hyperplasia of benign bone marrow B-cell precursors (hematogones) can mimic increased blasts, although morphologically the more homogeneous and hyperchromatic nuclei of these B-lymphoid precursors differ from the more reticular chromatin structure of myeloid-lineage blasts, and flow cytometric immunophenotyping can distinguish between the B-lineage of hematogones and the myeloid lineage of the blasts in MDS. Difficulty can arise in differentiating blasts from promyelocytes, especially from dysplastic promyelocytes. The most helpful morphologic feature in this situation appears to be the presence of a pale Golgi zone, at least faintly visible, in promyelocytes.

Prognostic Classification

Many individual prognostic factors have been studied in MDS in an attempt to stratify patients into various risk groups for the purpose of prescribing the appropriate level of medical management and therapeutic intervention for each patient. In addition, various models have been developed for the purpose of predicting outcomes.

The IPSS was proposed in 1997 and based on a meta-analysis of 816 patients with untreated primary MDS with a long follow-up period. At the time of the study, patients with 20% to 30% marrow blasts—now considered as having AML—were also included. In this scoring system, patients with MDS were stratified into four different risk groups according to a combined score that considered the percentage of BM blasts, type of cytogenetic abnormalities, and the number of cytopenias. Patients in the lowest risk group had a median survival of 5 to 6 years with only approximately 20% progressing to AML when followed for as long as 19 years. In contrast, essentially all patients in the highest risk group had developed AML by 3 years, and the median survival in this group of patients was less than 1 year.

Published 15 years later, the revised IPSS (IPSS-R) was culled from a much larger data set of over 7000 patients from multiple international patient databases and incorporated several additional features. Instead of only 6 specific abnormalities forming 3 cytogenetic subgroups in the original IPSS, there are 16 specific abnormalities forming 5 distinct cytogenetic subgroups in IPSS-R. There is an additional marrow blast category to separate patients with 2% or more marrow blasts from those with fewer than 5% but more than 2% marrow blasts. Finally, new thresholds for cytopenias take into consideration depth of cytopenias, not just number of cytopenias (Table 18.4). Age, performance status, serum levels of ferritin, lactate dehydrogenase (LDH), and β2-microglobulin were not formally incorporated in the IPSS-R scoring system, but they were identified as significant features for predicting survival, although not acute leukemia transformation. With five instead of four prognostic risk groups, the IPSS-R provided greater discriminating power than the original IPSS.

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TABLE 18.4

Revised International Prognostic Scoring System (IPSS-R) for Myelodysplastic Syndromes[a]

Prognostic variable	0.0	0.5	1.0	1.5	2	3	4
Marrow blasts (%)	≤2	na	>2% to <5%	na	5%–10%	>10%	na
Karyotype[b]	Very good	na	Good	na	Intermediate	Poor	Very poor
Cytopenias							–
Hemoglobin (g/dL)	≥10	na	8 to <10	<8	na	na	na
Platelets ($\times 10^9$/L)	≥100	50–<100	<50	na	na	na	na
Absolute neutrophil count ($\times 10^9$/L)	≥0.8	<0.8	na	na	na	na	na

[a]Revised International Prognostic Scoring System Risk Groups: Very low (risk score ≤1.5); low (risk score >1.5–3); intermediate (risk score >3–4.5); high (risk score >4.5–6); and very high (risk score >6)
[b]Karyotype: "Very good" includes -Y and del(11q); "Good" includes normal, del(5q), del(12p), del(20q), and double including del(5q); "Intermediate" includes del(7q), +8, +19, i(17q), and other single or double independent clones; "Poor" includes -7, inv(3)/t(3q)/del(3q), double including -7/del(7q), and complex (3) abnormalities; "Very poor" includes complex (>3) abnormalities.
na, Not applicable.

Transfusion dependency has been demonstrated to be associated with a shorter survival and an increased risk of leukemic evolution in MDS, but it is not a feature in either the IPSS or the IPSS-R schemes. It can be considered as a reliable indicator of the severity of the disease, although it may partly reflect the presence of comorbid conditions and also is subjected to regional differences in thresholds for red blood cell transfusion. A prognostic model was developed that incorporated the 2001 WHO classification MDS categories, cytogenetics, and transfusion dependency. This WHO Classification-based Prognostic Scoring System (WPSS) has been proposed as a dynamic prognostic model for predicting survival and leukemic evolution that could be applied to patients with MDS at any time during their clinical course. Compared to the four risk groups as defined by the IPSS, the WPSS was able to identify five risk groups of patients with different survival profiles, with the best improvement in prognostic accuracy over what the IPSS observed among patients without excess blasts. The WPSS was also shown to be of value in stratifying the outcome of patients undergoing allogeneic stem cell transplantation. Of note, the WPSS has been recently been modified such that "transfusion dependency" has been replaced by severe anemia, defined as hemoglobin less than 9 g/dL for men and less than 8 g/dL for women.

In addition to the IPSS, R-IPSS-R, and WPSS schemes, other risk stratification models have evaluated the prognostic value of other variables, such as patient age, performance status, degree of anemia, BM fibrosis, immunophenotypes of myeloid progenitor cells, serum levels of lactate dehydrogenase, and new cytogenetic risk categories.

More important, recent attempts to incorporate gene mutations have demonstrated the considerable potential of additional prognostic information, with *EZH2* mutation identifying a subgroup of lower risk MDS with worse-than-expected prognosis, whereas mutations in *RUNX1, TP53, EZH2, ETV6*, and *ASXL1* predicted poor overall survival independent of other established risk factors. This area of investigation is active, and new information on the optimal way to incorporate mutational analysis into prognostic scoring systems including the IPSS-R will undoubtedly be forthcoming. Beyond gene mutation data, others have suggested incorporating gene expression profiling to further improve prognostic modeling in MDS.

Prognosis and Therapy

Previously, therapeutic options in MDSs have typically entailed either supportive care to alleviate symptoms related to the cytopenias or cytotoxic chemotherapy followed by hematopoietic stem cell transplantation with curative intent. More recently, several therapeutic options have become available that provide strategies at achieving disease stabilization. Decisions regarding the best treatment plan typically represent a balanced consideration of the patient's MDS subtype and its prognosis, the patient's age, symptomatology and any clinical comorbid conditions, and the goal of any intended therapeutic intervention. A detailed discussion of such deliberations on the best appropriate treatment strategy is beyond the scope of this chapter; the reader is referred to the National Comprehensive Cancer Network website at www.nccn.org for the most up-to-date and complete guidelines. The following is a brief and general summary of current treatment strategies in MDS.

Hematopoietic Growth Factor Support

The goal of hematopoietic growth factor administration in MDS is to reduce the symptoms related to the cytopenias. As such, this therapeutic approach is supportive and not

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curative. Approximately 20% of patients with MDS who are symptomatic from anemia respond to erythropoietin therapy with or without concurrent G-CSF administration. Patients with low (<500 mU/mL) endogenous erythropoietin levels and without a del(5q) abnormality are more likely to respond. Although administration of G-CSF or GM-CSF to patients with neutropenia resulting from MDS can improve the neutrophil count, the use of these growth factors is not routine because no survival advantages have been shown in multicenter randomized phase III studies and, in fact, there was a suggestion that G-CSF/GM-CSF therapy might have accelerated progression to acute leukemia. Although thrombopoietic agents such as romiplostim and pegylated eltrombopag have appeared promising either alone or in combination with hypomethylating agents in early preliminary studies, they are currently not routinely prescribed for patients with MDS.

Immunosuppression

As discussed above, increased proliferation and apoptosis of hematopoietic precursors characterize MDS, and proinflammatory cytokines such as tumor necrosis factor α (TNF-α) appear to play a role in the apoptosis. Therapeutic trials using agents such as the anti–TNF-α blockers etanercept in an attempt to modulate or block the inflammatory cytokines have been small in patient sample size. Without data from larger controlled trials, the role of such immunomodulatory agents as a mainstay in the treatment of MDS seems doubtful, at least in the foreseeable future. Based on reported improvements of cytopenia and survival in patients younger than 60 years and with lower IPSS or intermediate-1–risk MDS, or those with hypocellular BM, HLA-DR15 histocompatibility type, and PNH-type cells, immunosuppressive therapy with equine antithymocyte globulin or cyclosporin is a therapeutic option for these subsets of patients.

Immunomodulation: Lenalidomide

For patients with low-risk MDS and with del(5q) as a sole cytogenetic abnormality, the use of lenalidomide as a single agent has resulted in high erythroid responses, with approximately two thirds of the patients showing an improvement of their hemoglobin levels for a median duration of 2.2 years. In addition, partial and complete cytogenetic responses have been reported in the range of 73% and 45%, respectively. Subsequent to the initial publication of these results and with further follow-up, it remains unclear whether there is improved overall survival with long-term lenalidomide therapy; clinical and cytogenetic relapse have been reported in 50% of patients, possibly because of persistence of a del(5q) stem-cell population that had evaded or resisted therapy. By applying high-throughput sequencing to a series of bone marrow specimens collected from patients with MDS over time, Mossner and colleagues showed a highly complex and dynamic clonal landscape even among patients with MDS del(5q) who had initially shown clinical response to lenalidomide, with persistent clonal hematopoiesis and emergence of additional independent clones. For these reasons, the long-term effects of lenalidomide on the treatment of patients with MDS require further study. The use of this agent in patients with MDS without the del(5q) abnormality is currently not part of the NCCN guidelines.

Epigenetic Therapies: Hypomethlating Agents

Originally designed as a pyrimidine nucleoside analogue of cytidine to overcome cytarabine arabinoside resistance, 5′-azacitidine has since been shown to act as a DNA methyltransferase inhibitor. In a series of multicenter randomized controlled trials, patients with MDS receiving 5′-azacitidine experienced statistically significant longer median time to leukemic progression, longer median survival, and improved quality of life when compared to patients receiving supportive care only. A recent re-analysis using the stricter response criteria of the International Working Group reaffirmed these results. In the current NCCN guidelines, 5′-azacytidine is part of the treatment for patients with either low- or high-risk (IPSS) MDS. In two non-randomized studies, decitabine (another hypomethylating agent) and 5′-azacytidine appeared comparable in terms of response rates and survival, although some have suggested a preference for the latter because of the improved survival with this agent among higher risk patients.

Epigenetic Therapies: Histone Deacetylase Inhibitors

Results of a few phase II studies using valproic acid as a single agent showed response rates ranging from 6% among patients with MDS with increased blasts to approximately 52% among patients with MDS without increased blasts. The value of combining histone deacetylase inhibitors with other agents such as all-*trans* retinoic acid, another hypomethylating agent, or other agents requires future studies.

Cytotoxic Chemotherapy

For patients with MDS with increased blasts approaching the level of acute leukemia, an induction chemotherapy regimen that is typically used in the setting of acute myeloid leukemia is an option. However, remissions tend to be short-lived and are achieved in only 30% to 50% of cases. Consolidation chemotherapy, hematopoietic stem cell transplantation, or both, are often necessary after the induction therapy.

Allogeneic Hematopoietic Stem Cell Transplantation

Allogeneic hematopoietic stem cell transplantation is the only curative therapy for patients with MDS, but its use is limited by the typically older age of patients with MDS, who are often ineligible for many hematopoietic stem cell transplantation protocols. Recent advances in supportive care have enabled some transplantation programs to raise the age of eligibility to 65 years or older. The introduction of reduced-intensity and non-myeloablative conditioning regimens in

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the past several years has provided another potential avenue to hematopoietic stem cell transplantation for older patients with high-risk MDS. However, even with increased use of allogeneic stem cell transplantation in older patients, for a disease in which more than 80% of affected patients are older than 60 years, many patients with MDS remain ineligible for this curative therapy.

In addition to age and associated comorbid conditions, timing of transplantation in MDS is another issue for consideration. An analysis by Cutler and colleagues suggests that, whereas delayed BM transplantation is associated with maximal life expectancy for patients with MDS in the IPSS risk categories of low and intermediate-1, immediate transplantation should be considered for those in the intermediate-2 or high-risk categories.


The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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SECTION 4

Disorders of Histiocytes, Mast Cells, Plasma Cells, Spleen, and Ancillary Techniques

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19

Disorders of Histiocytes*

■ Jennifer Lee Picarsic, MD ■ Kudakwashe Chikwava, MB, ChB

■ DERIVATION OF HISTIOCYTES

The term *histiocyte* has come to encompass two cell lines, the monocyte-macrophages and the specialized antigen-presenting dendritic cells (DCs). Although a long-held belief that myeloid-derived monocytes were the immediate precursors of all macrophages and dendritic cells, the understanding of the mononuclear phagocytic system (MPS), which includes DC, monocytes, and macrophages, is undergoing continual refinement, including the nomenclature, cellular origin, and overlapping functional capabilities. Guilliams and colleagues, in 2014, proposed a unified nomenclature based on ontogeny in which the MPS could be better understood as a dynamic monocyte-macrophage system, with both differences and redundancy in origin, function, and morphology, that includes three broad cell families—namely, embryonic-derived macrophages, common DC precursor (CDP)-derived DCs, and monocyte-derived cells (including both monocyte-derived macrophages and monocyte-derived DCs), based first on their ontogeny and secondarily on their functional and phenotypic properties (Fig. 19.1).

Under the influence of cytokine macrophage colony-stimulating factor (M-CSF or CSF1) embryonic precursors of the yolk sac and fetal liver migrate to the fetal peripheral tissues where they maintain a self-renewing population of tissue-resident macrophages throughout adult life (e.g., splenic red pulp macrophages, bone osteoclasts, brain microglia, liver Kupffer cells, renal mesangial cells, pulmonary alveolar macrophages, placental Hofbauer cells, serosal macrophages, the interstitial histiocytes of the skin and connective tissues). Within this nomenclature, the epidermal Langerhans cells, derived from embryonic precursors and possessing self-renewing properties, would also be classified as resident tissue "macrophages" (see Fig. 19.1). The lymph nodes have their own resident macrophages that reside within the sinuses, the follicular germinal centers, and the nodal paracortex, and there is functional heterogeneity of the various macrophages. The spleen has follicular, sinus, and cordal macrophages.

DCs, on the other hand, are believed to be derived from hematopoietic stem cells of the bone marrow, distinct from classic monocytes (see Fig. 19.1). The common dendritic cell precursor (CDP) gives rise to either the classic/conventional DCs (cDC) or plasmacytoid DCs (pDC). The marrow, under the influence of the cytokine FMS-like tyrosine kinase 3 ligand (FLT3), can give rise to the CDP that can either circulate as a preclassic DC or function as an intermediate stage within the bone marrow as a pre-plasmacytoid DC. The cDC may be further divided into cDC1 or cDC2 based on developmental pathways with differentiation achieved in tissues, as opposed to the pDC (plasmacytoid monocyte/interferon-producing cell) in which terminal differentiation occurs in the bone marrow and then populates the lymph node in steady-state or other tissues in inflammatory states. Studies have also suggested that some of the pDC may also be derived from lymphoid bone marrow precursors. Tissue DCs are found in the tissues during steady state (e.g., lymph node interdigitating and follicular, spleen, thymus). Only the brain appears to lack an intrinsic DC pool. Of note, despite their dendritic morphology, antigen-presenting capabilities, and phenotypic expression as DCs, epidermal Langerhans cells (LCs) may be better classified under the embryonic-derived "tissue macrophage" grouping, given their shared embryonic ontogeny and self-renewal properties (vide supra). The germinal center follicular DCs are the only members of the DC family not to have a myeloid origin and are most likely derived from fibroblast-like mesenchymal cells in various tissues. Their nonmyeloid origin now serves as the basis for the exclusion of follicular DC tumors from current histiocytic disorders/neoplasia classifications (Box 19.1).

The monocyte-derived cells are the third group of the dynamic MPS that serve as a pool of trafficking and inflammatory-mediated cells, derived from so-called common monocyte progenitor cells (cMoPs) of the bone marrow, which can give rise to a variety of functional cell types dependent on the local microenvironment. This includes both the monocyte-derived macrophages and monocyte-derived DCs (see Fig. 19.1). In human tissues, there is still debate as to

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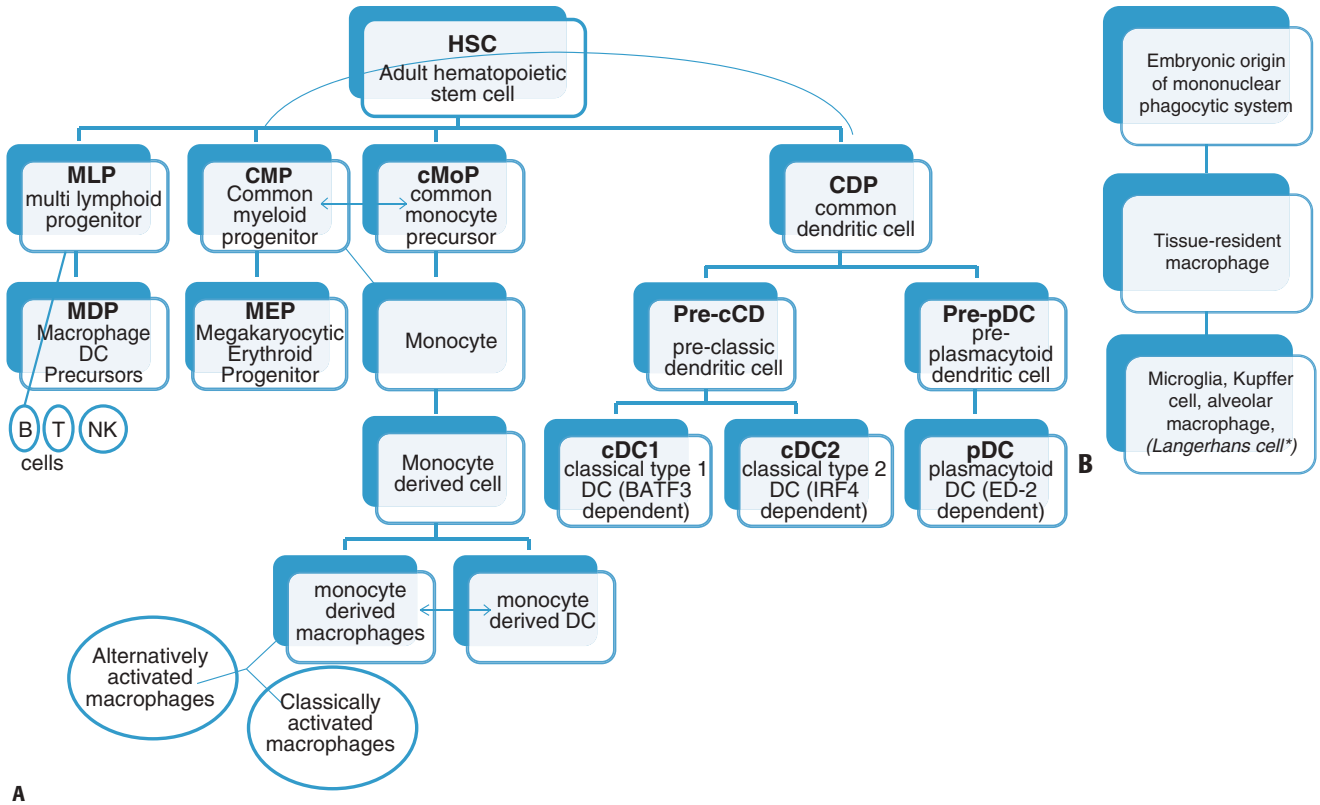


FIG. 19.1

A, Under steady-state conditions, the adult hematopoietic stem cell (HSC) gives rise to multiple precursor of the mononuclear phagocytic system. The multiple-lymphoid precursor can give rise to a macrophage-dendritic cell. Under stable conditions, monocytes and dendritic cells derive from a presumed common adult HSC with differentiation based on distinct transcriptional factor and cytokine control. Under inflammatory conditions, the common monocyte precursor (cMoP) and/or granulocyte-monocyte progenitor can augment the pool with inflammatory macrophages and dendritic cells. This model does not require transdifferentiation to explain phenotypic changes between T cells, B cells, or histiocytes. B, New understanding of the tissue macrophages in steady-state condition is that most are derived from embryonic hematopoietic cells of the extraembryonic yolk sac or fetal liver, *including the epidermal Langerhans cells. Note that there are a few tissue macrophages without self-renewal properties, believed to be bone marrow derived (e.g., heart, pancreas, gastrointestinal, dermis). *(A, Data from Geismann F, Manz MG, Jung S, et al: Development of monocytes, macrophages, and dendritic cells,* Science *327:656–661, 2010; Doulatov S, Notta F, Eppert K, et al: Revised map of the human progenitor hierarchy shows origin of macrophages and dendritic cells in early development,* Nature Immunol *11:585–593, 2010; and Guilliams M, Ginhoux F, Jakubzick C, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny.* Nat Rev Immunol *2014;14(8):571–578. B. Data from B. Ginhoux F, Guilliams M. Tissue-resident macrophage ontogeny and homeostasis.* Immunity *44(3):439–449, 2016.)*

what cells are actually "monocyte" derived. However, a subset of tissue macrophages of the gastrointestinal tract, dermis, heart, and pancreas appear to be derived from circulating adult cMoPs rather than embryonic precursors. In mouse models these bone marrow–derived tissue macrophages do not exhibit the same self-renewal properties as embryonic-derived tissue macrophages. Human blood monocytes are typically classified as $CD14^+/CD16-$ classic type, $CD14^+/CD16^+$ intermediate type, and $CD14^-/CD16$ nonclassic type. Studies have demonstrated that the plasticity of the $CD14^+/CD16^+$ monocytes is dependent on the local tissue environment, which, when stimulated with macrophage cytokine M-CSF (CSF1), induces high expression of CD163. Stimulation with dendritic cell cytokines, granulocyte-macrophage colony-stimulating factor (GM-CSF or CSF2), and IL4 results in much lower CD163 expression.

In general, the "macrophage," both of the resident tissue cell (embryonic-derived) and monocyte-derived bone marrow (BM) cell, is an active cell that is at the front line of the innate immune system. Both tissue-resident macrophages and monocyte-derived macrophages play a role during tissue injury and repair and are often hard to differentiate in active inflammatory states since they share tissue markers. For example, only recently has a microglia-specific marker, anti-Transmembrane 199 (Tmem199), made claims to selectively differentiate microglia from BM-derived monocytes in inflammatory CNS lesions. In basic terms the "macrophage" has an extensive repertoire of pattern recognition motifs, and can phagocytose, digest, and destroy pathogens; become immune activated; and interact with DCs, B cells, and others to induce specific and acquired immunity, both cellular and humoral. The "macrophage" can modulate responses to intrinsic and extrinsic stimuli. Individuals, by virtue of their genetic heterogeneity, can vary in their ability to handle similar insults. Polymorphisms in interleukin (IL)-12R, for example, will determine a variety of granulomatous responses

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Box 19.1 Classification of Histiocytic Disorders

2016 Revised Classification of Histiocytoses and Neoplasms of the Macrophage-Dendritic Cell Lineages Based on Clinical, Radiographic, Pathologic, Phenotypic, Genetic, and/or Molecular Features[a]

L group histiocytoses
- Langerhans cell histiocytosis (LCH)
- Indeterminate dendritic cell tumor (ICH)
- Erdheim-Chester disease (ECD)
- Mixed LCH/ECD

C group: Non-Langerhans cell histiocytosis of skin and mucosa

Cutaneous non-Langerhans cell histiocytosis
- Xanthogranuloma family
  - Juvenile xanthogranuloma (JXG)
  - Adult xanthogranuloma (AXG)
  - Solitary reticulohistiocytoma (SRH)
  - Benign cephalic histiocytosis (BCH)
  - Generalized eruptive histiocytosis (GEH)
  - Progressive nodular histiocytosis (PNH)
- Non-xanthogranuloma family
  - Cutaneous Rosai-Dorfman disease
  - Necrobiotic xanthogranuloma (NXG)
  - Cutaneous histiocytosis not otherwise specified

Cutaneous non-Langerhans cell histiocytosis with a major systemic component
- Xanthogranuloma family Xanthoma disseminatum
- Non-xanthogranuloma family: Multicentric reticulohistiocytosis (MRH)

R group: Rosai-Dorfman disease and miscellaneous noncutaneous, non-Langerhans cell histiocytoses
- Familial Rosai-Dorfman disease
- Sporadic Rosai-Dorfman disease
  - Classic (nodal) Rosai-Dorfman Disease
  - Extranodal Rosai-Dorfman disease
  - Neoplasia-associated Rosai-Dorfman disease
  - Immune disease–associated Rosai-Dorfman disease

M group: Malignant histiocytoses (histiocytic malignancy)
- Primary malignant histiocytoses: localization and subtype (histiocytic, Langerhans cell, interdigitating, indeterminate cell, or not specified)
- Secondary malignant histiocytoses (following or associated with another hematologic neoplasia)

H group: Hemophagocytic lymphohistiocytosis and macrophage activation syndrome (HLH/MAS)
- Primary HLH: Monogenic, mendelian inherited conditions leading to HLH
- Secondary HLH (apparently non-mendelian HLH)
- HLH of unknown/uncertain origin

Proposed 2016 WHO Classification of Mature Lymphoid, Histiocytic, and Dendritic Neoplasms[b]

Histiocytic and dendritic cell neoplasms
- Histiocytic sarcoma
- Langerhans cell histiocytosis
- Langerhans cell sarcoma
- Indeterminate dendritic cell tumor
- Interdigitating dendritic cell sarcoma
- Follicular dendritic cell sarcoma
- Fibroblastic reticular cell tumor
- Disseminated juvenile xanthogranuloma
- Erdheim-Chester disease[c]

[a]Emile JF, Abla O, Fraitag S, et al:. Revised classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages, *Blood* 127(22):2672–2681. Note: Follicular dendritic cell tumor/sarcoma is no longer considered a part of histiocytic/dendritic cell classification.
[b]Swerdlow SH, Campo E, Pileri SA, et al: The 2016 revision of the World Health Organization (WHO) classification of lymphoid neoplasms, *Blood* 127:2375–2390, 2016.
[c]Changes from the 2008 classification.

to mycobacterial infections. Macrophage production from monocyte-derived marrow precursors is driven by IL-1, IL-3, M-CSF, and GM-CSF. Similarly, DC replenishment can occur during periods of need from adult stem cell precursors and/or monocyte-derived DC, regulated, in part, by IL-4, GM-CSF, and tumor necrosis factor alpha (TNF-α). DCs exist in the periphery as sentinel cells, sampling the internal or external environment. When they encounter a danger signal through their cytokine or toll-like receptors, the cells become activated and proceed to mature. DCs can change their phenotype from one adapted to antigen acquisition (pinocytic, phagocytic) to one more suited to antigen presentation, which occurs in

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the central lymphoid tissues, lymph nodes, or spleen. DCs present antigen in a major histocompatibility complex (MHC)-restricted manner to a variety of cell types, T lymphocytes, B cells, and natural killer (NK) or NKT cells to induce immune or tolerizing effects. There is plasticity in the system, and both macrophages and DCs can be driven in the direction of the other by various stimuli; cells with dendritic phenotype can be converted to macrophage phenotype by IL-10 or M-CSF. It is therefore not surprising that there are gray zones in which the distinction of macrophages from DCs is not absolute and that histiocytic lesions commonly contain a mix of monocytes, macrophages, and DCs, even if one cell type dominates. Macrophage fusion is a common phenomenon resulting in multinucleated giant cells. Under certain circumstances, such as incubation with IL-4, macrophages can assume an epithelioid appearance.

IDENTIFICATION OF HISTIOCYTES

Histiocytes, DCs, and macrophages can be followed along their life cycle from precursors, through activation to mature functional cells by a wide panel of markers, surface and cytoplasmic, and by some of their histochemical characteristics. Table 19.1 lists some of these molecules, many of which are detected by flow cytometry, in situ by immunohistiology, or on frozen sections. A significantly limited panel is available for the recognition of DC and macrophages in fixed embedded tissues, and the panels can also be informative regarding maturation of the histiocytes. For example, PU.1, an Ets family transcription factor, stains nuclei of both macrophages and DCs but also myeloid cells and their leukemias. Few histiocyte-related molecules are informative on their own and are best used as panels of markers for tissue histiocytes. Table 19.2 shows antibodies that react almost exclusively with macrophages, those that are common to both macrophages and DCs (though the amount and pattern can differ), and the antibodies that are more exclusive to DCs. Some are more informative regarding subsets, such as CD1a and Langerin for Langerhans cells and factor XIIIa for dermal and interstitial macrophages. Although CD163 is a more specific tissue marker for the tissue macrophage than CD68, which is a lysosomal marker, epithelioid histiocytes, as seen in epithelioid granulomas and follicular tingible body macrophages, have low to negative CD163 expression. The Th1-induced proinflammatory markers interferon-γ (INF-γ) and TNF-α downregulate CD163 expression on the epithelioid histiocytes, which have high lysozyme expression and variably strong CD68 expression. CD163, however, is not a reliable marker of M2 versus M1 macrophages in tissues, as IL4 and IL13 cytokines in the Th2 response can also downregulate CD163, whereas $CD163^{+}$ macrophages are invariably present in both Th1- and Th2-associated diseases. One should also be aware of the clone type used for identifying CD68, especially in the evaluation of marrow macrophages as the CD68 KP1 clone also labels myeloid/granulocyte cells

TABLE 19.1
Selected Antibodies Informative for Histiocytes

Cluster	Cell Function	Predominant Histiocyte
CD1a, b, c	T-cell response to nonpeptide lipids and glycolipids	DC
CD4	MHC class II/HIV receptor	M, DC
CD11b	Complement C3b receptor	M
CD11c	CD11/CD18 receptor	M, DC
CD14	Lipopolysaccharide receptor	M
CD25	IL-2 receptor	M
CD31	PECAM-1	M
CD32	Fc IgG receptor, low affinity	M
CD33	Sialoadhesin	M
CD49	Integrin receptors	M
CD64	Fc IgG receptor, high affinity	M, DC
CD68	Macrosialin, lysosome-associated membrane glycoproteins	M, DC
CD83	Ig superfamily	DC
CD86	CD28/CD152 ligand	DC
CD91	Low-density lipoprotein related protein-1	M
CD103	Integrin α E	DC
CD116	GM-CSF receptor α	M, DC
CD123	IL-3 receptor α	PDC
CD163	Hemoglobin/haptoglobin scavenger receptor	M
CD169	Sialoadhesin	M
CD204	Macrophage scavenger receptor	M
CD205	DEC 205	M, DC
CD206	Macrophage-mannose receptor	M, DC
CD207	Langerin	DC
CD208	DC-LAMP	DC
CD209	DC-SIGN	DC, M
CD254	RANK-L	Tumor necrosis factor ligand M
CD283	TLR3	Toll-like receptor D
CD284	TLR4	Toll-like receptor M
CD303	CLEC4	C-type receptor PDC

NOTE: Many of the molecules are not unique to the histiocytes but are expressed as indicated on monocytes, macrophages, or dendritic cells as well as other cells. *DC,* Dendritic cell; *GM-CSF,* granulocyte-macrophage colony-stimulating factor; *Ig,* immunoglobulin; *IL,* interleukin; *M,* monocyte, macrophage; *MHC,* major histocompatibility complex; *PDC,* plasmacytoid dendritic cell.

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unlike the CD68 PGM1 clone. A strong diffuse cytoplasmic staining for CD68 KP1 in extramedullary lesions with absent to weak CD68 PGM1 expression serves as a diagnostic clue for myeloid sarcoma. CD123, the interleukin 3 receptor α, identifies the plasmacytoid DCs. Other molecules are more informative about DC maturation: CD83, DC-LAMP, and hi-fascin indicating an activated and mature phenotype. S100 has long been used as a DC marker when present in high amounts, but it is variably expressed in subpopulations of activated macrophages and is thus of limited discretionary power.

TABLE 19.2

Antibodies and Enzymes Informative for Histiocytes in Tissues

Cell Type	Marker
Macrophages	CD14, CD163, acid phosphatase, nonspecific esterase
Macrophages and dendritic cells	CD68 (PGM1 in bone marrow), HLA-DR, S100
DCs	CD1a, Langerin
Mature DCs	CD83, DC-LAMP, hi-fascin
Dermal macrophages	Factor XIIIa, CD163
Plasmacytoid DCs	CD123
Follicular DCs	CD21, CD35, clusterin

DC, Dendritic cell.

PHYSIOLOGIC EXCESS OF HISTIOCYTES

MACROPHAGES

Macrophages derived from recruited monocytes will accumulate at sites of infection and inflammation wherever tissue damage and destruction occur. Scavenging, phagocytosis, and digestion of debris are major functions, although macrophages are also active participants in the subsequent repair and immune reactions. Lymph node reticular sinus histiocytes accumulate largely in response to local draining stimuli, inflammation, or tissue damage. Follicular tingible body macrophages are characterized by the presence of apoptotic debris within the cytoplasm and increase in the presence of apoptotic activity within the dark portion of the germinal center. Normal macrophages can be overloaded by increasing the amount of substrate they are expected to handle. Collections of xanthomatous (yellow) macrophages are also part of the repair and scavenging process following tissue injury, such as perforation of the appendix or ruptured cyst (Fig. 19.2). Excessive turnover of cells with uptake of membrane-derived complex lipids leads to accumulation of ceroid–lipochrome-rich "sea-blue histiocytes," a term derived from their appearance on Wright- or Wright-Giemsas–type stains. Sea-blue histiocytes are an accompaniment of myeloproliferative disorders in the bone marrow or high platelet phagocytosis in the spleen. Ceroid histiocytosis is also the explanation for most pigmented macrophages in the gastrointestinal mucosa and in chronic granulomatous disease. Intravenous alimentation, especially when lipid is added, can lead to the accumulation of ceroid-filled macrophages in the liver, spleen, and lungs. The atheromatous plaque in large arteries has

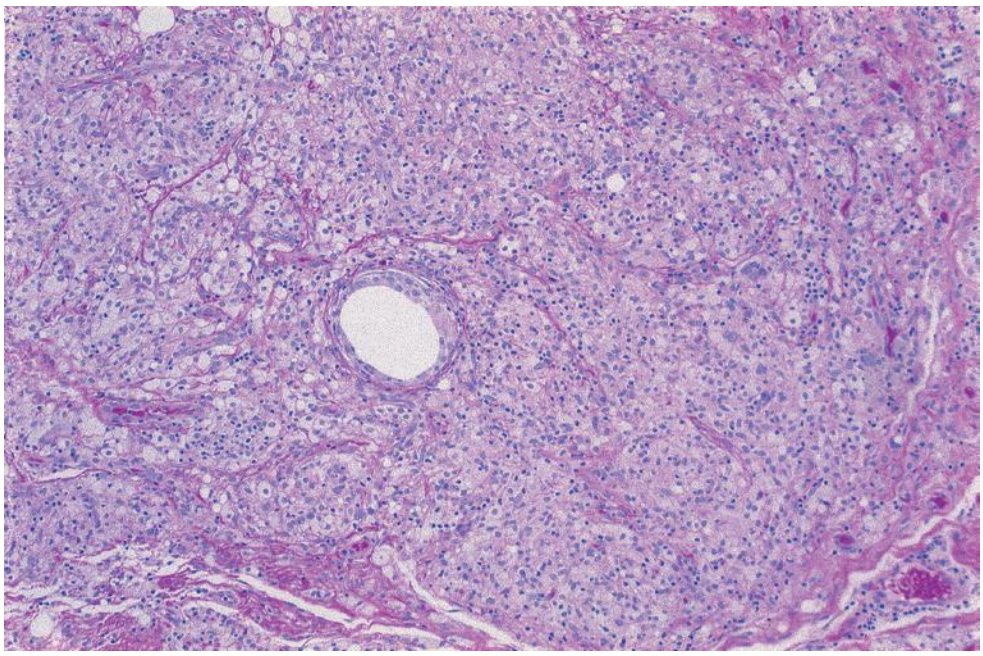


FIG. 19.2

Xanthomatous inflammation. Aggregates and sheets of foamy macrophages surround a central cavity, all that remains of a ruptured thyroglossal duct cyst.

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lipoproteins deposited in excess, and the accumulation of foamy lipid-filled macrophages is characteristic.

Dendritic Cells

Germinal center responses require binding of antigen to follicular DCs that produce IL-6, leading to germinal center expansion, a process inhibited by corticosteroids. Follicular hyperplasia is common in childhood infections and autoimmune and rheumatic disorders. Cytologically atypical follicular DCs are seen in multicentric hyaline-vascular type Castleman disease, probably driven by human herpesvirus 8–derived viral IL-6.

Accumulation of normal sentinel DCs can be seen along their migratory pathway when their numbers are increased in inflammatory processes. The best described is the paracortical DC hyperplasia known as *dermatopathic lymphadenopathy* but is not unique to that condition. Increased numbers of Langerhans cells from the periphery accumulate in the nodal paracortex in which they can form large, confluent, and nodular aggregates. Because these DCs are maturing or fully mature, they will have the phenotype of interdigitating DCs, $S100^+$, fascin-hi, $CD83^+$, DC-$LAMP^+$ with interspersed $CD1a^+$/$Langerin^+$ Langerhans cells in the paracortex, in contrast to LCH, which is a sinus disease (see Fig. 19.3). A diffuse paracortical DC hyperplasia in tonsils or lymph nodes can be an accompaniment of other infiltrating processes and can be misleading by diverting attention from the infiltrate, usually a leukemia or lymphoproliferative disorder.

Pathologic Excess of Macrophages

Infection and Foreign Materials

Macrophages can accumulate at sites of infection or tissue destruction as part of their physiologic role in repair. Osteomyelitis is an example in which the presence of excess macrophages can sometimes mimic that of a neoplastic overgrowth such as Langerhans cell disease or other histiocytosis (Fig. 19.4). Infection in the immune-suppressed individual can also be characterized by unconventional organisms and unusual responses. Atypical mycobacterial disease with mycobacterium avium intracellulare in particular has sheets of macrophages that harbor the organisms. *Mycobacterium tuberculosis* not only evades phagocytosis but inhibits apoptosis of infected macrophages, presumably by preventing mitochondrial damage and initiating plasma membrane repair. Malakoplakia with the typical Michaelis-Guttmann bodies is a disordered macrophage response to *Escherichia coli*. There are organisms that find safe haven in macrophages, shielded from other elements of the immune system, best characterized by mycobacterial spindle cell diseases including histoid leprosy and Whipple disease (*Tropheryma whippelii*), leishmania, and rhinoscleroma (Mikulicz cells containing *Klebsiella rhinoscleromatis*).

Many organisms can induce epithelioid transformation of macrophages and a granulomatous response, some with a more acute inflammatory response, such as *Yersinia, Tularemia, Bartonella,* and *Brucella* spp. Other organisms such as *Mycobacterium, Pasteurella,* and *Burkholderia* spp. (the last causing glanders and melioidosis) have a more chronic course and innate epithelioid transformation, often with a giant cell component. Sarcoidosis is the prototype of the noninfectious epithelioid granuloma, but nonsarcoidal conditions such as common variable immune deficiency (CVID) and Blau syndrome (*NOD2* mutations) can also manifest as granulomatous disease. Giant cell formation is also seen in a number of noninfectious circumstances with collections of epithelioid histiocytes at various tissue sites, with sarcoidosis and Crohn disease as common examples. Beryllium exposure is less frequent. Foreign bodies of many kinds can lead to macrophage accumulation, often with a giant cell component, and some aggregates may be epithelioid. Talc, silicone, starch, and detritic granulomas from prostheses are examples of exogenous material, but crystal-storing histiocytes may accumulate endogenous crystals that are derived from immunoglobulin (Fig. 19.5A). Drugs such as paclitaxel (Taxol) can lead to accumulation of histiocytes with periodic acid–Schiff (PAS)-positive inclusions, whereas clofazimine produces histiocytes containing red crystals (see Fig. 19.5B and C). Some, but not all, foreign bodies are birefringent, and polarization is mandatory when confronted with a histiocytic aggregate of unknown type.

Xanthomas and Xanthomatous Inflammation

The xanthomatous (yellow) macrophage response in tissue repair is a feature when inflammatory cells and tissue necrosis are being scavenged. There are sites where the xanthomatous inflammation can be excessive and even simulate tumor in its extent. Xanthogranulomatous pyelonephritis is a chronic pyelonephritis of adults in which an exuberant xanthomatous process can replace much of the kidney and even extend into the retroperitoneum. It is rare in children. A similar process can affect the gallbladder, and bacteria can be isolated from most. There are instances of so-called necrobiotic xanthogranulomas in soft tissue, orbit, and rarely viscera, sometimes in association with paraproteinemia. These xanthogranulomas are not infectious in nature. A xanthoma is a mass or nodule composed almost entirely of lipid-rich foamy macrophages. Most are associated with hyperlipidemias, both primary inherited forms and secondary hyperlipidemic states such as diabetes mellitus, cholestatic liver disease, or nephrotic syndrome. Xanthomas occur at various sites on the skin in crops as eruptive xanthoma, xanthelasma around the eyes, and tendinous xanthoma around the ankles, knees, hands, and elbows. The larger xanthomas may be associated with a center that contains fat

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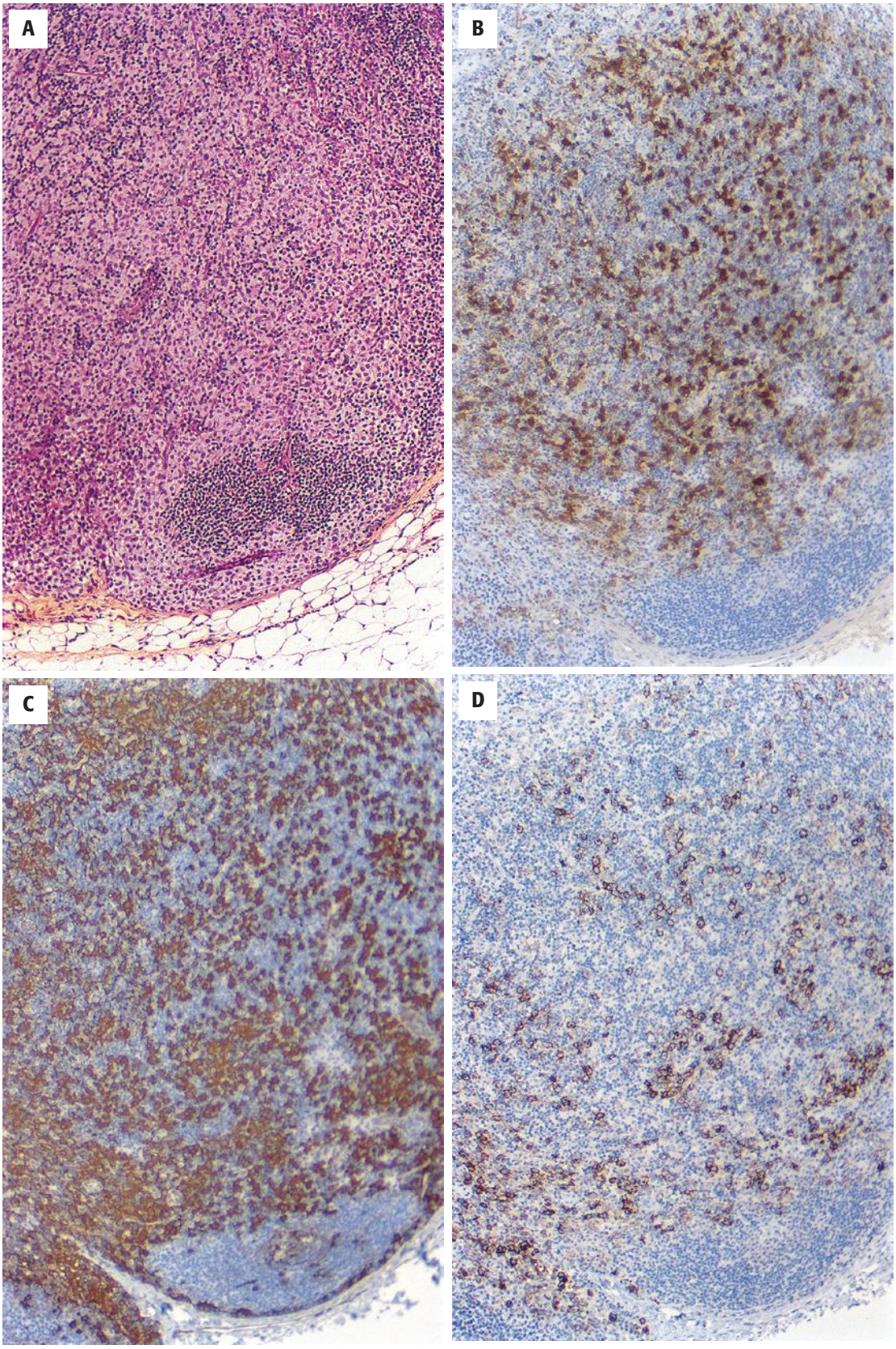


FIG. 19.3

Aggregation (hyperplasia) of paracortical nodal dendritic cells (DC). Dermatopathic lymphadenopathy, although the same pattern is seen in nodes draining non-dermal sites. A, The paracortex is replaced by light-staining dendritic cells that have interdigitating DC phenotype. B, High S100. C, High fascin expression. D, Numbers of CD1a-positive Langerhans cells. Contrast with LCH, which is a sinus disease.

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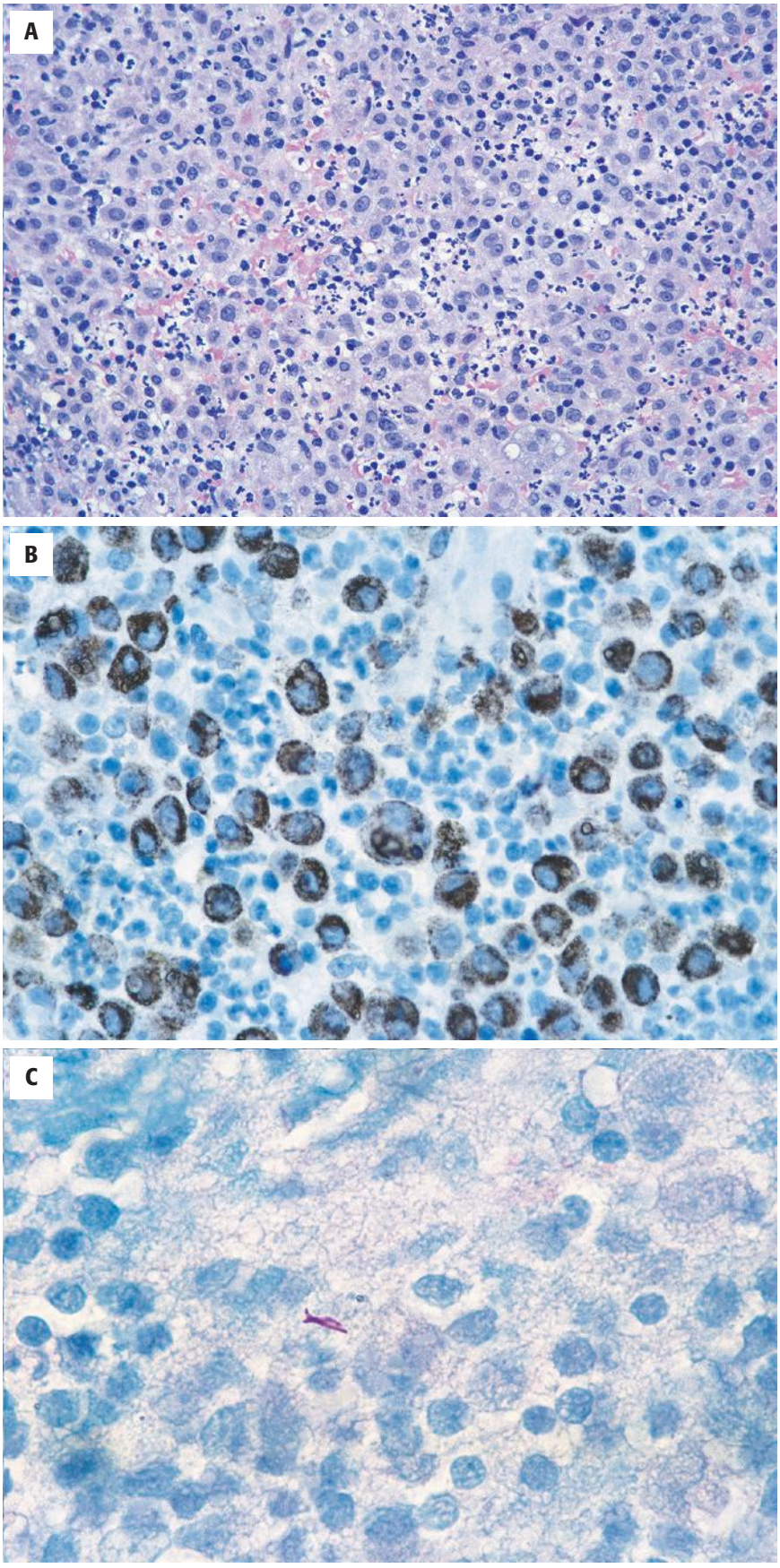


FIG. 19.4

Bone with histiocyte-rich lesion. A, The histiocytes stain strongly for CD68 (PGM-1) and were CD1a-negative (B). C, Acid-fast mycobacterial organisms are demonstrated.

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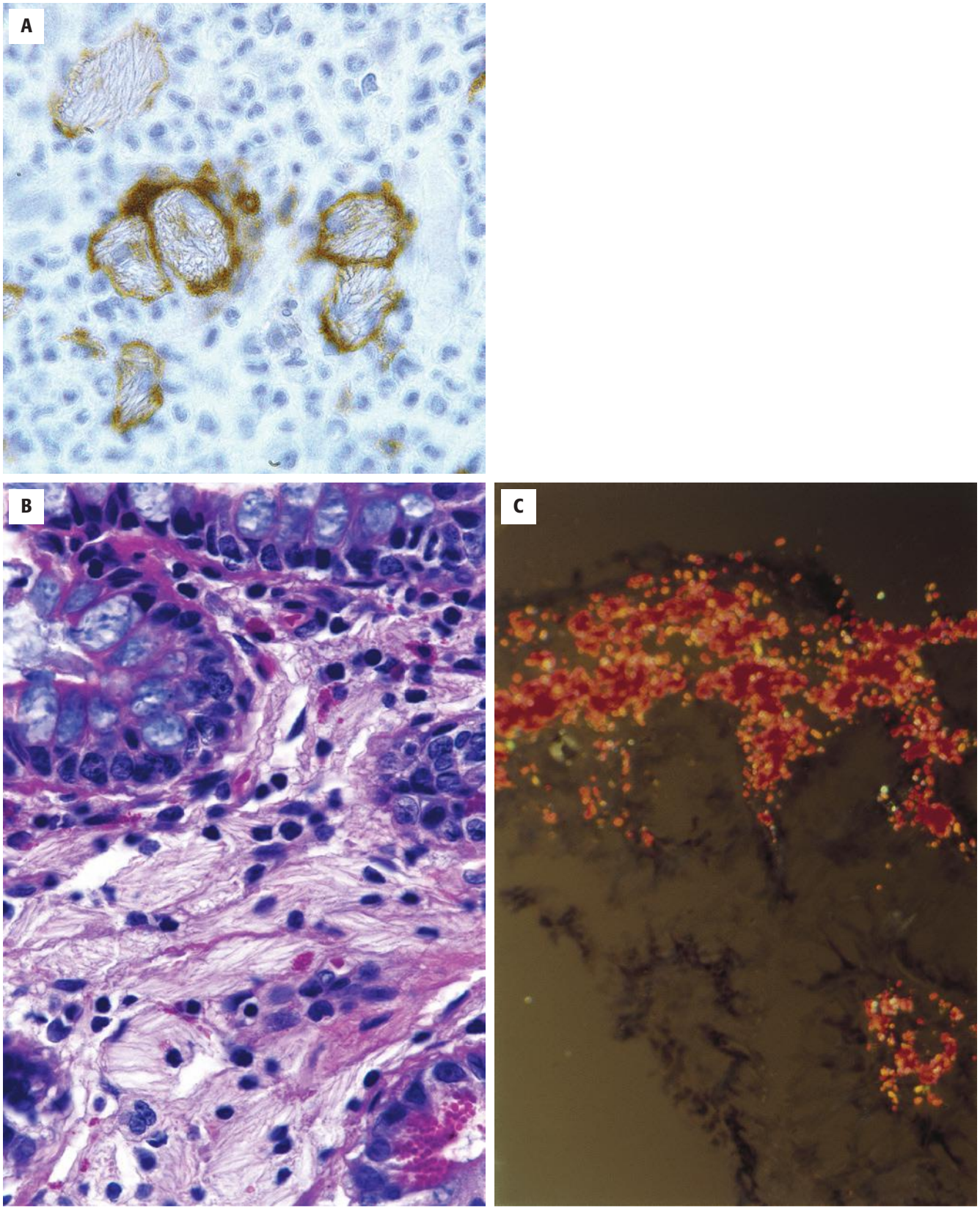


FIG. 19.5

Crystals in macrophages. A, Bone marrow with refractile immunoglobulin crystals in macrophages stained for CD163, an example of crystal storing histiocytosis. B, Small bowel with intestinal macrophages that have a striated cytoplasmic appearance. C, Polarization of a frozen section of the bowel in B reveals the red crystals of clofazimine.

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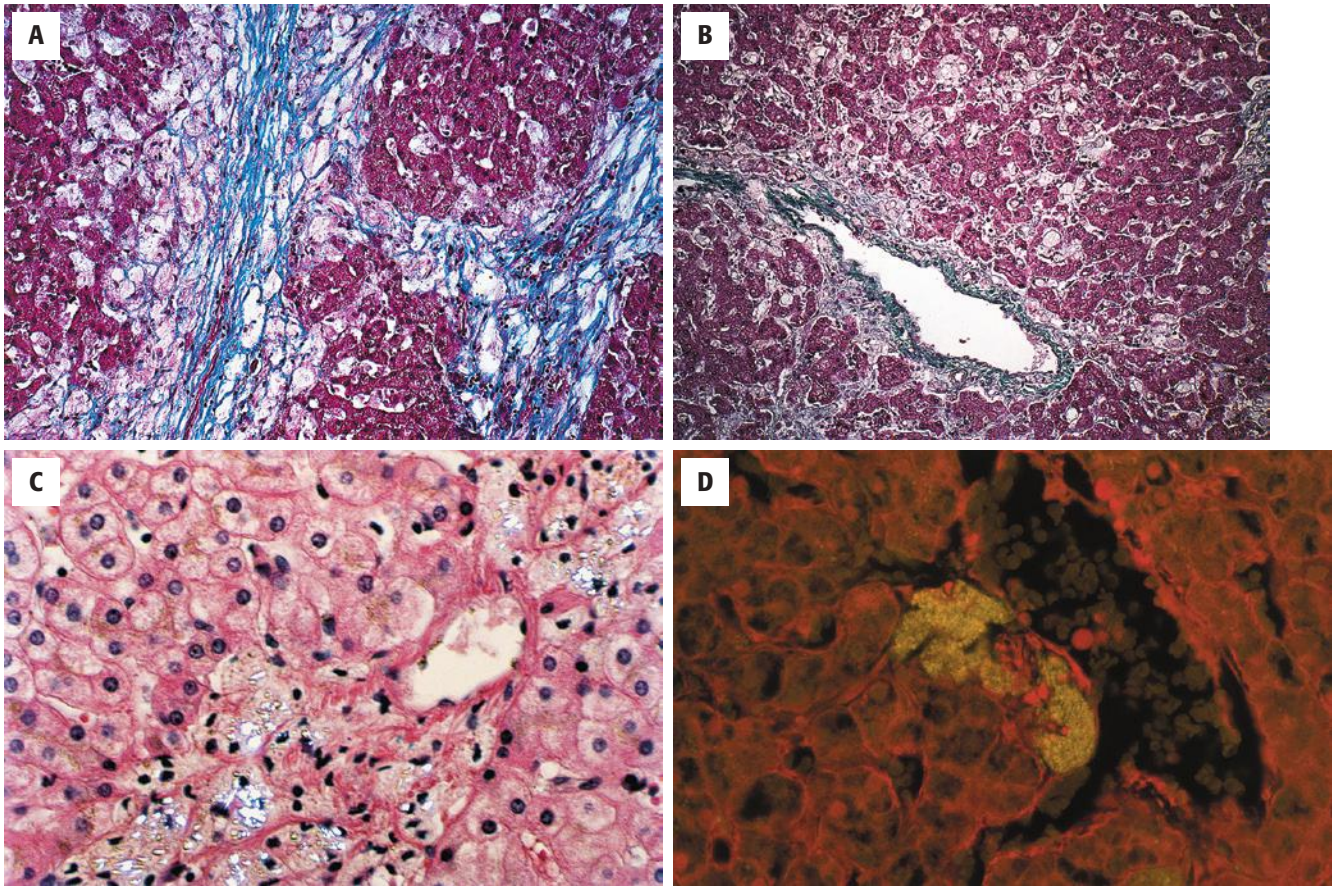


FIG. 19.6

Liver with storage diseases that affect the sinusoidal macrophages. A, Gaucher disease in a Mallory trichrome stain has sheets of perifibrous macrophages with pale, striated cytoplasm. B, Niemann-Pick in a Masson trichrome has foamy pericentral macrophages. C, Cystine crystals are seen in sinusoidal macrophages by polarization. D, Hermansky-Pudlak disease has ceroid-filled macrophages seen as yellow autofluorescence.

necrosis, cholesterol crystals, and a surrounding foreign-body type giant cell reaction. Atherosclerotic lesions are characterized by xanthomatous macrophages. A history of hyperlipidemia is the rule in true xanthomas.

FUNCTIONAL DEFECTS OF HISTIOCYTES

Because of their specialized peculiar functions, macrophages are rich in lysosomes. In a number of inherited defects of the lysosomal apparatus, there is a deficiency of enzymes needed to degrade macromolecules or to transport the degraded substance out of the lysosome, leading to accumulation of naturally occurring metabolites within lysosomes (lysosomal storage disorders). The classes of molecules involved include the mucopolysaccharidoses (MPS I-VII), glycoproteinoses, glycogenosis (type II), sphingolipidoses, lipidoses, the multiple enzyme deficiency disorders, and the lysosomal transport defects. Each of the defects included within these categories will have a peculiar tissue distribution depending on the substrate that fails to be catabolized or transported. The accumulation of the various substances will produce highly different phenotypic expression when viewed by light or electron microscopy, and both of these modalities have been combined with histochemistry to categorize the various disorders (Fig. 19.6). On occasion, discovery of the storage cells in tissues might be the first clue to the presence of a metabolic disorder, and the physical characteristics of the storage material can lead to the biochemical or genetic testing needed for confirmation.

Considering the numerous functions that macrophages have in health and disease, it seems remarkable that so few defects have been documented that are restricted to these cells. Apart from the inherited lysosomal storage disorders, some examples often share the deficiency with other cells. Autosomal-recessive osteopetrosis affects osteoclasts from the same hematopoietic precursors as macrophages. When the responsible gene is *TCIRG1,* it affects the vacuolar proton pump of osteoclasts and gastric parietal cells. Fewer instances are caused by the *CLCN7* gene mutation that encodes a chloride channel. Hematopoietic stem cell transplant in the *TCIRG1* but not the *CLCN7* form can arrest the disease by providing functional monocytes and osteoclasts. The effect of these mutations on other macrophages, if any, is not known.

Leukocyte adhesion deficiency due to defects in β-integrins hinders the ability of granulocytes and monocyte-derived macrophages to accumulate at infection sites. Chronic

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granulomatous diseases involve the inability to provide an oxidative burst important in microbial killing. The responsible defects resulting in abnormal electron transfer from cytoplasmic nicotinamide adenine dinucleotide phosphate (NADPH) to molecular oxygen are common to neutrophils and macrophage abnormalities of $gp91^{phox}$ and include a macrophage-specific component of NADPH oxidase.

Chronic granulomatous disease (CGD) can develop for the first time as lymphadenopathy, and nodal involvement is seen in 50% of patients. Active chronic inflammation and granulomas occur, and occasionally pigmented macrophages might provide a clue to the diagnosis. The pigment is lightly PAS positive and has the features of ceroid, the ultrastructural equivalent being lysosomal debris. The most frequent organisms in current experience are *Staphylococcus aureus,* followed by *Burkholderia cepacia, Serratia marcescens, Nocardia* species, *Aspergillus* species, *Salmonella* species, and bacille Calmette-Guérin. Confirmation of the diagnosis of CGD is by direct measurement of superoxide production, ferricytochrome c reduction or dihydrorhodamine oxidation.

Hermansky-Pudlak syndromes with oculocutaneous albinism and platelet storage disease are caused by defects in intracellular protein trafficking that can affect lysosomes in some macrophages and melanocytes. Alveolar macrophages may be targeted in Hermansky-Pudlak syndrome type 1, in which progressive pulmonary fibrosis occurs.

The emerging field of toll-like receptors (TLR), their normal variation and defects, is of importance because of their widespread expression on macrophages. Recent reviews provide insights into research investigating the expression of TLR in macrophages of patients with autoimmune disease, which may be implicated as potential targets of therapy. For example, increased TLR5 was found in the synovium and synovial-associated macrophages in patients with rheumatoid arthritis, as compared to control subjects. MicroRNAs, small non-coding RNAs that are a class of gene expression regulators, may also play a role via their induction and regulation by TLR stimulation in macrophages, which appears altered in some autoimmune disease.

Kikuchi-Fujimoto disease (histiocytic necrotizing lymphadenitis) is a self-limiting condition characterized by proliferation and accumulation of histiocytes of unclear origin. The benign lymphadenopathy is associated with systemic symptoms and fever. Paracortical areas are filled with histiocytes, plasmacytoid DCs, $CD8^+$ T cells, and karyorrhectic debris. Later changes are more xanthomatous. The diagnosis is histopathologic by exclusion of other causes of inflammatory lymphadenopathy.

Tangier disease owing to high-density lipoprotein deficiency leads to the accumulation of cholesterol esters in lymphoid tissues, tonsils, lymph nodes, liver, and spleen. Functional effects on macrophage physiology have been documented for Gaucher disease. Hemophagocytic syndromes are also a serious consequence of the Griscelli syndrome, Chediak-Higashi syndrome, and Hermansky-Pudlak syndrome diseases (see below under Hemophagocytic Lymphohistiocytosis and Macrophage Activation Syndromes).

DC dysfunction has been identified in the X-linked Wiskott-Aldrich syndrome in which Wiskott-Aldrich syndrome protein is functionally defective. Macrophages, DCs, and osteoclasts are affected and have disordered motility because of their inability to form podosomes. Common variable immune deficiency and CD40 defective hyperimmunoglobulin M disease may have diminished DC function, but it is not established if this is the primary defect. Because DCs can act as the portal for viral infection, they may be increased or selectively depleted in some viral infections, notably human immunodeficiency virus (HIV).

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

Hemophagocytic lymphohistiocytosis (HLH) is a severe, potentially fatal systemic inflammatory activation disorder (not a primary histiocytic disorder) characterized by a regulatory disorder of T cells leading to unbridled macrophage activation and inflammatory cytokine upregulation. Despite the name, the pathologic features of hemophagocytosis are neither specific nor sensitive in the overall diagnosis and constitute only one of the eight diagnostic criteria. Historically HLH has been classified as primary or familial based on an underlying genetic defect in lymphocyte cytotoxic defects. However, in the recent revised classification of histiocytoses, an expansion of this group to include other mendelian inherited conditions associated with HLH and immune impairment was proposed. Secondary or acquired HLH is now known as apparently non-mendelian HLH, which includes infection-related HLH, malignancy-associated HLH, transplant-related HLH, HLH associated with a defined rheumatologic condition, and HLH of unknown/uncertain origin. Historically macrophage activation syndrome (MAS) has been noted as a symptom of HLH occurring in a patient with an underlying rheumatic disorder (e.g., systemic juvenile idiopathic arthritis and its adult equivalent, adult-onset Still disease, systemic lupus erythematosus, and Kawasaki disease). Recognition of the clinical features of MAS belonging to the spectrum of HLH has led to the proposal of the term MAS-HLH for this "secondary" or so-called acquired HLH.

SECONDARY HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS INCLUDING MACROPHAGE ACTIVATION SYNDROME

Secondary HLH, including rheumatic-driven MAS-HLH, is thought to be incited by an acquired (i.e., infectious, malignancy, drug) or non-mendelian source, unlike primary HLH, in which the etiology is derived from a genetically programmed defect in immune regulation, most notably of the cytotoxic lymphoid system. However, secondary HLH is now best considered as "apparently non-mendelian HLH" as an

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increasing number of cases in this group are revealing genetic mutations that lead to at least partial immune/cytokine function.

Although macrophage functional patterns have been described as type 1 (Th1-driven) or type 2 (Th2-driven), a wide variety of microenvironmental factors result in a large number of different and overlapping functional and phenotypic subtypes that are constantly being modulated. Macrophages can be immune activated by soluble factors, such as cytokines, with interferon-gamma (INF-γ) being the most classic example, but TNF, IL-1, IL-2, and macrophage migratory inhibitory factor (MIF) can also cause proinflammatory activation, as can lipopolysaccharide. These classically activated macrophages are referred to as *M1 macrophages*. Activated M1 macrophages participate in both antimicrobial immunity, by enhancing opsonization and phagocytosis, as well as in cellular immunity by stimulating or inactivating lymphoid receptors, largely through their active cytokine production and other proinflammatory mediators. Deactivation of M1 can be mediated by IL-4, IL-10, IL-13, and TGF-β. IL-4 and IL-13 can also induce an alternative anti-inflammatory activation, mediated by M2 macrophages, that enhances major histocompatibility complex class II expression and mannose receptor-mediated endocytosis. Alternatively activated (i.e., M2) macrophages are heterogeneous in terms of the cytokines that induce their differentiation; they play a role in tissue remodeling and repair, resistance to parasites, immunoregulation, and tumor promotion. Macrophages in HLH and MAS-HLH exhibit many characteristics of M2 macrophages, including the membrane and cytoplasmic expression of scavenger receptor CD163, but, as stated previously, CD163 is not a reliable M2 marker as tissue stains cannot reliably differentiate between M1 and M2 macrophages

In response to a number of different stimuli, a systemic inflammatory or macrophage activation syndrome can develop. Viral infections are the best known, but bacterial infections, rheumatologic disorders, especially the systemic form of juvenile idiopathic arthritis, cancers, lymphoproliferative disorders, intravenous alimentation, and multiple organ failure are among other causes. Although the pathogenesis is unclear, in general persistently activated macrophages appear to be caused by a defect in granule-mediated cell cytotoxicity, leading to an unrestrained lymphocyte–NK cell–driven macrophage stimulation that leads to disseminated overactivity (e.g., enhanced antigen presentation) of the macrophages throughout the body, possibly mediated by TNF-α, ineffective deactivation through depressed NK and cytotoxic CD8 T cells, and repeated INF-γ–dependent stimulation of TLR. Also, defects in perforin and FAS systems lead to unbridled immune activation via their loss in maintaining homeostasis of DC cells and T-cell activation by antigen presentation. An increase in the number and size of endogenous macrophages, with or without hemophagocytosis, is a feature of this condition that is best seen in the bone marrow but also in spleen, liver, and lymph nodes (Figs. 19.7, 19.8, and 19.9). The exaggerated inflammatory response leads to a hypersecretion of proinflammatory cytokines (INF-γ, TNF-α, IL-1, IL-4, IL-6, IL-8, IL-10, IL-18) (e.g., "cytokine storm"). In its most severe expression, there are significant functional effects of the cytokine storm, with bone marrow depression, hepatomegaly with hepatocellular damage and raised hepatocellular enzymes, and effects on the clotting cascade. Treatment is centered on halting the cycle of inflammation that is best abated when the inciting condition is treated or disappears. In severe instances, without resolution, it can lead to progressive organ failure and can be fatal. Thus treatment measures include supportive care, directing the underlying inciting event, and variable success with high doses of steroids, cyclosporine (CSA), or agents aimed at counteracting the effects of TNF-α, such as infliximab and etanercept. The combination of etoposide (VP-16), a cancer chemotherapeutic drug with potent cytotoxicity against activated T cells, and dexamethasone is standard of care based on HLH-94, the international prospective clinical trial sponsored by the Histiocyte Society. In the subsequent HLH-2004 trial, preliminary results did not see additional benefit in adding CSA to the VP-16/dexamethasone backbone during the first 8 weeks of therapy.

GENETIC FORMS OF HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS, INCLUDING FAMILIAL HLH

Genetic forms of HLH (formerly "familial HLH") refer to a group of disorders with clinical and pathologic manifestations of an acute and often fatal form of uncontrolled macrophage activation owing to defective apoptosis, cytotoxic T or NK function, or inappropriate inflammasome function. Traditionally, familial hemophagocytic lymphohistiocytosis (fHLH) has been referred to a small group of inherited disorders that have the clinical and pathologic manifestations of an acute and usually fatal form of uncontrolled macrophage activation owing to defective apoptosis or cytotoxic function of cytotoxic cells (e.g., cytotoxic T cells, T-regulatory cells, or NK cells). This group has recently expanded to include select genetic disorders that also display features of macrophage activation via inappropriate inflammasome or inflammation activation and are now collectively referred to as genetic (mendelian inherited) conditions leading to HLH.

Clinical Features

The clinical features are similar to secondary HLH, dominated by the effects of a hypercytokine state with a wide range of clinical severity. Diagnostic guidelines were originally developed in the context of a pediatric cohort by the Histiocyte Society that include clinical, laboratory, and histopathologic findings because the constellation was important, but definitive molecular diagnosis is now available for some. A family history of parental consanguinity or prior sibling death may be elicited. Prolonged fever and cytopenias, most commonly

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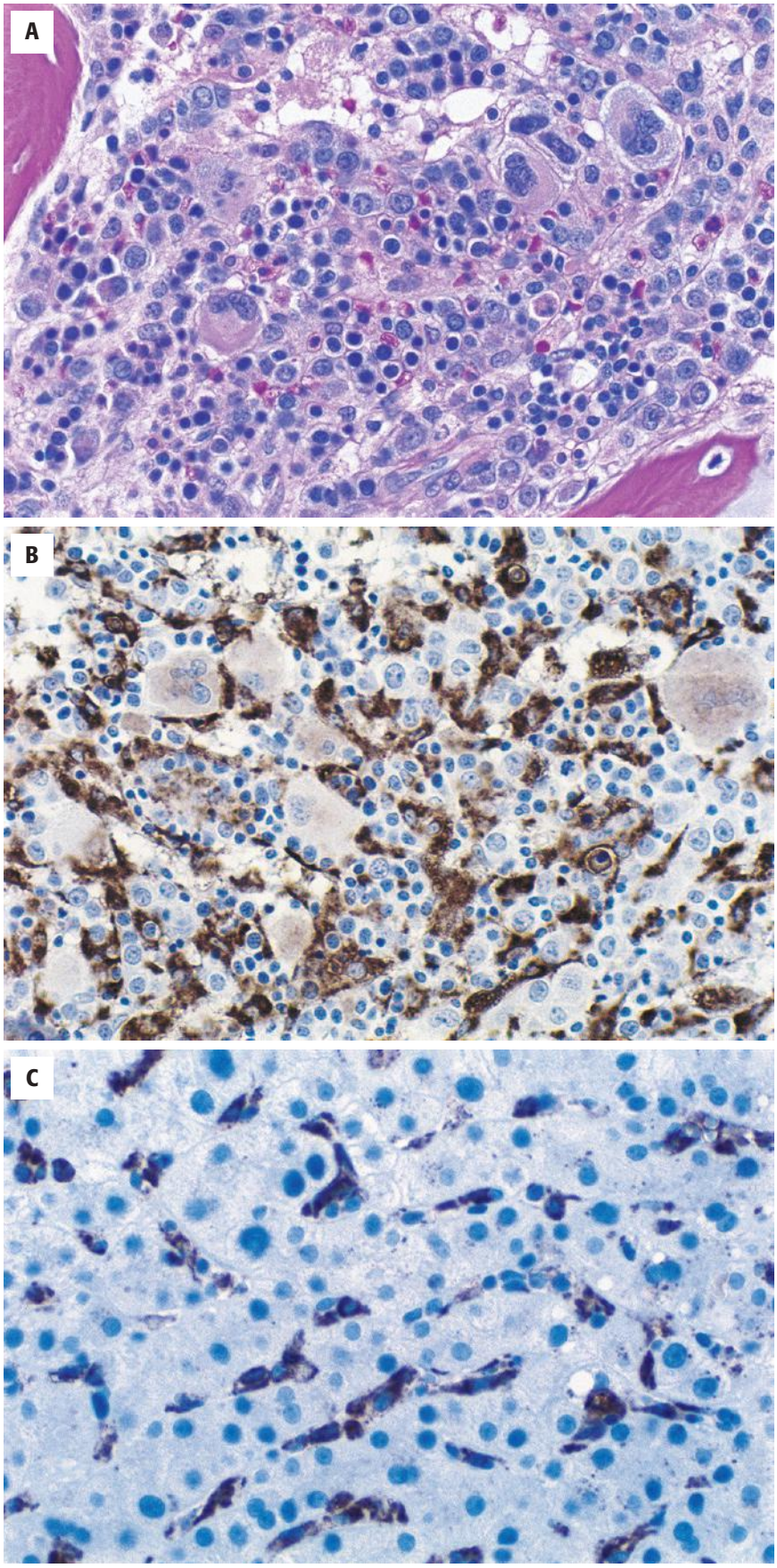


FIG. 19.7

Macrophage activation syndrome. Hemophagocytic lymphohistiocytosis (MAS-HLH): juvenile rheumatoid arthritis. A, A cellular marrow has mild maturational changes, but the histiocytosis is not obvious. B, Immunostain for CD68 (PGM-1), which does not cross-react with myeloid cells, reveals abundant and large macrophages, with some displaying hemophagocytosis. C, Liver enzymes were abnormal, but the only change was the prominence of the sinusoidal Kupffer cells with little hemophagocytosis.

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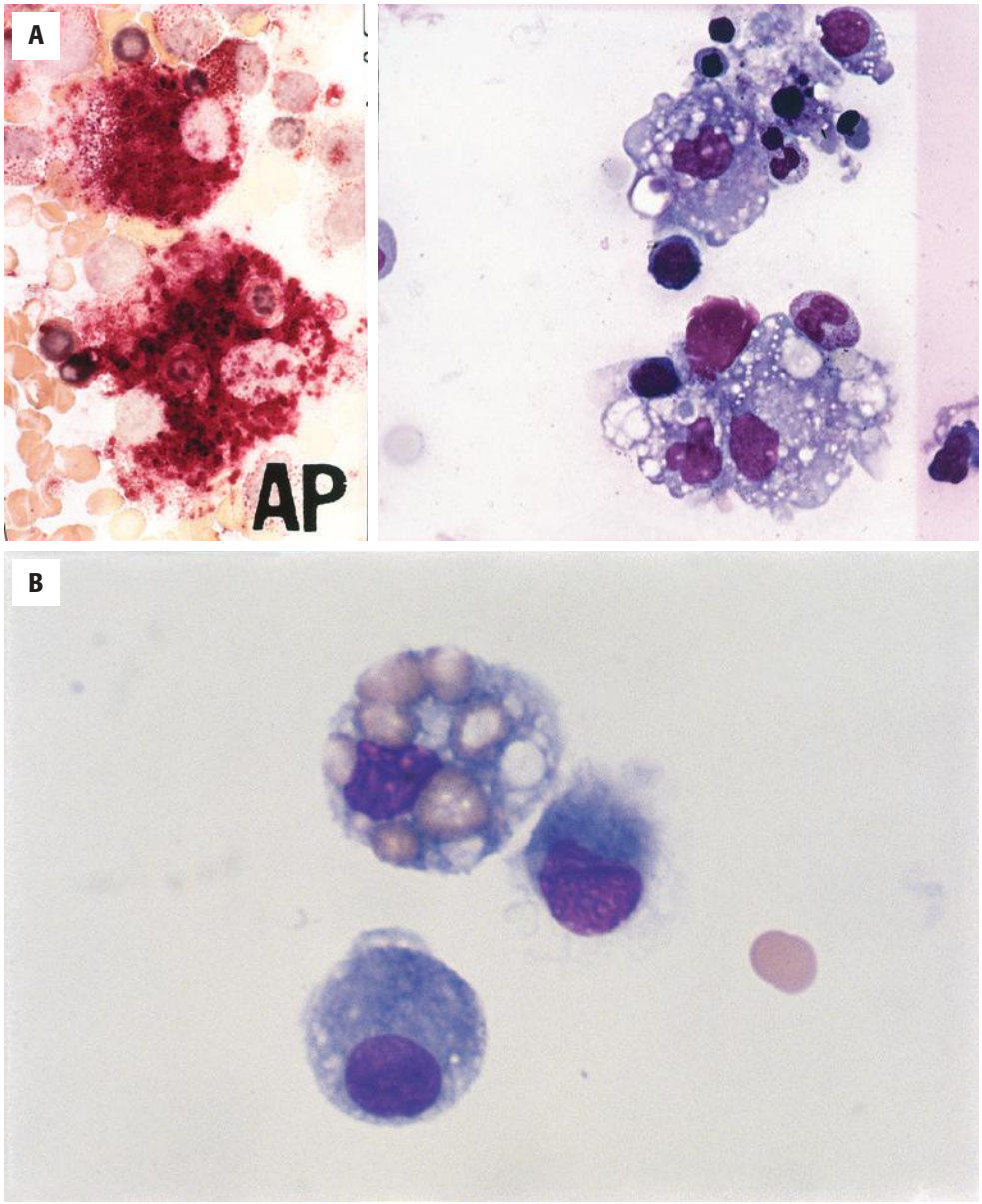


FIG. 19.8

Hemophagocytic lymphohistiocytosis. The bone marrow in active phases will have large, actively phagocytic macrophages that may contain other formed blood elements. A, Acid phosphatase (AP) is demonstrable in the cytoplasm. B, Similar cells that have intracytoplasmic erythrocytes are present in spinal fluid.

anemia and thrombocytopenia within the first 2 years of life, may be associated with neurologic and meningeal signs and symptoms, hepatomegaly with evidence of hepatocellular dysfunction, splenomegaly, and sometimes a skin rash. Laboratory features include hypofibrinogenemia alone or with hypertriglyceridemia, hyperferritinemia, and high levels of circulating soluble CD25 (IL-2 receptor). NK cell function is low or absent.

Pathologic Features

Gross Findings

Gross findings are nonspecific and are limited to hepatomegaly and splenomegaly.

Microscopic Findings

An excess of activated macrophages is the hallmark, usually with prominent hemophagocytosis. These cells can be found in the bone marrow, spleen, and lymph nodes. Cerebral spinal fluid with pleocytosis, including mononuclear cells and activated macrophages, with variable hemophagocytic cells may be found in central nervous system (CNS) involvement (see Fig. 19.8). The macrophage activation of hemophagocytosis can be cyclical, and intervening biopsy specimens (e.g., marrow) may be negative at first examination or during troughs of activity with gradual replacement of the marrow by increasing contents of large phagocytic histiocytes and progressive depletion of hematopoiesis. In the liver, the infiltrate is largely portal with the accompaniment of T lymphocytes (i.e., lymphohistiocytosis). Liver pathology in cases of fHLH associated with cytotoxic defects includes

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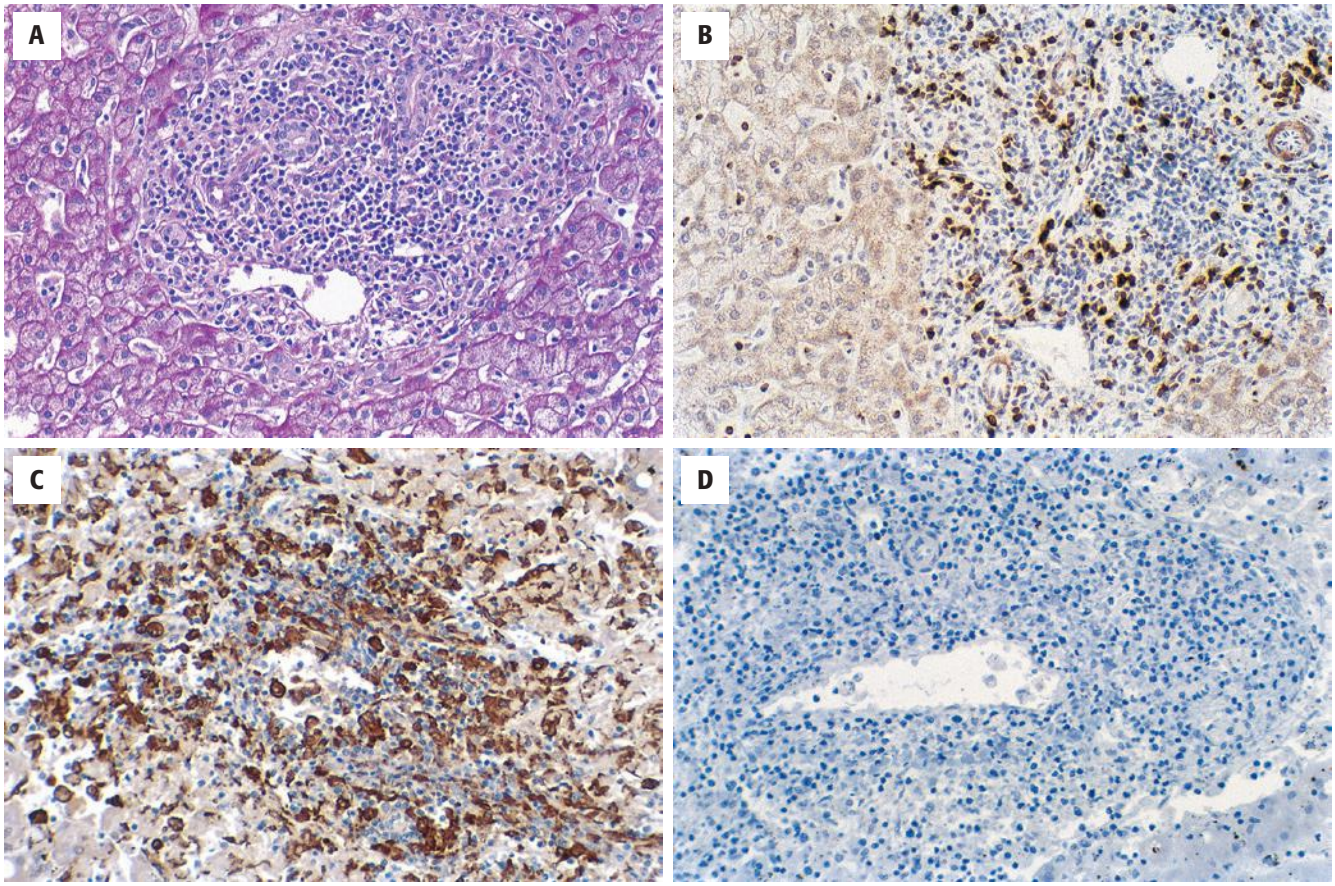


FIG. 19.9

Liver in familial hemophagocytic lymphohistiocytosis (fHLH). A, The infiltrate is mostly portal and consists of a mix of macrophages and lymphocytes. B, In more than half of the familial cases, CD3/CD8 will be demonstrable on the lymphocytes, and perforin is present. C, In instances of fHLH2, the vast numbers of portal macrophages are seen with CD163, but in D, perforin is not detectable on the lymphocytes.

endothelialitis of the portal and central veins, often with free-floating macrophages in lumen of the veins. Lymphocyte-mediated bile duct injury is also a typical feature. In addition to this chronic hepatitis-like appearance, three other histopathologic patterns have been described in fHLH with cytotoxic defects. These are a leukemia-like pattern, a histiocyte storage-like pattern, and a neonatal giant cell hepatitis-like pattern. Other genetic forms of HLH and secondary HLH may or may not display these same hepatic features.

Ancillary Studies

Molecular and Cytogenetic Features

The genetic forms of HLH associated with cytotoxic defects (fHLH) include a handful of known genetic disorders. FHL1 (Online Mendelian Inheritance in Man [OMIM] no. 267700) is mapped to 9q21.3-22, but the genetic defect is not yet determined. Approximately 30% to 50% of patients have mutations in the perforin/*PRF1* gene FHL2 (FHL2, OMIM no. 603553), which is mapped to 10q22.1 and whose function is to induce apoptosis. Most defects lead to reduced or absent perforin expression on peripheral blood cells, a feature that can be documented by flow cytometry. FHL3, mapped to 17q25, is the form characterized by mutations in the *Munc*13-4/*UNC13D* gene (FHL3, OMIM no. 608898), which is important for vesicle priming for perforin transport as well as docking and fusion of cytotoxic granules with the cytoplasmic membrane, but perforin content is normal. Together FHL2 and FHL3 tend to make up over half of fHLH cases. A smaller frequency will have FHL4, mapped to 6q24 (OMIM no. 603552), which is caused by mutations in the Syntaxin-11/*STX11* gene, important for vesicle transport. FHL5, mapped to 19p13 (OMIM no. 613101) is caused by mutations in the Munc18-2/syntaxin-binding protein-2 gene (*STXBP2*), which is important for vesicle trafficking and release, which can be associated with colitis, bleeding tendency, and hearing loss. Three additional fHLH resulting in lymphocyte cytotoxic defects include Griscelli syndrome type 2, mapped to 15q21, caused by a mutation in the RAS-associated protein/*RAB27A* gene, one of the MUNC 13-4 effector molecules important for signal transduction and that results in partial albinism. The second is Chediak-Higashi syndrome mapped to 1q42.1-42.2, caused by a mutation in the lysosomal trafficking regulator/*LYST1* gene, which is important for vesicle transport, resulting in partial albinism, susceptibility to infection, and neuropathy. The third is X-linked

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GENETIC FORMS OF HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS—FACT SHEET

Definition

- A group of inherited conditions resulting in uncontrolled macrophage activation caused by defective apoptosis or cytotoxic function of lymphocytes (e.g., T or NK cells), with newer cases now including genetic abnormalities of inappropriate inflammasome activation.

Incidence and Location

- 0.12 to 0.15/100,000 children per year (Sweden systemic)

Morbidity and Mortality

- Mortality is 100% untreated, around 50% overall 5-year survival with bone marrow transplantation protocols (HLH-94/HLH-2004)

Gender, Race, and Age Distribution

- Male-to-female ratio = 1, although a slight male predominance may be related to X-linked lymphoproliferative disorders
- Race distribution unknown but appears to occur in all races and ethnic groups
- Median age 2 to 3 months; 90% <2 years
- Rare in older children or adolescents, but late occurrences likely attributed to one or more HLH-associated gene sequence variants

Clinical/Laboratory Features

- A molecular diagnosis consistent with HLH diagnosis
- Abnormalities of cytotoxic defects
  - FHL1: Gene unknown 9q21.3-22
  - FHL2: *PRF1* gene 10q22.1
  - FHL3: *Munc*13-4/*UNC13D* gene 17q25
  - FHL4: Syntaxin-11/*STX11* gene 6q24
  - FHL5: Munc18-2/*STXBP2* gene 19p13
  - X-linked lymphoproliferative syndrome type 1 (XLP-1): *SAP/SH2D1A* Xq25
  - Griscelli syndrome type 2: *RAB27A* gene 15q21
  - Chediak-Higashi syndrome: *LYST1* gene 1q42.1-42.2
- Abnormalities of inflammasome overactivation: XLP2/*BIRC4* and *NLRC4*.
- Abnormalities affecting inflammation *SLCA7* gene
- Flow cytometry with elevated granzyme B and either undetectable perforin, diminished signaling lymphocyte activation molecule (SLAM)-associated protein (SAP), or diminished X-linked inhibitor of apoptosis (XIAP) protein expression (*SAP and XIAP only in male patients*)
- Diminished or absent CD107a degranulation assay (normal in FHL-2, XLP, and secondary HLH)
- In the absence of genetic information, diagnosis of HLH requires five of the following eight features[a]
  - Fluctuating fever
  - Hepatosplenomegaly
  - Bicytopenia, especially thrombocytopenia (hemoglobin <9 g/L, neutrophil $<1.0 \times 10^{e9}$/L, platelet $<100 \times 10^{e9}$/L)
  - Hyperferritinemia (>500 μg/L; although >3000 μg/L concerning and >10,000 μg/L higher sensitivity and specificity)[a]
  - Hypertriglyceridemia (≥3 SD fasting values) and/or hypofibrinogenemia (≤3 SD of normal for age)
  - Low or absent NK-cell function
  - Increased soluble CD25 (>2400 U/mL or >2 SD)
  - Hemophagocytic histiocytosis (bone marrow, liver, spleen, cerebrospinal fluid)

[a]See also Jordan et al: *Blood* 2011;118(15):4041–4052. When considering "familial" or genetic HLH without direct evidence of a genetic mutation, presence of malignancy or other known trigger must also be excluded. If clinical criteria are met, a provisional diagnosis of "familial (apparently mendelian) HLH" of unknown/uncertain origin can be made.

GENETIC FORMS OF HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS—PATHOLOGIC FEATURES

Gross Findings

Gross findings are nonspecific and are limited to hepatomegaly and splenomegaly

Microscopic Findings

- Hemophagocytic histiocytosis in bone marrow, spleen, lymph nodes, liver, cerebrospinal fluid
- Liver with features of chronic hepatitis, portal lymphohistiocytosis, and Kupffer cell activation with hemophagocytosis, noted in fHLH abnormalities of cytotoxic defects
- Early: Bone marrow possibly cellular and active in the face of cytopenias; Late: progressive depletion of hematopoiesis and increasing content of large phagocytic histiocytes
- Cerebral spinal fluid with pleocytosis (mononuclear cells and activated macrophages)

Fine-Needle Aspiration Biopsy Findings

- Histiocytosis with hemophagocytosis on bone marrow or spleen

Immunohistochemical Features

- Increased numbers of marrow histiocytes CD68 (PGM-1) and CD163
- Increased macrophages in spleen and liver CD68, CD163

Differential Diagnosis

- Secondary HLH will show similar pathologic features and cannot be distinguished by pathologic features alone. Viral infection, especially EBV, rheumatologic conditions (MAS-HLH), and T-cell malignancies, should always be excluded
- Hemophagocytosis and erythrophagocytosis in minor transfusion reactions

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lymphoproliferative syndrome (XLP) type 1 (XLP-1) mapped to Xq25, which is caused by a mutation in the *SAP/SH2D1A* gene, which is important in signal transduction and which results in hypogammaglobulinemia, with a heightened sensitivity for Epstein-Barr virus (EBV) infection and lymphoma.

A small but steadily increasing number of additional mutations not related to lymphocyte cytotoxic defects may also lead to primary HLH-like disorders causing hyperinflammation through an overstimulated, innate immune system and macrophage activation. These include two mutations with abnormalities of inflammasome overactivation (XLP2/*BIRC4* and *NLRC4*). Gain-of-function mutations discovered in the inflammasome gene, *NLRC4*, have also demonstrated HLH-like findings, including macrophage activation, with some showning an earlier (e.g., neonatal) onset including bowel inflammation. The inflammasome is a complex of innate immune system receptors that integrate danger recognition signals and caspase-1–induced inflammation to stimulate the release of proinflammatory cytokines IL-1β and IL-18. X-linked inhibitor of apoptosis protein/X-linked lymphoproliferative (XIAP/XLP-2), mapped to Xq25, results in mutations of the XLP2/*BIRC4* gene. This mutation has been known to be a genetic variant of HLH, often EBV driven, causing mild and recurrent HLH and colitis symptoms with splenomegaly and hypogammaglobulinemia in some.

Other genetic forms of HLH that result in mutations affecting inflammatory pathways include abnormalities in the lysinuric protein intolerance/*SLCA7* gene, detected in infants with elevated plasma ammonia after a protein-rich meal with failure to thrive and muscle hypotonia.

Whereas there are reports of HLH-like features in other primary immunodeficiencies, including Hermansky-Pudlak-2 syndrome (OMIM no. 608233) and AP3 complex/*AP3B1* gene, which results in congenital neutropenia and immunodeficiency, it is generally considered that these HLH-like syndromes are not true primary HLH because they are often triggered by EBV with B-cell lymphoproliferation.

Immunohistochemistry

The demonstration of an excess of activated macrophages is key to the recognition of the condition, but a definitive diagnosis requires genetic documentation and/or appropriate clinical/laboratory criteria. Increased and activated marrow macrophages in HLH are demonstrated using CD163 or anti-CD68 PGM-1 antibody that has little cross-reaction against hematopoietic precursors, allowing identification of hemophagocytosis. At all other sites, CD68 (KP-1 or PGM-1) and CD163 will demonstrate the macrophages effectively, including in the portal areas and sinusoids of the liver. In patients who have a perforin mutation, the CD3/CD8 cells do not stain for perforin by immunohistochemistry, if available (see Fig. 19.9).

Fine-Needle Aspiration Biopsy

Marrow is the most commonly examined aspirate, although splenic puncture is more commonly done in Europe. In both instances, the presence of great numbers of large cytoplasm-rich but bland macrophages that have hemophagocytosis, especially erythrophagocytosis, is typical during active episodes.

Differential Diagnosis

The most important differential diagnosis is distinguishing a genetic primary form of HLH from the secondary HLH syndromes that are treated by targeting the inciting condition: infection (e.g., viral, bacterial, fungal, parasite), rheumatologic condition (HLH-MAS), malignancy (hematologic, solid tumor), treatment related (chemotherapy, hyperalimentation with fat overload syndrome), or transplant related. An established molecular diagnosis, when available, is definitive for primary HLH, but some cases of secondary HLH may be related to partial impairments of known mutations in cytolytic function. This has made the distinction between primary and secondary HLH less definitive. Furthermore, the clinicopathologic features in individual instances are so variable, and infection can serve as the trigger for a crisis in the primary genetic forms of HLH, mimicking a secondary infection-associated HLH. It should also be noted that the most common cause of erythrophagocytosis is not hemophagocytic syndrome but a minor transfusion reaction.

Prognosis and Therapy

The genetic forms of HLH are rapidly fatal, with a mean survival of 2 months unless treated effectively, including bone marrow transplantation. The HLH-94 protocol (now HLH-2004), which is based on fHLH of cytotoxic defects, stabilizes patients using immunosuppression and chemotherapy (steroids, etoposide, and anti-T–cell agents) and follows that with bone marrow transplantation, the 5-year post-transplant survival probability is approximately 54% ± 6%. In genetic forms of HLH, the standard of care is hematopoietic stem cell transplantation, as it remains the only curative therapy in these patients. More recently, reduced intensity chemotherapy with alemtuzumab (a monoclonal antibody targeting CD56 on mature lymphocytes but sparing stem cells) is given 2 weeks prior to bone marrow transplant and has resulted in improved survival. Treatment of HLH-like conditions with mutations outside of classic lymphocyte cytotoxic defect group (i.e., inflammasome disorders) may require further evaluation of IL-1 and IL-18 blockage.

CYTOPHAGIC HISTIOCYTIC PANNICULITIS

Cytophagic histiocytic panniculitis appears to be a localized expression of HLH. Some cases of cytophagic histiocytic

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panniculitis are associated with instances of HLH, including a recent association with *STX11* (FHL4), but the local effect may predominate. Other instances appear to be examples of infection-associated secondary HLH, most commonly harboring EBV in the panniculitis, confirmed by in situ hybridization staining of EBV-encoded small RNA (EBER) probe and sometimes also associated with a clonal T-cell proliferation. However, both primary HLH and infection-associated HLH must be separated from subcutaneous panniculitis-like T-cell lymphoma (SPTCL) in which the lymphoma drives the histiocytic component and atypical lymphocytes encircle the fat vacuoles. Most are examples of the systemic SPTCL-αβ with clonal αβ rearranged T cells that are $CD3^+CD8^+$ cytotoxic cells. There is loss of CD2, CD5, and CD7 in up to 50% of cases.

CLASSIFICATION OF THE HISTIOCYTIC PROLIFERATIONS

Box 19.1 lists the most updated classification of these disorders by the Histiocyte Society and a more recent but limited classification by the World Health Organization. Of note, although the 2016 revised classification of histiocytosis and neoplasms of the macrophage-dendritic cell lineages includes HLH, as noted above, HLH is not a primary histiocytic disorder but rather a regulatory disorder leading to unbridled macrophage activation and hyperinflammatory cytokine upregulation.

LANGERHANS CELL HISTIOCYTOSIS

Langerhans cell histiocytosis is a disorder characterized by clonal proliferation of abnormal histiocytic cells that while having the phenotypic expression of Langerhans cells (LC) ($CD1a^+$/$Langerin^+$) share no common precursor with the similarly named epidermal cells. Gene expression profiling comparing LCH cells to epidermal LCs and immature myeloid DCs has suggested that LCH arises from myeloid dendritic cell precursors. LCH cells have high expression of markers associated with myeloid DC at various stages of differentiation (CD33, CD13, CD11c, CD11b, CD66c, CD200LF), and less epithelial marker expression (EpCAM, E-cadherin, and CD36) as compared to normal epidermal LCs. A bone marrow-derived myeloid/monocyte precursor has been implicated in LCH based on mouse models and human data by Berres and colleagues (2014). This has led the field in the Histiocyte Society to recognize LCH as an inflammatory myeloid-derived neoplasm with demonstration of *BRAF*-V600E mutations in bone marrow hematopoietic precursors and circulating blood myeloid/monocytes. LCH pathogenesis is now believed to be based on timing of the mitogen-activated protein kinase/extracellular signal-regulated kinases (MAPK/ERK) pathway activation in myelopoiesis, which may help explain the different clinical phenotypes in LCH (Fig. 19.10). More detailed reviews on the cell of origin, pathogenesis, and molecular aspects of LCH are beyond the scope of this chapter and available elsewhere.

Clinical Features

Langerhans cell histiocytosis affects children primarily, but there is an increasing incidence in adults. In children, the disease can be limited to a single site unifocal (e.g., bone, soft tissue, skin lesions) or involve multiple foci in a single system (multifocal bone or multiple lymph node lesions), or there may be multisystem (MS) disease that involves two or more organ systems. Involvement of some organs confers a higher risk of morbidity and mortality (MS-LCH high risk); these are the liver, bone marrow, and spleen. In children, pulmonary disease occurs in 25% of cases with multisystem LCH; however, it is no longer considered an independent risk organ. Penetrating skull lesions of the craniofacial bones and temporal vault carry an increased risk of future CNS sequelae, including diabetes insipidus (e.g., "CNS-risk" sites). Mortality in these higher risk children with multisystem disease, often younger than 2 years, has improved, with 3- and 5-year survival of 84% following the LCH III protocol, with therapy intensification after 6 weeks if active disease persists, along with prolonged therapy of 1 year rather than 6 months.

In adults, the disease often affects the lungs of smokers, although bone and skin lesions occur. Common but nonspecific B-type symptoms can include fever, weight loss, and fatigue. The specific clinical manifestations depend on the site of involvement. Bone involvement, monoostotic or polyostotic, is the most frequent presentation; it is commonly associated with pain, pathologic fractures, vertebral collapse (e.g., vertebra plana), and local soft-tissue extension. Skin involvement often involves flexures and the scalp with a seborrheic rash that may be petechial. Newborns have more papular lesions that commonly regress. Liver disease may present with abnormal liver function tests (e.g., gGTP, bilirubin), hepatomegaly, or jaundice. Clinically a biopsy may not be necessary to prove liver involvement if these features are present in the setting of known LCH disease. The liver involvement is typified by infiltration of the major bile ducts that causes a sclerosing cholangitis and biliary strictures and ends in a biliary cirrhosis with eventual need for liver transplantation. The lungs are more commonly involved as part of multifocal disease in children but may often be the only site in smoking adults. Interestingly, some children who have recovered from early childhood disease have developed pulmonary LCH when they began smoking as adolescents. Pulmonary involvement may lead to dry cough, dyspnea, pneumothorax, interstitial and peribronchial fibrosis, and respiratory compromise. CNS involvement by active LCH involves the hypothalamic-posterior pituitary axis, causing irreversible diabetes insipidus, or growth retardation, and

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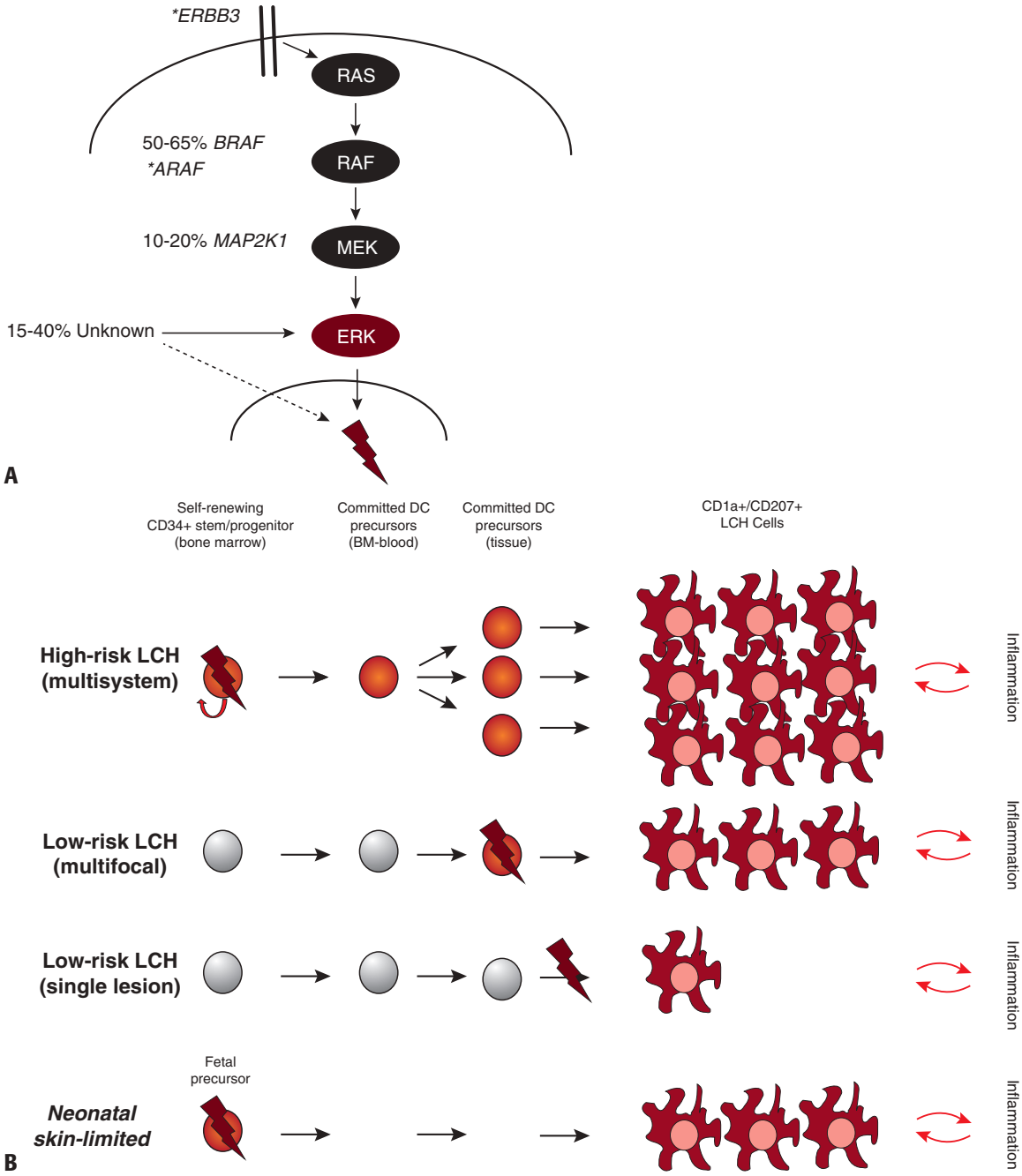


FIG. 19.10

Model of Langerhans cell histiocytosis (LCH) pathogenesis. Clinical phenotype of LCH may be related to timing of ERK activation during specific stage of myelopoiesis. A, Constitutive activation of MAPK pathway is implicated in LCH lesions with the majority of documented alterations found in *BRAF*, including point mutations and fusions. PIK3CA pathways (not shown) also implicated in LCH pathogenesis (* indicates case reports). B, Proposed misguided myeloid dendritic cells (DC) model of LCH pathogenesis in which somatic mutations *(lightning bolt)* and subsequent ERK activation *(red cell)* at specific stages of DC development determine clinical outcome. The hypothesized model is adapted from human and mouse studies (Berres ML, et al. 2014), leaving room for future refinement. In this model, activating mutations in hematopoietic stem cells or bone marrow progenitor cells result in multisystem high-risk disease; activating mutations in tissue-restricted precursors result in multifocal low-risk disease, and activating mutations in more differentiated tissue-restricted cells result in single lesion disease. *(Figure used with permission of Collin M, Bigley V, McClain KL, Allen CE: Cell(s) of origin of Langerhans cell histiocytosis,* Hematol Oncol Clin North Am *29(5):828, 2015.)*

it can involve the choroid plexus and meninges. Late CNS involvement, years later, has historically been described as indirect without active LCH; however, newer data may be challenging that notion with the possibility of a BRAF mutant precursor cell driving the late demyelinating progressive and debilitating neurodegenerative disease of LCH (ND-LCH). Clinicoradiographically ND-LCH is characterized by bilateral, usually cerebellar demyelinating foci resulting in progressive ataxia, dysarthria, nystagmus, hyperreflexia, dysdiadochokinesia, dysphagia, and blurred

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vision. Hematopoietic involvement is seen with bone marrow and spleen infiltration, but the cytopenias that are common are functional (possibly cytokine mediated) because the hematopoietic marrow is preserved in many sampled cases. Lymph nodes and the gastrointestinal tract can be involved, resulting in lymphadenopathy and sometimes life-threatening malabsorption, anemia, bloody diarrhea, and protein-losing enteropathy, whereas it may be asymptomatic or present as a polyp-type lesion in adults. The kidney and gonads are almost never affected. Because of the widespread and protean possibilities, LCH when first diagnosed at any site is evaluated by staging, a clinical survey that maps the extent of involvement. Referral centers also advocate mutational studies in the MAPK/ERK pathway, including sensitive polymerase chain reaction (PCR) *BRAF* studies on the lesion and, if *BRAF*-V600E mutation positive, upfront testing of peripheral blood for circulating *BRAF*-mutated precursor cells to help follow disease progression.

Radiologic Features

Bone and Adjacent Soft Tissue

Once a diagnosis is established, an age-appropriate survey of the skeleton to look for other lesions is often performed. More sensitive modalities such as whole-body fludeoxyglucose-positron emission tomography (FDG-PET) scans can localize new lesions and are more informative of disease activity. In the early stages, expanding new bone lesions may have a rapidly growing, lytic appearance with poorly defined margins that mimic malignant disease. The presence of pathologic fractures can confound the picture. Older and involuting lesions have an osteolytic center and sclerotic, sharply defined borders, and the differential diagnosis includes low-grade lesions such as chronic osteomyelitis. Cranial bone involvement with adjacent soft-tissue disease is characteristic (Fig. 19.11). Computed tomography (CT) or magnetic resonance

LANGERHANS CELL HISTIOCYTOSIS—FACT SHEET

Definition
- LCH is a heterogenous group of disorders, which is now considered by the Histiocyte Society as a myeloid inflammatory neoplasia with accumulation of bone marrow–derived Langerhans cell histiocytosis (LCH cells)
- Multisystem (MS-LCH): Two or more organs involved (e.g., bone and skin)
- Multifocal LCH: Multiple sites within one organ system (e.g., multiple bone lesion)
- Single system (SS-LCH): Single site (e.g., bone)

Incidence and Location
- 0.54 to 0.9 per 100,000 children (Denmark, Sweden) and unknown in adults; probably underestimates the true incidence since many are not reported
- Rare familial cases
- Bone and soft tissue (most common), skin, lymph node, liver, bone marrow; lung in smoking adults

Morbidity and Mortality
- Highest mortality in those younger than 2 years (30% to 50%) who have "risk organ" involvement (e.g., liver, marrow, spleen), but treatment according to LCH III protocol has shown improved survival
- High risk of diabetes insipidus and neurodegenerative sequelae with "CNS risk" involvement (e.g., craniofacial bones, temporal skull bone)
- Morbidity high for multiple bone lesions and adult multisystem or lung disease (91% 5-year survival)
- Unifocal bone disease and some cases of isolated congenital skin lesions (*BRAF*-V600E wild type) usually self-limiting if staging reveals no other involvement

Gender, Race, and Age Distribution
- Variable male predominance 1–2 : 1 in children, 1.4 : 1.2 in adults
- Age range: from neonates to adults
- Neonatal skin-only papular disease, may be self-limited ("Hashimoto-Pritzker disease")
- Systemic disease (MS-LCH), <2 years, often with risk organ involvement ("Letterer-Siwe disease")
- Multiple bone, soft tissue disease (MS-LCH), 2 to 10 years ("Hand-Schüller-Christian disease")
- Solitary bone or soft tissue disease (SS-LCH), 5 to 15 years ("eosinophilic granuloma")
- Adult disease, mean age 33 years (SD = 15 years), 30% single system, 70% multisystem

Clinical Features
- Symptoms vary according to site and organs involved
- Skeletal pain, soft tissue mass in bone lesions
- Extraosseous manifestations: diabetes insipidus, seborrheic skin rash, pulmonary abnormalities, lymphadenopathy, hepatosplenomegaly, and pancytopenia

Radiologic Features
- Osteolytic intramedullary bone lesions, poorly demarcated in early and active lesions
- Osteolytic intramedullary bone lesions, well demarcated by sclerotic rim in older and regressing lesions
- Peribronchial and interstitial lung changes progressing to honeycombing in some
- Loss of normal pituitary signal and thickening of the pituitary stalk
- Variable symmetric magnetic resonance imaging (MRI) signal intensity changes of the cerebellum, basal ganglia, and/or pons along with dilated Virchow-Robin spaces in neurodegenerative LCH disease

Prognosis and Treatment
- Localized skin and bone lesions can be observed or resected, treated intralesionally or topically with steroids, or given low-dose radiation to bone
- Multisystem LCH given systemic chemotherapy according to LCH III protocol
- Recurrent or refractory disease; 2 chlorodeoxyadenosine (2CDA) or bone marrow transplantation; newer studies investigating clofarabine and BRAF inhibitor therapy
- Adult lung disease: cessation of smoking, corticosteroids

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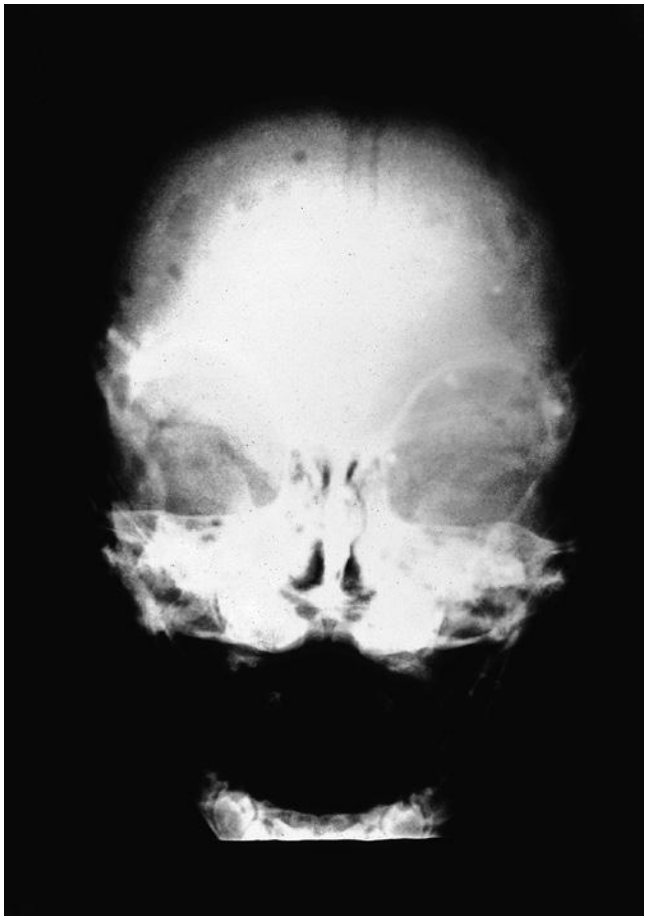


FIG. 19.11

Langerhans cell histiocytosis. A radiograph of the head of a 4-month-old child who had widespread active diseases shows the presence of bilateral punched-out parietal lesions in the calvarium. The sclerotic rims, not seen here, are more characteristic of chronic or long-standing disease.

imaging (MRI) may help to ascertain the extent and character of the bone lesion before biopsy. Early lung involvement is best demonstrated by high-resolution CT that reveals a delicate interstitial and cystic change that is bronchocentric and spares the intervening lung. Late disease is nodular, fibrotic, scarring, and honeycombing on imaging studies. Pleural bullae are responsible for the bouts of pneumothorax. Imaging of the liver may demonstrate the features of a sclerosing cholangitis. Central nervous system disease in the early infiltrative phase is characterized by posterior pituitary stalk involvement, and there can be space-occupying choroid or meningeal masses. Late disease is seen best by MRI that reveals the symmetric neurodegenerative foci of the cerebellum and basal ganglia.

Pathologic Features

Gross Findings

Bone lesions may be sharply demarcated or gray to yellow depending on lipid content. Lung and thymic lesions if rarely

LANGERHANS CELL HISTIOCYTOSIS—PATHOLOGIC FEATURES

Gross Findings
- Bone lesions may be sharply demarcated or gray to yellow, depending on lipid content
- Lung and thymic lesions may be cystic

Microscopic Findings
- Cells are oval and measure 15 to 25 μm, usually nested in sheets or clusters
- Nuclei are oval or grooved or have complex foldings
- Nucleoli are inconspicuous
- Cytoplasm is pale
- Binucleated and multinucleated forms are common, with osteoclast-like cells in bone and contiguous soft-tissue lesions
- Mitoses are variable, never atypical; necrosis may be present
- Eosinophils are variable and may be absent to overwhelming, with microabscesses filled with Charcot-Leyden crystals
- Plasma cells are rare within in the histiocytic aggregates

Immunohistochemical Features
- Oval histiocytes with CD1a/Langerin and high sensitivity and specificity
- S100 nuclear/cytoplasmic, sensitive not specific; vimentin
- CD68 and HLA-DR-low, paranuclear
- VE1 dark cytoplasmic granular staining in *BRAF*-V600E mutated cases (~50% of cases)

Molecular Testing
- Mutations in *BRAF*-V600E (50-64%), *MAP2K1* (10% to 20%), case reports of *BRAF* in frame deletions, *ERBB3, RAS, ARAF*, and *PIK3CA-AKT,* with about 15% of cases still unknown
- *BRAF* gene fusions now being discovered
- *BRAF*-V600E detection should include sensitive molecular methods for accurate detection, especially given the variable low content of histiocytes in some cases

Ultrastructural Features
- The LCH cells have sparse organelles with Birbeck granules required for ultrastructural confirmation; however, this has been replaced by Langerin (CD207) immunohistochemistry
- Classic dendritic cells and intervening macrophages

Fine-Needle Aspiration Biopsy Findings
- Bone, lymph node, thyroid, and hypercellular lesions with histiocytes having moderately abundant pale cytoplasm and a grooved or folded nucleus
- Eosinophils possibly not obvious

Differential Diagnosis
- Bone osteomyelitis, especially chronic recurrent culture-negative
- Soft-tissue, inflammatory, and granulomatous processes
- Hodgkin lymphoma with eosinophils
- Foci of $CD1a^+$ DC hyperplasia can occur in lymph nodes containing lymphoma, simulating LCH, but extranodal disease never seen in these instances

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resected may be cystic, but there are virtually no early or late changes in any of the organs that will be suggestive of LCH.

Microscopic Findings

Diagnosis of LCH is made by identifying the lesional cells as LCH cells with the Langerhans cell phenotype and the correct pattern of tissue involvement. The cells are moderately large (20 to 25 μm), oval, and not dendritic in shape, with a grooved (coffee bean) or complex folded nuclear profile. Nuclei are commonly single or two to three per cell, and there are osteoclast-type multinucleated cells in many sites that may harbor the folded LCH nucleus (Fig. 19.12), which are phenotypically different from the intermixed CD1a–/Langerin-/CD68^{+} osteoclast giant cells resorbing the bone. Cytoplasm is abundant and pale and may have few fine granules. The gold standard for the diagnosis remains the phenotypic confirmation of cells that look cytologically appropriate for LCH. In some sites, most notably bone, eosinophils can be interspersed often in large numbers, albeit unevenly. Although the lesions were formerly called *eosinophilic granulomas,* eosinophils are not required for the diagnosis, which rests on identifying the LCH cell. In bone and soft tissue, early lesions contain sheets of LCH cells with inflammatory cells, mostly T cells largely restricted to the periphery. Phagocytic macrophages and osteoclast-type giant cells can be present or even dominate the picture. The LCH cells disappear as lesions regress, and it is possible to biopsy a late, healing lesion from which the LCH cells have disappeared and therefore be unable to confirm the diagnosis. In the skin, the process is usually epidermotropic, hugging the epidermis and filling the papillary dermis. The cells are characteristically large and oval. Lymph node involvement is strictly sinus in distribution at first, spilling over into the paracortex and thus following the normal migratory pathway for sentinel DCs reaching the lymph node (Fig. 19.13). Liver involvement is almost uniquely biliary, with LCH cells in the epithelium of large-caliber bile ducts where they cause a sclerosing cholangitis (Fig. 19.14). The cells can migrate peripherally within the biliary tree, usually between the basement membrane and the biliary epithelial cells. When there is extensive involvement, parenchymal nodules can occur. Lung involvement is peribronchial with extension of LCH cells into the peribronchial alveolar walls. Bone marrow involvement is often subtle; first because there may only be a few LCH cells in small clusters and second because there may be a reactive macrophage histiocytosis that obscures the

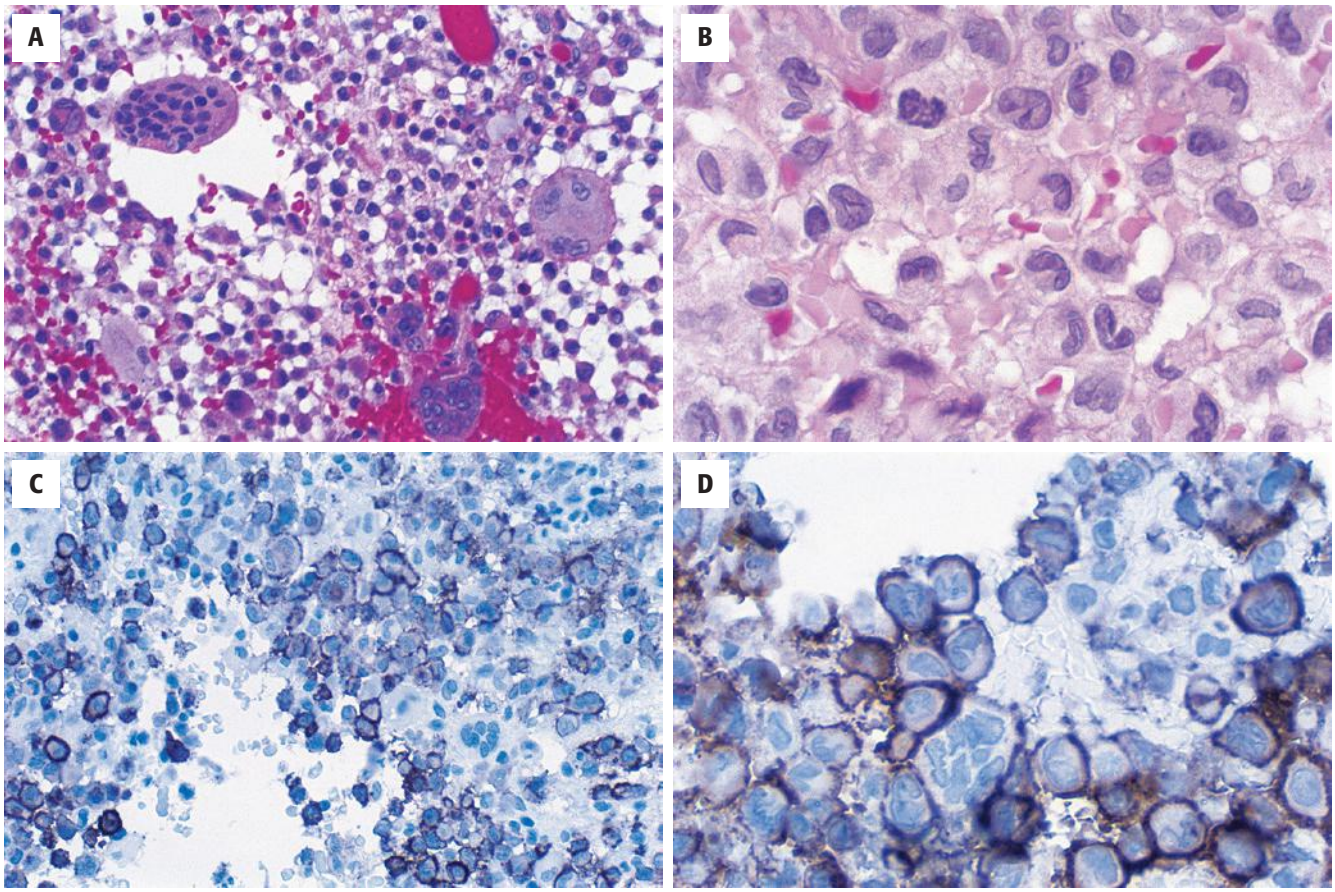


FIG. 19.12

Langerhans cell histiocytosis (LCH): bone. This lesion has few eosinophils, but osteoclast-type giant cells are present. A, The LCH cells predominate. B and C, Strong membrane staining for CD1a is demonstrable. D, Mononuclear and multinucleated LCH cells have CD1a staining, and the angular nuclear profile is typical. Langerin stains the LCH cells.

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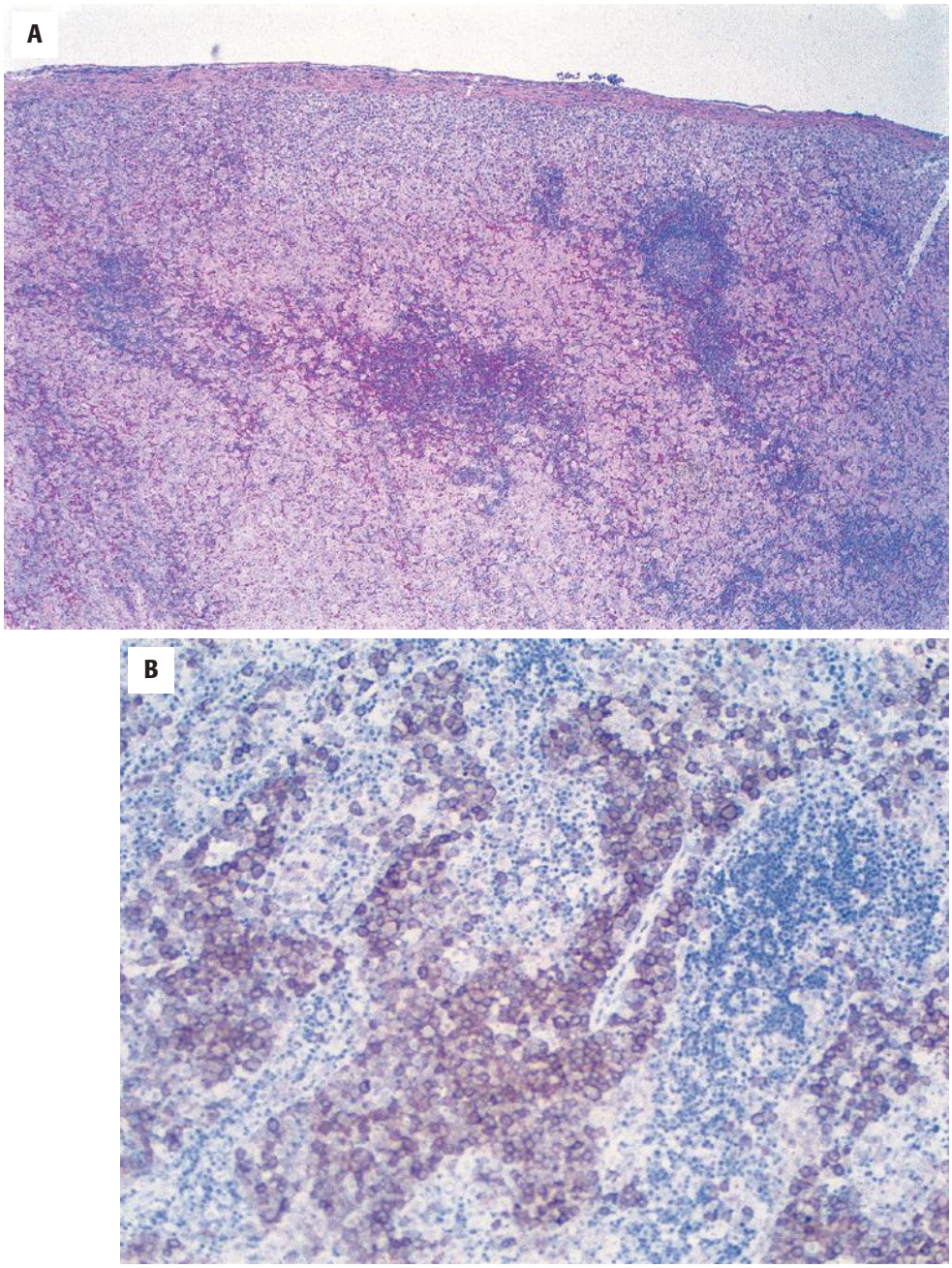


FIG. 19.13

Langerhans cell histiocytosis (LCH): lymph node. A, The lymph node is replaced by sheets of LCH cells, and no particular distribution can be discerned. Few residual follicles remain. B, CD1a immunostain reveals that in the lymph node, sinus LCH has strong CD1a presence, whereas the paracortical cells have little to no CD1a expression but enhanced HLA-DR surface pattern, suggesting limited maturation.

picture. In these cases demonstration of *BRAF*-mutated cells by PCR studies (e.g., aspirate) would further support the diagnosis, especially if the patient has a known *BRAF*-mutated lesion. Thymic involvement, although rare, shows discrete morphologic patterns including solitary and/or cystic LCH lesions with thymic gland disruption; more variable medullary or diffuse thymic disease in multisystemic LCH; and rarely, a mixed histiocytic lesion with a concurrent LCH and juvenile xanthogranuloma-like proliferations. Posterior pituitary, leptomeningeal, and choroid involvement has the characteristic large, oval LCH cells. CNS-LCH lesions have been described based on small case series that include (1) circumscribed granulomatous lesions including variable numbers of $CD1a^+$ cells with a robust surrounding xanthogranulomatous or macrophage-rich inflammatory response (most commonly in circumventricular sites, leptomeninges, and the pituitary); (2) $CD1a^+$ cell–rich lesions in infundibular sites extending to the hypothalamus and adjacent CNS parenchyma with variable inflammation and parenchymal injury including demyelination and gliosis; and (3) CD1a cell poor wherein lesions, most often in the cerebellum, are associated with ill-defined leukoencephalopathy and neurodegenerative features with a fibrotic/gliotic response. Few neurodegenerative (ND-LCH) cases have undergone pathologic investigation. Current work is in progress to better delineate the pathophysiology of late ND-LCH disease. Splenic involvement can also be difficult to define except in those patients who form LCH nodules in the spleen. Gastrointestinal involvement

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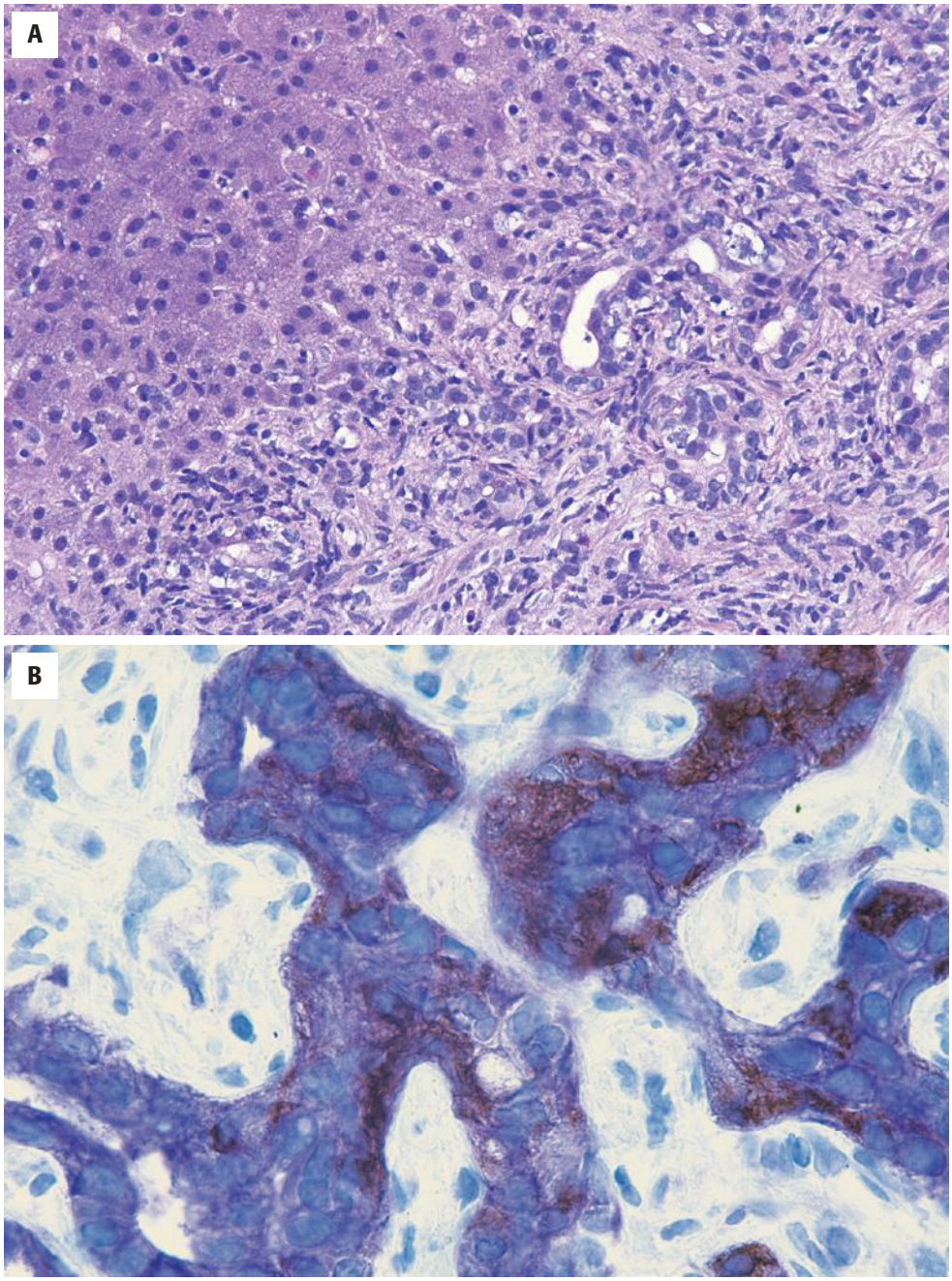


FIG. 19.14

Langerhans cell histiocytosis (LCH): liver. A, The liver has biliary obstructive features with prominent bile duct dilatation and ductular proliferation, but no infiltrate is detected. B, Double stain for epithelial cells (cytokeratins AE1/AE3, *blue*) and CD1a *(brown)* show the subtle presence of LCH cells percolating between the epithelial cells and the basement membrane.

fills the lamina propria and can be subtle until one recognizes that the usual cell mix is replaced by a single population of LCH cells. Thyroid and thymus can be involved with sheets of oval LCH cells that have the diagnostic phenotype.

Ancillary Studies

Immunohistochemistry

The cells of LCH have a stable phenotype: CD1a$^+$, Langerin$^+$, S100$^+$, and vimentin$^+$. They have small, paranuclear intracytoplasmic accumulations of CD68 and HLA-DR. In sites such as the paracortex of lymph nodes, the LCH cells can show some evidence of limited maturation in which CD1a is focally lost and membrane HLA-DR expression is increased. The VE1 antibody will show a dark, cytoplasmic granular staining pattern in the clonal histiocytes with the *BRAF*-V600E mutation (see Fig. 19.17B).

Molecular and Cytogenic Features

In the past (early 2000s), loss of heterozygosity (LOH) of possible tumor-suppressor genes (e.g., 9p and 22q adult/pulmonary and 1p and 7 in children/bone) was the only

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described molecular aberration in LCH. However, since the last edition, a molecular transformation has commenced in LCH starting with the identification of *BRAF*-V600E in 2010. Since that time, advances in genetic techniques have led to new findings and better understanding of the molecular changes in LCH. Frequency of *BRAF*-V600E mutations has been described in 50% to 64% of adult and pediatric patients with LCH in several studies, highly dependent of the method of testing. Highly sensitive qPCR methods are recommended in order to detect small allelic fractions (e.g., down to ~1% mutant), which are common in LCH, as cells are often diluted with a mixed inflammatory milieu. Activated *BRAF* stimulates constitutive transcription and cellular proliferation through the *RAS/RAF/MEK/ERK* pathway. Case reports of *BRAF*-V600E wild-type LCH have also shown *MAP2K1* (10% to 20%) and fewer reports of *ERBB3*, *RAS*, *ARAF,* and *MAP3K1* activating mutations that also work on the same signaling pathway with universal ERK pathway activation (see Fig. 19.10). New reports of *BRAF* exon 12 in-frame deletions and *FAM73A-BRAF* gene fusions have been issued. Also point mutations in the phosphoinositide 3-kinases-Akt murine thymoma (*PIK3CA-AKT*) pathway has also been implicated in LCH in rare cases. In particular, *BRAF* mutations have clinical significance as they are therapeutic targets and appear to drive the varied pathogenesis of LCH. A bone marrow–derived myeloid/monocyte precursor has been implicated in LCH based on mouse models and human data. Although the identification of *BRAF*-V600E mutations have been noted in both single lesion (e.g., low-risk) and multisystem (e.g., high-risk) LCH, the landmark study by Berres and colleagues in 2014 showed for the first time that MS-LCH also harbored the *BRAF*-V600E mutation in $CD34^+$ bone marrow hematopoietic precursors and $CD11c^+$/$CD14^+$ circulating blood myeloid/monocyte fractions, unlike low-risk LCH. The human data was further supported by mouse models showing differential LCH-like 'high risk' and 'low risk' phenotypes dependent on the enforced expression of *BRAF*-V600E, driven either by CD11c-Cre (high-risk phenotype) or Langerin-Cre promotors (low-risk phenotype). These findings have provided initial data for a model of LCH pathogenesis. The timing of MAPK/ERK activation in myelopoiesis may help explain the clinically distinct LCH risk groups (see Fig. 19.10) and support a new classification of LCH as a *myeloproliferative neoplasm* or rather an *inflammatory myeloid neoplasia*, given the likely contribution of the inflammatory milieu to its pathogenesis. For the first time, a recent large French registry study has correlated *BRAF*-V600E mutations with high-risk LCH and poor short-term response to first-line chemotherapy. Although the Histiocyte Society has not officially recommended upfront molecular testing for all LCH, still under the prevue of "experimental" testing, data are accumulating in support of mutational testing. Furthermore, if *BRAF*-V600E positive, reflex qPCR *BRAF*-V600E on blood or bone marrow is also recommended to help follow disease course when applicable.

Ultrastructure Features

The Birbeck granule was originally identified in Langerhans cells of the skin, then in adult pulmonary LCH, and later in cutaneous and bone lesions of childhood. The structure forms where the C-type lectin Langerin (CD207) accumulates and the Birbeck granule appears to be related to endosomal trafficking. The use of Langerin immunostain has largely supplanted the need for ultrastructural analysis. The Birbeck granule is a rod-shaped bilaminar disk with an internal zipper-like pattern of striations, often with a bulbous dilatation at one end, like a tennis racquet. The granules are found wherever Langerhans cells are seen in the body but are not unique to Langerhans cells and have been described in other cells (Fig. 19.15). LCH cells have few cytoplasmic organelles and filopodia at the cell surface.

Fine-Needle Aspiration

Lesions that are aspirated are commonly mixed in nature, and the LCH cell must be identified. Presumptive diagnosis rests on the cytologic features of large, bland, oval histiocytes that are nonphagocytic and have pale cytoplasm and folded nuclei. Immunocytology for CD1a or Langerin is definitive but must be interpreted in the correct clinicopathology setting (Fig. 19.16). S100 is soluble and does not survive without fixation. Pulmonary involvement can be inferred when the imaging is compatible and more than 12% of the larger bronchoalveolar cells are $CD1a^+$ or display Langerin.

Differential Diagnosis

The differential diagnosis depends on the site of involvement. Skin lesions of mastocytosis (urticaria pigmentosa), early type juvenile xanthogranuloma, and melanocytic nevi ($S100^+$) can simulate LCH. An increase in perivascular $CD1a^+$ Langerhans cells that are spindled or dendritic in shape is a feature of many chronic dermatoses, most notably chronic scabies. The thymus and thyroid can contain microscopic collections of hyperplastic LCH-like cells in thymectomies and thyroidectomies of patients without LCH disease. Skin infiltrates with follicular hyperplasia (i.e., cutaneous pseudolymphoma) may also harbor small hyperplastic LCH-like collections without LCH disease.

Applying immunohistochemistry to a reactive lymph node with dermatopathic-like changes will reveal an increased number of dendritic cells in the paracortex that can form large, confluent, and nodular aggregates of T cells and DCs, including Langerhans cells. Because the DCs are maturing or fully mature, they will have the phenotype of interdigitating DCs ($S100^+$, fascin-hi) with interspersed Langerhans cells ($CD1a^+$, $Langerin^+$, $S100^+$) in the paracortex. In some cases the Langerhans cells may be exuberant and cluster but should not be diagnosed as LCH, which is a sinus disease (see

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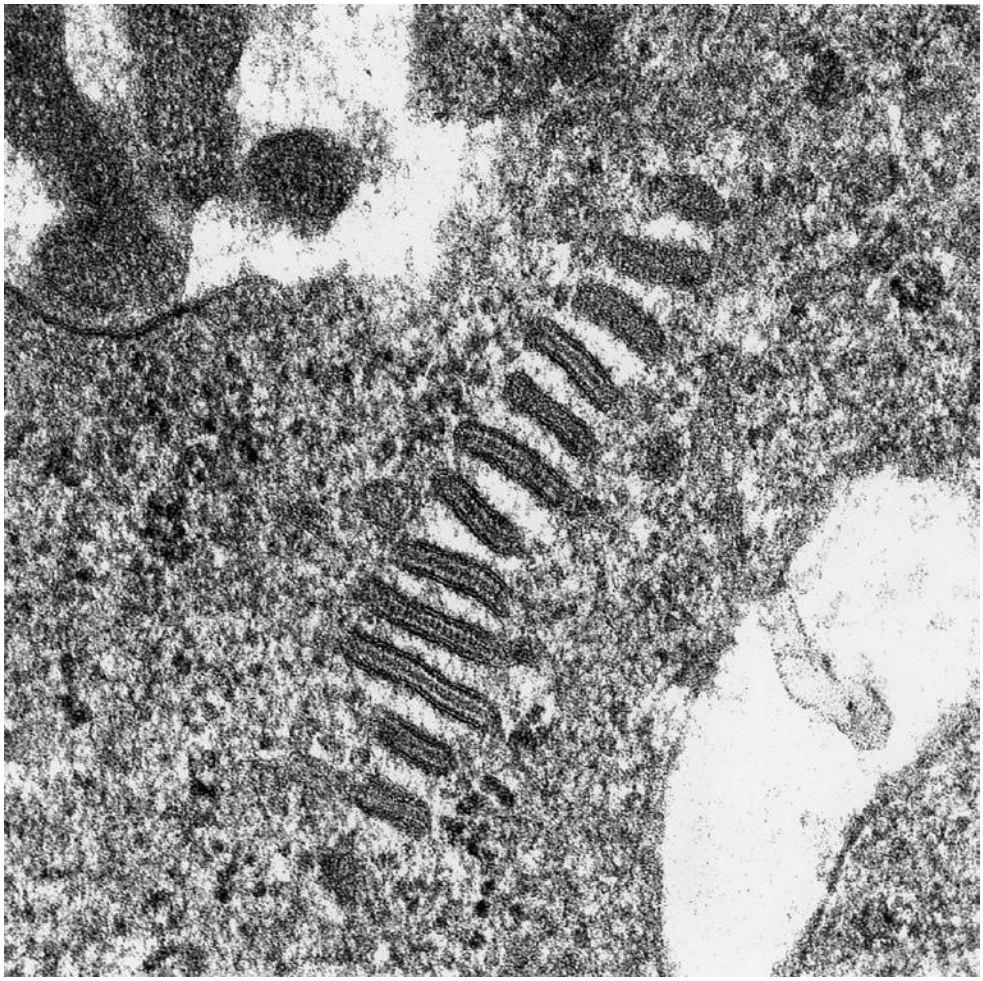


FIG. 19.15

Langerhans cell histiocytosis (LCH): Birbeck granules. Electron microscopic examination of an LCH lesion reveals an unusual palisade of Birbeck granules with the laminar membranes and the central zipper (original magnification, ×135,000).

Fig. 19.3). A diffuse paracortical DC hyperplasia in tonsils or lymph nodes can be an accompaniment of other infiltrating processes and can be misleading by diverting attention from the infiltrate, usually a leukemia or lymphoproliferative disorder.

Bone lesions are most likely to be confused with osteomyelitis, especially chronic recurrent multifocal culture-negative disease. LCH has few plasma cells in between the lesional LCH cells but may have more in the surrounding inflammatory reaction. Hodgkin lymphoma can contain eosinophils and scattered $CD1a^+$ cells, and Rosai-Dorfman disease can also produce bone lesions. In late and healing lesions where LCH cells are few, fibrotic or fibrohistiocytic lesions enter the differential diagnosis. In these cases, demonstration of mutant *BRAF* can support LCH involvement, but its absence cannot exclude it.

Lung lesions that are fibrotic may have few LCH cells and can resemble fibrosing interstitial diseases with honeycombing in the most severe instances. Alveolar macrophages can simulate LCH cells but are not $CD1a^+$; however, smokers may have increased numbers of LCs without LCH. Spontaneous pneumothorax can be associated with an eosinophilic pleural reaction.

Bone marrow involvement by LCH is obscured by the common finding of a marrow macrophage histiocytosis. Only $CD1a^+$/$Langerin^+$ large cells can be considered informative, but PCR (on aspirates) for *BRAF*-V600E has identified a mutation in the absence of CD1/Langerin staining, and this more sensitive molecular technique should supersede immunohistochemistry in such instances. S100 can be confounding in the bone marrow by staining stromal cells, fat cells, and some T cells, and activated macrophages.

Liver involvement is simulated by macrophage activation in children with widespread disease who may have hepatomegaly and hypoalbuminemia. An identifiable peribiliary infiltrate, $CD1a^+$/$Langerin^+$, is diagnostic, but biliary obstructive changes on a biopsy specimen are presumptive evidence of LCH involvement downstream and may not be demonstrated in a biopsy. Juvenile xanthogranuloma lesions also have a portal distribution but have a different phenotype and spare the bile ducts.

Pituitary lesions must be distinguished from germ cell tumors. The late demyelinating CNS lesions do not have a typical LCH cell presence. Choroid plexus and dural lesions of LCH acquire large numbers of xanthoma cells and can simulate reactive histiocytosis or even juvenile xanthogranuloma.

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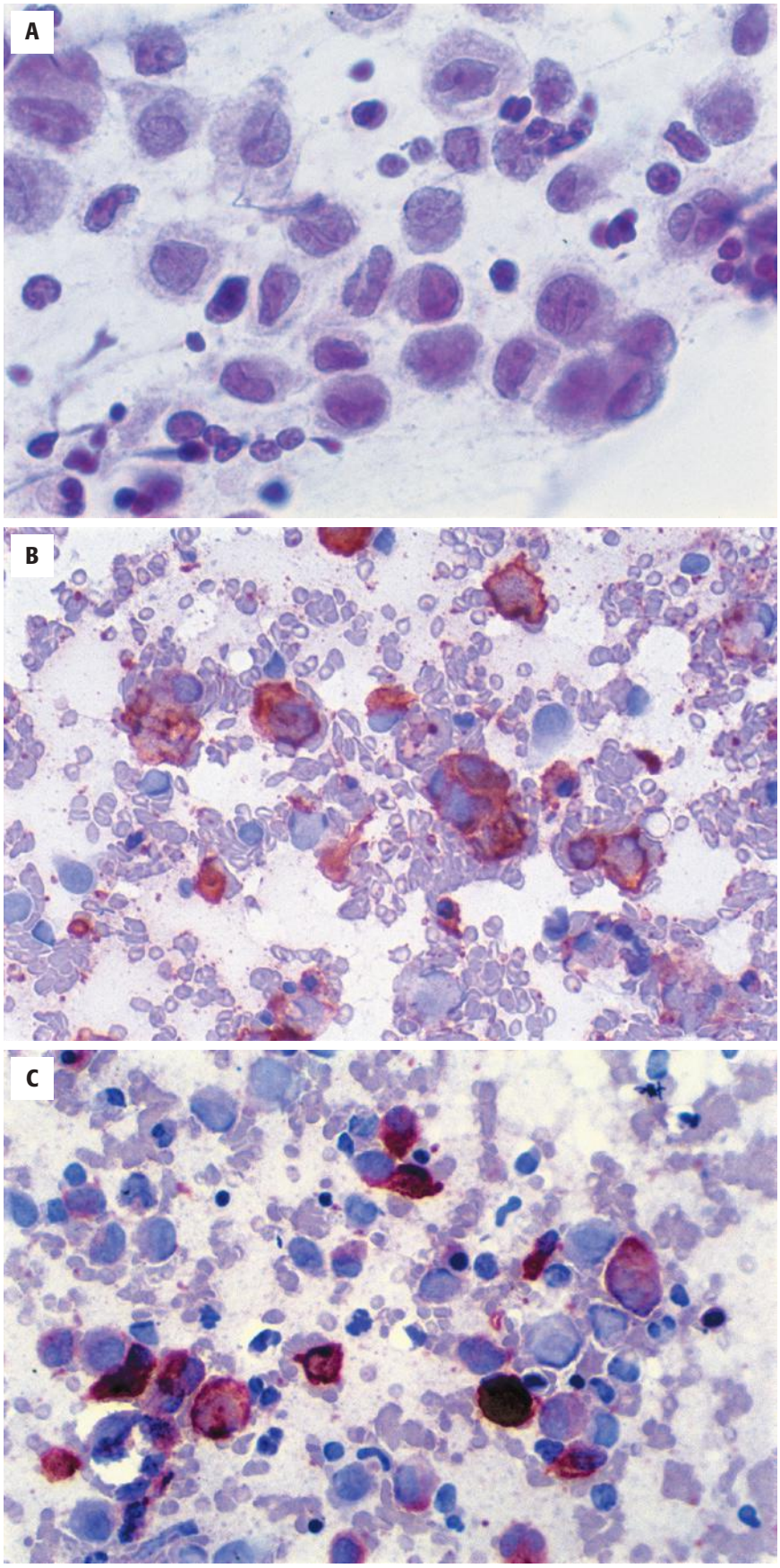


FIG. 19.16

Langerhans cell histiocytosis: aspirate. Aspiration of a temporal soft-tissue mass has a mixed cell population. A, Shown are the angular nuclear profiles with the complex grooving pattern. Immunostains for CD1a (B) and Langerin (C) confirm the diagnosis.

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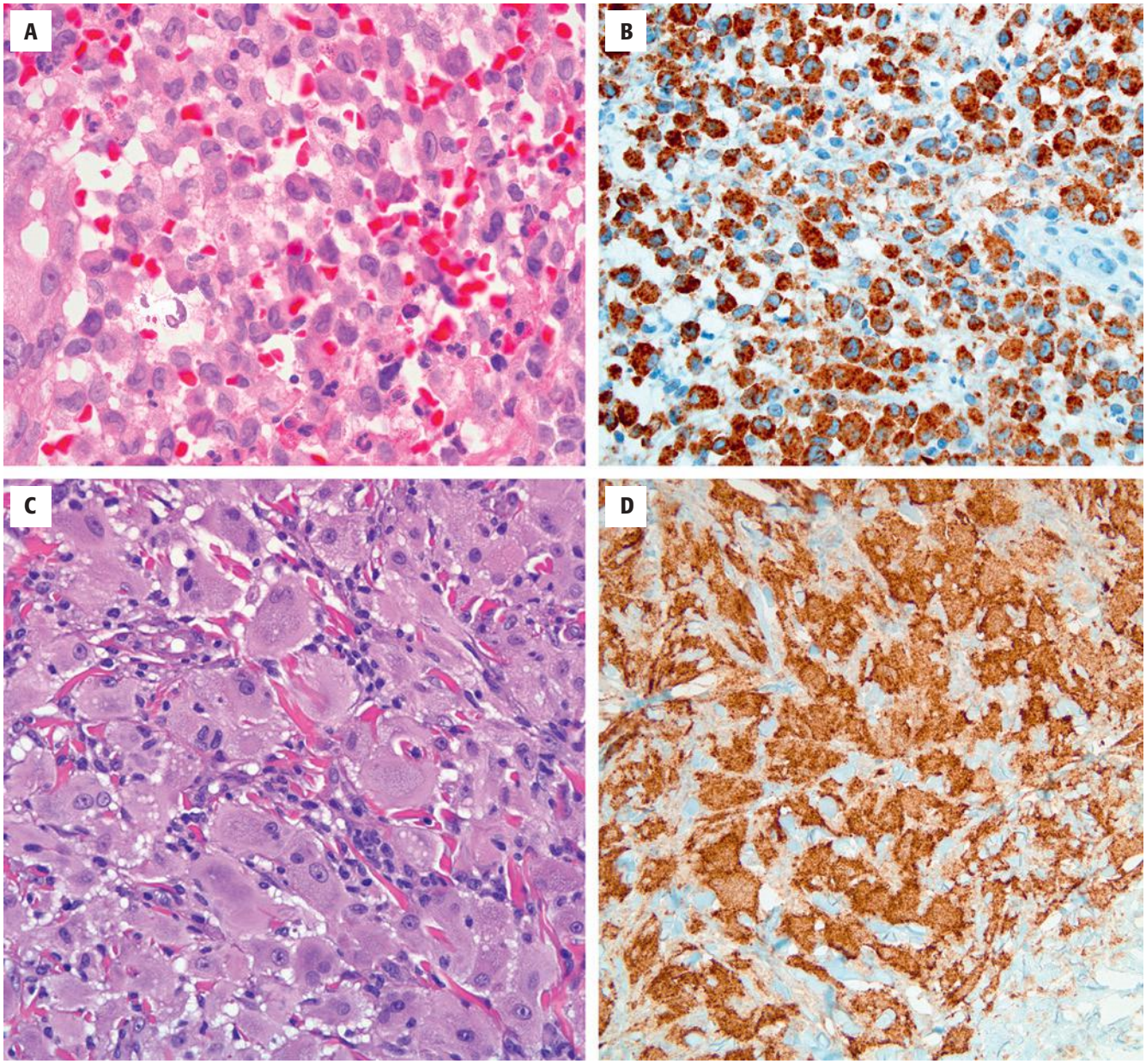


FIG. 19.17

Cutaneous Langerhans cell histiocytosis and subsequent Erdheim-Chester disease at different locations in the same patient. A, The LCH infiltrate was present first with typical morphology and phenotype, H&E. B, VE1 antibody showed strong granular cytoplasmic staining. C, One year later another skin lesion was biopsied showing features of reticulohistiocytoma subtype of xanthogranuloma family lesion with CD163, CD68, and factor 13a staining (not shown). D, The oncocytic cells stained strongly for VE1 immunostain, which was correlated with *BRAF*-V600E mutation.

Prognosis and Therapy

The prognosis depends on the site and extent of disease, with the high-risk organ sites having a poorer outcome. Diabetes insipidus (DI) is often permanent. The greater the number of organ systems involved in small children, the worse the outcome, with the highest mortality for those with high-risk organ involvement (e.g., bone marrow, liver, spleen) and for those who have disease progression during the first 12 weeks of therapy. High risk of long-term CNS effects (i.e., DI, endocrinopathies, ND-LCH) is associated with certain "CNS-risk sites" including the temporal bone, maxillofacial bones, and orbital bone involvement. Poor initial response to chemotherapy in the systemic form also portends a poor outcome and was the impetus for therapy intensification in LCH III protocol after 6 weeks.

Adults with smoking-related lung disease have variable outcomes; about half have disease regression with smoking cessation and with steroids, but others require chemotherapy or progress. Monoostotic bone lesions often regress no matter what modality is used but can leave impairment at the site (e.g., vertebral collapse). From a management standpoint, the pathologist should realize that excising an LCH lesion

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with "negative" margins is not required, and attempts to do so may result in more extensive surgery than necessary. Indomethacin or intralesional steroids are used with polyostotic bone lesions and low-dose radiation or chemotherapy with vinblastine-prednisone have been used. In the LCH-III study 66% of patients with risk organ involvement and 86% of patients without risk organ involvement demonstrated a response to corticosteroids and vinblastine by 6 weeks. Therapy intensification (addition of etoposide or mercaptopurine) was added for nonresponders and high-risk MS-LCH in the initial 12 weeks with improved outcomes. Second-line, salvage therapy includes immune suppression with cyclosporine, low-dose cladribine, intermediate-dose cytosine-arabinoside (cytarabine), or clofarabine (purine analogue used in acute myeloid leukemia), which has been used with variable success. Persistent high-risk MS LCH despite various salvage treatments may be cured with bone marrow hematopoietic stem cell transplantation. Lung or liver transplantation in some instances has been used in refractory disease. For the first time, *BRAF*-V600E has also been suggested as a prognostic marker, correlating with high-risk LCH and increased resistance to first-line therapy in a French LCH cohort. Targeted therapy with MAPK/BRAF inhibitors has been under active investigation, with case reports highlighting improved outcomes in refractory disease; however, larger series with longer follow-up are needed to assess efficacy and toxicity.

Note that transition from bona fide LCH to later Langerhans cell sarcoma (dendritic sarcoma, Langerhans' phenotype) is exceptionally rare, and there is reason to believe that the LCH in these instances was cytogenetically different to begin with (see Langerhans Cell Sarcoma).

NON–LANGERHANS CELL HISTIOCYTOSIS

Erdheim-Chester Disease, Including Adult Xanthogranuloma Family Lesions

Clinical Features

Like the juvenile xanthogranuloma family, adult xanthogranuloma can vary from single lesions to multiple lesions, xanthoma disseminatum, and "systemic-form" Erdheim-Chester disease (ECD). Localized lesions are common around the orbit, where a number of clinical variants are recognized: adult-onset xanthogranuloma, necrobiotic xanthogranuloma, adult-onset asthma, periocular xanthogranuloma, and the ocular involvement of Erdheim-Chester disease. Historically, ECD has been grouped under the category of the systemic xanthogranuloma (XG) family given its shared immunophenotype, but it has been long recognized that it has a distinct clinical and radiographic presentation. Accumulation of data over the past few years has shifted our understanding of ECD toward a clonal inflammatory myeloid neoplasm, similar to LCH. ECD is now recognized as a distinct entity in the updated 2016/2017 WHO tumors of hematopoeitic and lymphoid tissues. An increasing number of reports link LCH and ECD, either within the same lesion/site or at different sites within the same patient during his or her lifetime, while also sharing clonal mutations in the mitogen-activated protein kinase (MAPK) pathway. These are the drivers that led to the recent proposal in classifying LCH and ECD together within the "L" (Langerhans) group of the revised classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages (see Box 19.1). For the purposes of this chapter we opt to leave ECD as its own category, acknowledging the shared molecular phenotype with LCH and shared immunophenotype with the XG family of lesions.

ECD is characterized by symmetric bony sclerosis on x-ray and 99T bone scintigraphy and organ involvement of the CNS, orbit, lung, retroperitoneum, kidney, heart, and skin. The biology is chronic and often aggressive, depending on involvement site. "High-risk" disease is seen with severe involvement of any one organ. ECD has a predilection for the hypothalamic-pituitary axis with the development of diabetes insipidus, much like Langerhans cell histiocytosis, and in a number of instances it has been associated with LCH at various sites.

Radiologic Features

Solitary and localized forms have no distinctive imaging features, but ECD is, in part, a radiologic diagnosis characterized by polyostotic sclerosis. This disease involves the bones around the knee, less frequently the elbow. The diaphyses and metaphyses have a coarsely increased trabecular pattern of medullary sclerosis and cortical thickening on radiography (Paget's disease–like pattern). Bone scintigraphy is said to show pathognomonic bilateral and symmetric increased uptake affecting both diaphysis and metaphysis of the femur and tibias. Computed tomography demonstrates increased density, and MRI highlights low-signal marrow replacement on fat-suppressed T1-weighted images, mixed signals on T2, and some enhancement after gadolinium contrast. FDG-PET has been used to identify new lesions and to monitor response to therapy.

Pathologic Features

Gross Findings

There are no gross characteristics other than the appearance of the skin lesions, as described for the juvenile xanthogranuloma family.

Microscopy Findings

The histopathology and phenotype are identical to those of juvenile xanthogranuloma. The dominant cell is oval in shape or slightly spindled and has an oval nucleus without

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ERDHEIM-CHESTER DISEASE—FACT SHEET

Definition
- Clinicoradiographic findings in the context of a histiocytic proliferation of epithelioid to xanthomatous cells with an XG phenotype; now considered as an inflammatory myeloid neoplasm

Incidence and Location
- True incidence unknown, 500 to 550 case reports
- Bone, retroperitoneum, including perinephric and periaortic; lung, including pleural, pericardial, skin, orbit; and CNS, including frequent posterior pituitary involvement

Morbidity and Mortality
- Chronic disease with remissions and progression dependent on sites of involvement
- High morbidity and mortality depending on extent of organ involvement

Gender, Race, and Age Distribution
- Adults 40 to 80 years of age, with male predominance; few pediatric cases reported

Clinical Features
- Constitutional: fevers, fatigue, night sweats; CNS manifestations, including ataxia, diabetes insipidus, endocrinopathy, double vision, exophthalmos, and psychiatric features; dyspnea; bone pain; xanthelasma and rash

Radiographic Features
- Polyostotic sclerosis, classically of the distal long bones with symmetric involvement by 99Tc bone scintigraphy
- Retroperitoneal fibrosis-like infiltrate
- Perinephric stranding "hairy kidney," often with ureteral involvement and hydronephrosis
- Periaortic infiltration "coated aorta" and pericardial thickening or effusion
- Pulmonary septal thickening, ground-glass opacities, or centrilobular opacities
- Enhancing lesions of the meninges, intra-axial lesions of the cerebellum and pons
- Enlargement of the pituitary gland and stalk

Prognosis and Treatment
- Five-year survival at 68% in those on interferon therapy
- Radiation, immunotherapy using INF-α, and chemotherapy with cladribine
- Vemurafenib, a *BRAF*-V600E inhibitor, therapy shows promising responses without short-term resistance; however, long-term efficacy and toxicity are still being investigated

the complexity of the LCH nucleus. Cases range from a cellular infiltrate of plump to slightly spindled cells with eosinophilic cytoplasm having variably vacuolated to "xanthomatous" or foamy-appearing histiocytes, including a variable number of Touton giant cells. In most cases the histiocytic proliferation is within a densely fibrotic stroma admixed with plasma cells, lymphocytes, and rare granulocytes in the background (Fig. 19.18). ECD shows the classic juvenile xanthogranuloma phenotype. In the orbit, the adult xanthogranuloma and "asthma-associated xanthogranulomas" are identical. The necrobiotic xanthogranulomas have areas of central tissue necrosis, and the phenotype has proved to be more variable, which may reflect evolution over time and may be considered as a biologically distinct process altogether.

Erdheim-Chester Disease Pathologic Features

Ancillary Studies

Immunohistochemistry

The immunophenotype is shared with JXG and other adult XG family lesions, which is that of a dermal macrophage.

ERDHEIM-CHESTER DISEASE—PATHOLOGIC FEATURES

Gross Findings
- No distinct gross findings

Microscopic Findings
- Morphology and phenotype are identical to those of xanthogranuloma family lesions
- The dominant cell is oval in shape or slightly spindled with variable vacuolated to xanthomatous cytoplasm
- Most cases have a densely fibrotic stroma with a variable mixed inflammatory infiltrate

Immunohistochemical Features
- Phenotype of JXG family: Surface staining for CD14 and surface/cytoplasmic CD163, granular cytoplasmic CD68, cytoplasmic factor XIIIa and fascin
- Little or no S100 and negative CD1a and Langerin
- VE1 dark cytoplasmic granular in *BRAF*-V600E mutated cases

Ancillary Molecular Testing
- Mutations in *BRAF*, *MAP2K1*, *NRAS*, *PIK3CA*, and gene fusions identified
- *BRAF*-V600E testing should include sensitive molecular methods for accurate detection, especially given the variable low content of histiocytes in some cases
- Confirmation of negative *BRAF*-V600E using another genotyping modality and/or genotyping from a different site (e.g., urine, blood) may be considered in *BRAF* wild-type bone lesions

Differential Diagnosis
- Skin: Hyperlipidemic eruptive xanthomas with serum lipid elevation
- IgG4-sclerosing lesions: Increased IgG4/IgG plasma ratio >0.4
- Other fibrohistiocytic processes including "burned-out" LCH and RDD lesions
- Other histiocytic-rich lesions including xanthogranulomatous inflammatory processes and lymphomas

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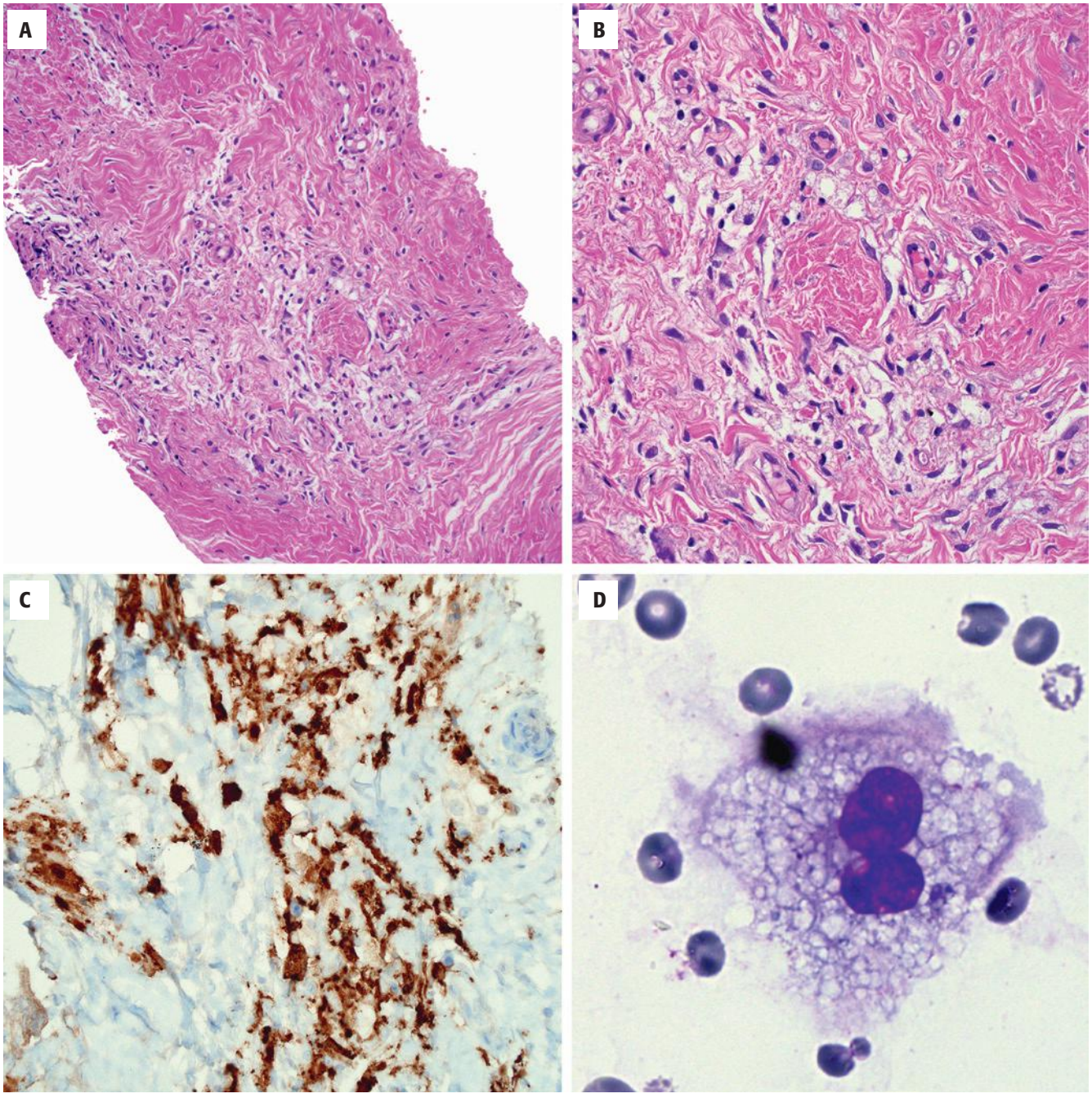


FIG. 19.18

Erdheim-Chester disease. Core needle biopsy and aspirate of retroperitoneal fibrosis of same patient with LCH/ECD. A and B, Dense fibrosis with intermixed histiocytes, some foamy to xanthomatous on H&E, which are highlighted with cytoplasmic factor 13a (C) along with CD68 and CD163 (latter stains not shown). D, The Wright-Giemsa–stained aspirate shows a foamy macrophage.

CD163 (a hemoglobin-haptoglobin scavenging receptor) shows membranous and cytoplasmic staining; CD14 (a monocyte or macrophage receptor that binds lipopolysaccharide) also has surface/membranous staining; CD68 (a lysosomal macrosialin) will have granular cytoplasmic staining. The cells will also show strong cytoplasmic staining for factor XIIIa (a tissue transglutaminase, formerly suggested to highlight monocyte-derived interstitial DCs, is now thought to highlight dermal macrophages, albeit microenvironmental factors likely affect normal activation states) and fascin (an actin bundling protein, which is associated with nodal interdigitating dendritic cells). There is typically little or no S100, CD1a, or Langerin expression. Of note, heavily xanthomatous cells tend to lose factor XIIIa expression, but a retained rim of peripheral staining in less xanthomatous cells is often seen.

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Molecular and Cytogenetic Features

Cases of ECD, with or without associated LCH, also have shown a high prevalence of *BRAF*-V600E mutations (up to 75% of cases). Similar to LCH, testing of ECD for *BRAF*-V600E should include sensitive molecular methods for accurate detection, especially given the variable low content of histiocytes in some cases. Confirmation of negative *BRAF*-V600E using another genotyping modality and/or genotyping from a different anatomic site (e.g., urine, blood) may be considered in BRAF wild-type (wt) bone lesions, as recommended by recent consensus ECD guidelines given its potential therapeutic implication. If properly validated, the VE1 antibody will show a dark, cytoplasmic granular staining pattern in the clonal histiocytes of those lesions harboring *BRAF*-V600E mutation. Other MAPK mutations including *MAP2K1* and *NRAS* will also show pERK antibody expression. In addition, mutations in *PIK3CA* have been shown in rare cases of both *BRAF*-wt and concurrent V600E mutations. Additional gene fusions (e.g., *KIF5B-ALK* and *LMNA-NTRK1*) have also been identified in rare cases.

Differential Diagnosis

We ascribe to the idea that $CD68^+$ xanthomatous cells alone are insufficient for a confident diagnosis of ECD, which requires a strict clinicopathologic correlation. Molecular confirmation of a *MAPK/BRAF*-V600E mutation in the tissue, blood, and even urine can be of further diagnostic assistance. Distinction from hyperlipidemic eruptive xanthomas is required. ECD, by virtue of its wide anatomic distribution, will have different regional patterns. In contrast to the IgG4-related sclerosing disease lesions, IgG4 content is not increased in the lesions. The combination of histopathologic features and phenotype must be taken in concert with the clinical and imaging findings for definitive diagnosis.

Prognosis and Therapy

Xanthoma disseminatum and progressive nodular histiocytosis can be locally aggressive and destructive but with some capacity to involute. ECD is a chronic, often debilitating disease with remissions and progression, all dependent of the site and extent of involvement. Older studies have shown dismal prognosis, with only 43% of patients alive after an average follow-up of 32 months; however, newer studies have shown a 68% survival at 5 years with interferon therapy. Radiation, immunotherapy (e.g., IFN-α with IL-1 suppression), and chemotherapy have been used with some success. In particular, cladribine has been shown to induce a significant response in patients with ECD involving long bones, pericardium, and retroperitoneum as well as patients primarily exhibiting neurologic symptoms. Anakinra, a recombinant IL-1R antagonist, has also been tried in small series. Combination sirolimus and prednisone therapy are being actively investigated in clinical trials, as are targeted kinase inhibitor therapy with vemurafenib and imatinib. *BRAF*-V600E–positive ECD cases, with or without concurrent LCH, have also shown good responses to vemurafenib without resistance. As in LCH, long-term efficacy and toxicity are still being investigated. Although direct mutations in *KIT*, *ABL*, or *PDGFR* have not been demonstrated in ECD, the high expression of PDGFR-β has led some to use imatinib mesylate with variable results in small series.

Juvenile Xanthogranuloma Family

Clinical Features

The juvenile xanthogranuloma (JXG) family of lesions has many parallels to LCH. There are small solitary lesions, mostly cutaneous, but also larger and deep lesions that, like the skin lesions, are amenable to slow regression. There are many clinical subtypes including benign cephalic histiocytoses, generalized eruptive histiocytosis, progressive nodular histiocytosis, xanthoma disseminatum, and solitary reticulohistiocytoma, which are included in the xanthogranuloma family of lesions. A small subpopulation of young children, mostly younger than 1 year, may have widespread systemic JXG with involvement of the skin, subcutaneous tissues, liver, spleen, lung, bone, meninges, eye (iris), and less commonly the brain. JXG does not involve the lymph nodes, and such involvement should rule out a histiocytic sarcoma with JXG phenotype. Reports of molecular aberrations in systemic JXG lesions have emerged, although as compared to LCH and ECD, systemic JXG is rare, and even fewer cases have been molecularly characterized.

Localized variants in children include deep JXG that can involve muscle or retroperitoneum and benign cephalic histiocytosis in which clusters of lesions occur on the head, face, or scalp. Xanthoma disseminatum, which is more common in young adults, involves a myriad of lesions that involve skin and mucosa with special predilection for the upper aerodigestive tract. As with LCH, pituitary-hypothalamic involvement with diabetes insipidus is described. Associations of JXG and neurofibromatosis, as well as JXG and juvenile myelomonocytic leukemia, are well known. Rare reports of occurrence in monozygotic twins suggest a genetic predisposition. Rare overlap with LCH occurs in the same patient, concurrent or asynchronous.

Radiologic Features

Most forms of the juvenile xanthogranuloma family have no unique imaging features beyond documenting a space-occupying lesion at a particular site.

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JUVENILE XANTHOGRANULOMA FAMILY—FACT SHEET

Definition
- Juvenile xanthogranuloma family of lesions is a histopathologic term that describes an accumulation of non-Langerhans cell histiocytes with a phenotype of dermal macrophages and encompasses a wide range of clinical phenotypes

Incidence and Location
- True incidence and prevalence of JXG remain unknown
- Cutaneous lesions most common, followed by subcutaneous fat or deep soft tissue
- Organ involvement is very rare, reported in the CNS, liver, bone, lung, spleen, eye (iris), pancreas, adrenal glands, intestines, kidneys, marrow, and heart
- Lymph nodes not typically involved, if so, histiocytic sarcoma with JXG phenotype should be considered

Morbidity and Mortality
- Cutaneous JXG prognosis is excellent; slow spontaneous regression is the rule
- Residual effects of regression are minor (residual hyperpigmentation, minimal atrophy, or loss of elastic fibers in the dermis)
- Extracutaneous JXG also spontaneously regresses; treatment focused on relieving possible mass effect symptoms
- Morbidity/mortality limited to rare congenital and infantile systemic cases, including involvement of CNS or massive hepatic involvement with cytokine-mediated liver failure

Gender, Race, and Age Distribution
- Solitary lesions: male-to-female ratio of 1.5 : 1, half are <1 year of age (range, 0 to 12 years)
- Multiple lesions: higher reported male prevalence 12 : 1

Clinical Features
- Cutaneous lesion: may have similar color to surrounding skin, erythematous, or yellowish
- Clinical forms of JXG family
  - Benign cephalic histiocytosis: young children (<2 years) with clusters of cutaneous mostly reddish lesions on the head, face, or scalp
  - Xanthoma disseminatum: young men (20 to 40 years) with cutaneous flexural and widespread mucosal involvement, including upper aerodigestive tract with many small lesions
  - Multicentric reticulohistiocytosis: older women (>50 years), with acral predilection and associated destructive arthritis, hyperlipidemia, and underlying malignancy or autoimmune disorder in up to half of patients
  - Progressive nodular histiocytosis: older patients (40 to 60 years) with micro- (<0.5 cm) and macronodular (1 to 5 cm) hard fibrotic, tan to brownish nodules predominantly of the trunk
  - Ocular JXG: left untreated can lead to hyphema, glaucoma, blindness
  - Adult (20 to 40 years) xanthogranuloma: with a variable clinical presentation (vascular, xanthomatous, fibrotic) predominantly in the head-neck-shoulder region
- Systemic symptoms are rare but can involve marrow with cytopenias
- Liver infiltration may cause elevation of liver enzymes, hypoalbuminemia, and an elevated erythrocyte sedimentation rate
- Pituitary involvement may lead to diabetes insipidus
- Associations with neurofibromatosis and juvenile myelomonocytic leukemia described

Radiographic Features
- No unique imaging features beyond documenting a space-occupying lesion at a particular site

Prognosis and Treatment
- Excellent prognosis as spontaneous regression is the rule for localized lesions
- Surgery for relief of mass effect
- Systemic JXG and xanthoma disseminatum may involute, but if infiltration at vulnerable sites (e.g., brain or larynx), may need low-dose LCH-type chemotherapy
- Rarely, death in the systemic forms, mainly of infants with fulminant hepatic failure or brain involvement

Pathologic Features

Gross Findings

There are no gross characteristics other than the appearance of the skin lesions. The nodules (1 to 10 mm in diameter) are described as being yellow to red-brown with overlying telangiectasia, solitary or multiple. Xanthoma disseminatum has myriads of skin and mucosal lesions that are raised and pink in early stages.

Microscopic Findings

All forms of the JXG family (much like LCH) are characterized by the presence of a dominant histiocyte with classic appearance and phenotype. Early lesions have small histiocytes that have folded bland nuclei and a moderate amount of faintly vacuolar cytoplasm (Fig. 19.19). Touton giant cells with a wreath of nuclei around a central eosinophilic core and a xanthomatous periphery are seen in approximately 85% of cases but not required for diagnosis. As the lesions age, the presence of xanthomatous cells becomes more prominent, and spindle cells also increase in number. Lymphocytes (T cells, CD3, CD43) are interspersed, and there may be eosinophils that are occasionally prominent. Reticulohistiocytoma (RH), with larger, more eosinophilic, and glassy cells, is characterized by oncocytic cells with abundant glassy pink cytoplasm. Whereas it has traditionally been classified as its own entity, microscopically it shares an immunophenotype with the xanthogranuloma family of lesions and may be classified as the oncocytic variant of the XG family of lesions.

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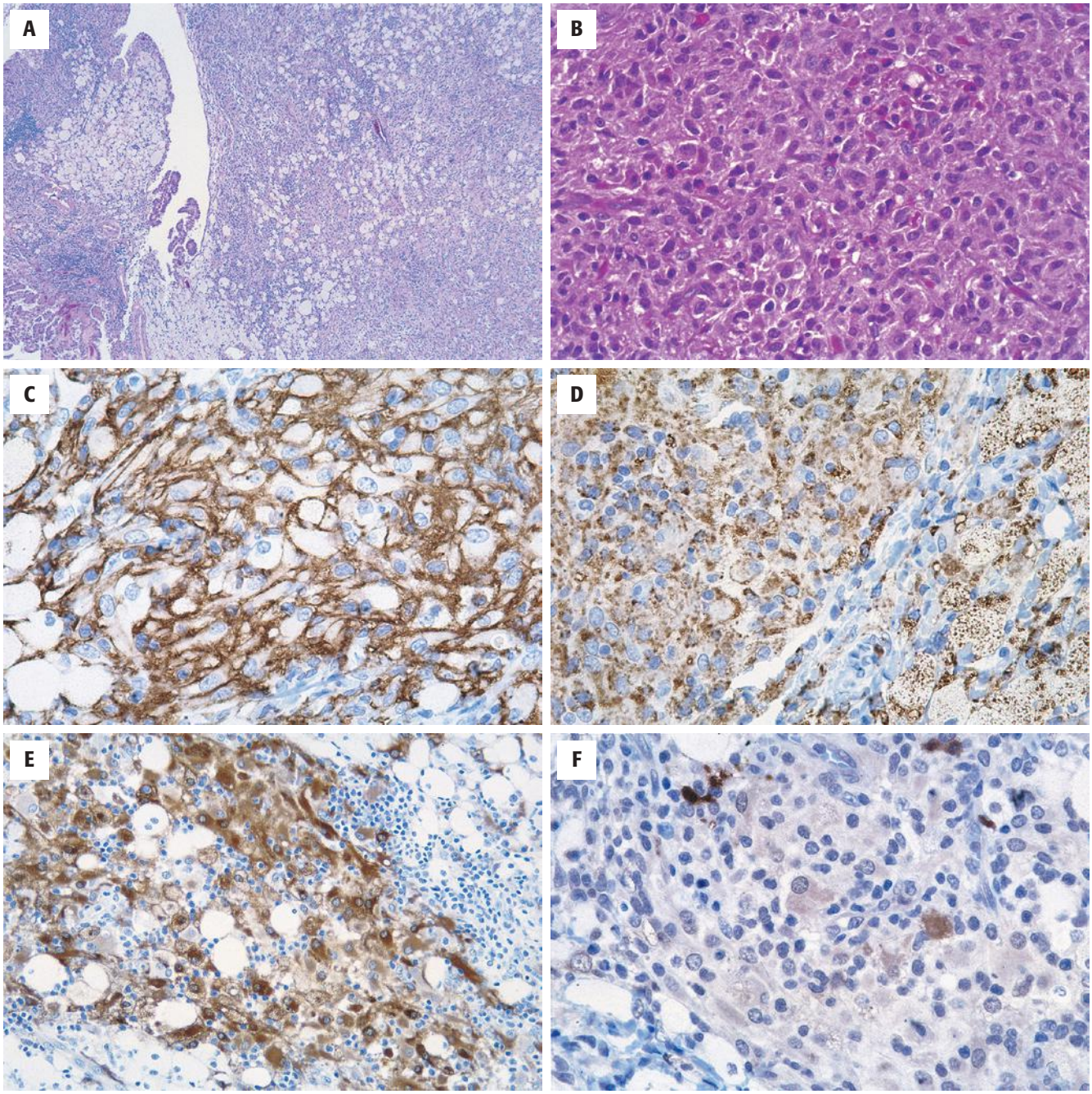


FIG. 19.19

Juvenile xanthogranuloma family. Lesions occur most commonly on the skin, but mucosal sites, especially the upper aerodigestive tract, are vulnerable. A, Laryngeal lesion. B, The cells have a bland cytoplasm-rich appearance similar to Langerhans cell disease. C, The cells have macrophage markers CD14 and CD68 (D). E, Cytoplasmic factor XIIIa is present, but S100 is generally low to absent (F).

Ancillary Studies

Immunohistochemistry

The phenotype of the histiocyte at the core of the entire range of lesions is highly characteristic. The lesions have expression of macrophage/monocytic markers CD163 (membrane/cytoplasmic) and CD14 (strong membrane). There is granular cytoplasmic staining with CD68 (KP-1, PGM-1). Stains for FXIIIa and fascin show cytoplasmic expression, but highly xanthomatous cells may lose staining for factor XIIIa, which is typically retained at the perimeter of lesions (vide supra, ECD immunohistochemistry [IHC]). CD1a and Langerin are absent, and S100 is either lacking or present in low-variable amounts in less than 30% of cases, by personal experience (see Fig. 19.19). Vimentin is also documented, but leukocyte common antigen (LCA) is typically absent.

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JUVENILE XANTHOGRANULOMA FAMILY—PATHOLOGIC FEATURES

Gross Findings
- No distinctive gross findings; yellow to red-brown nodules with overlying telangiectasia of skin

Microscopic Findings
- Early lesions: small mononuclear histiocytes that have bland nuclei and a moderate amount of vacuolated cytoplasm and Touton giant cells
- Older lesions: showing increasing amount of xanthomatous and spindle cells
- Reticulohistiocytoma (RH) subtype: large eosinophilic oncocytic-type cells

Immunohistochemical Features
- Surface staining for CD14 and surface/cytoplasmic CD163
- Granular cytoplasmic CD68
- Cytoplasmic FXIIIa and fascin
- Little or no S100 and negative CD1a and Langerin
- Rare systemic ALK$^+$ systemic histiocytosis with JXG morphology in young children

Ancillary Molecular Testing
- Systemic JXG case reports have shown point mutations in *ARAF, MAP2K1, NRAS, KRAS, PIK3CD*, and one *BRAF* gene fusion
- *TPM3-ALK* in rare ALK$^+$ histiocytosis
- Solitary JXG lesions have shown no consistent genetic abnormalities to date

Ultrastructural Features
- Lack Birbeck granule and usually have intracytoplasmic lipid droplets

Fine-Needle Aspiration
- Bland histiocytes rich in lipid droplets

Differential Diagnosis
- Langerhans cell histiocytosis, especially late burned-out LCH
- Eruptive xanthomas, associated with hyperlipidemias
- Dermal fibrous histiocytomas: SMA positive with interspersed factor XIIIa, CD163, and CD68 spindled histiocytes
- Rosai-Dorfman cells are larger with paler cytoplasm as compared to the oncocytic RH cell
- Melanocytic nevus, including Spitz: The Junctional component and S100 positivity should distinguish the diagnosis

Others have shown expression with CD4 and CD31, which also highlight monocytes and histiocytes, along with CD11c, a monocytic, histiocytic, and myeloid dendritic cell marker.

An ALK$^+$ variant has been described with *TPM3-ALK* fusions showing positive ALK immunohistochemistry in a membranous and cytoplasmic expression. The three index cases had a neonatal presentation with hepatosplenomegaly and sinusoidal infiltrates of ALK$^+$ histiocytes but had a self-limited presentation, distinct from the systemic/visceral JXG lesions. ALK staining may help separate these lesions.

Molecular and Cytogenetic Features

Case reports of systemic JXG have shown point mutations in *ARAF, MAP2K1, NRAS, KRAS, PIK3CD*, and one *BRAF* gene fusion with *RNF11*. Solitary JXG lesions have shown no consistent genetic abnormalities to date.

Ultrastructural Features

The cells are bland histiocytes that lack the Birbeck granule and usually have intracytoplasmic lipid droplets. A number of nonspecific intracytoplasmic findings such as dense bodies, wormlike bodies, and popcorn bodies are described.

Fine-Needle Aspiration Biopsy

The lesions are represented by bland histiocytes rich in lipid droplets, and only the phenotype, as described previously, is definitive when combined with the relevant clinical information.

Differential Diagnosis

The major differential diagnosis is from Langerhans cell histiocytosis. CD1a, Langerin, and S100 should discriminate between the two. Eruptive xanthomas, associated with hyperlipidemias, require documentation of the serum lipids. Unlike the JXG and ECD, the xanthoma cells express little factor XIIIa or fascin. It is important to keep in mind those heavily xanthomatous cells tend to lose factor XIIIa. Older JXG lesions that contain more spindle cells, especially the progressive nodular histiocytosis variant, simulate the fibrous histiocytomas, but the JXG-family phenotype is distinctive. The dermal fibrous histiocytomas (dermatofibroma) may have large numbers of interspersed factor XIIIa, CD163, and CD68 spindled cells. Rosai-Dorfman cells are much larger with paler cytoplasm and selective S100$^+$. As described in the following section, reticulohistiocytomas are now considered as part of the JXG family of lesions. Melanocytic nevus cells can confound the diagnosis, but the S100 stain will usually discriminate by being uniformly strong.

Prognosis and Therapy

The prognosis and therapy vary for the different clinical types at their characteristic ages. Solitary lesions in the young usually regress slowly. Systemic JXG and xanthoma disseminatum may involute but can involve vulnerable sites such as the brain or larynx and may need low-dose chemotherapy. Rarely, death in the systemic form has been associated with fulminant hepatic failure (possibly cytokine mediated) or brain involvement. Cases of aggressive

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systemic JXG following leukemia have been described to have identical gene rearrangements of the leukemia, which likely represent a biologically distinct histiocytosis (vide infra).

RETICULOHISTIOCYTOMA AND MULTICENTRIC RETICULOHISTIOCYTOSIS

Two forms of reticulohistiocytoma have been historically recognized: solitary reticulohistiocytoma (a cutaneous lesion also called *solitary epithelioid histiocytoma*) and multicentric reticulohistiocytosis (MRH; a cutaneous and rheumatologic condition). Both are characterized by infiltration by the same type of histiocyte, in which current classifications now list them under the heading of "C Group," cutaneous, non-LCH xanthogranuloma family of lesions (see Box 19.1). MRH may be considered as a cutaneous non-LCH with a major systemic component (see Box 19.1). Visceral symptoms and muscular, cardiopulmonary, ocular, thyroid, and submandibular involvement have been reported occasionally. Lymph node involvement is rare. The solitary lesion is seen in neonates and young people with the same phenotype as the more classic JXG lesions. MRH is more common in older females and can be associated with hyperlipidemia, underlying malignancy, or an autoimmune disorder in half the patients.

Pathologic Findings

The lesional histiocyte is large (50 to 100 μm) with one to three eccentric, oval, or grooved nuclei and abundant, deeply eosinophilic glassy cytoplasm that stains lightly with PAS and resists diastase. Early lesions may contain many macrophages and lymphocytes mimicking other histiocytoses. Phagocytosis of connective tissue or cellular components may be seen. The cells stain similar to JXG family lesions with CD163, CD14, and CD68 but not S100 or CD1a (Fig. 19.20). Fascin stains the lesional cells lightly and variably. Electron microscopy shows large mononucleated or multinucleated cells with numerous peripheral microvilli. Birbeck granules are absent. The differential diagnosis includes Rosai-Dorfman disease, but the cytoplasm of the MRH cells is neither pale nor does it contain emperipolesis. The cells are larger than those of the classic JXG family and have been confused for Spitz and melanoma cells, but S100 stain is negative. The non-neural (S100-negative) granular cell lesion can look similar, but other high-grade epithelioid lesions should be excluded.

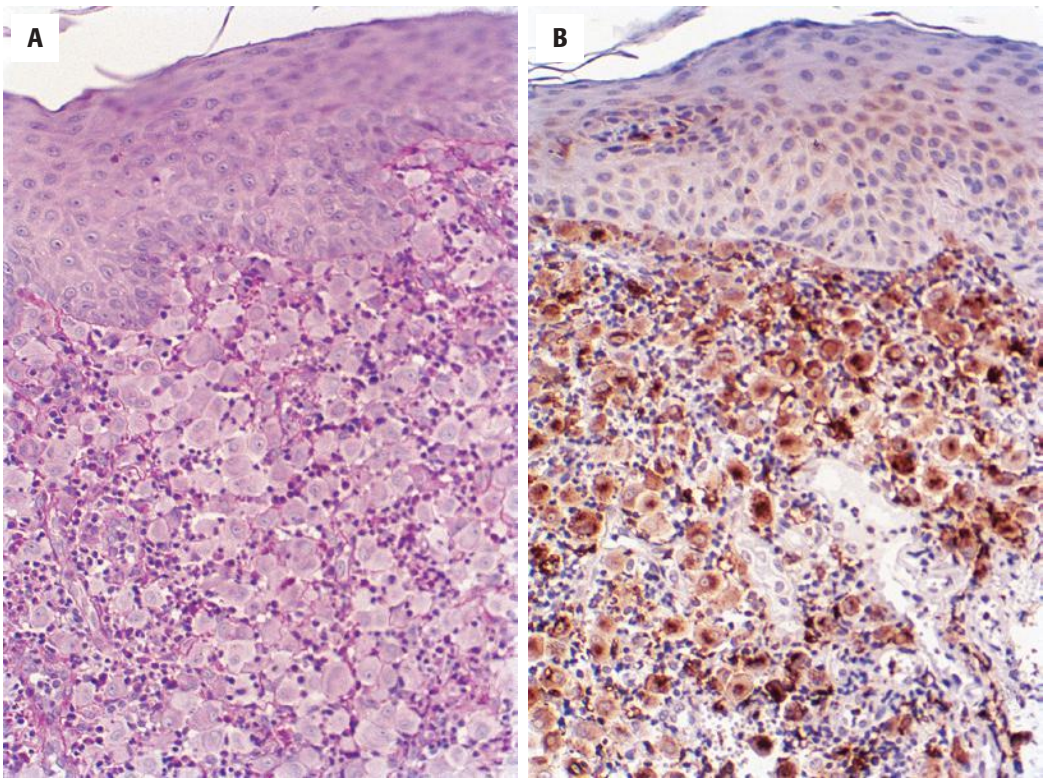


FIG. 19.20

Juvenile xanthogranuloma family: Reticulohistiocytoma type. A, The skin lesion contains large cells that have abundant glassy cytoplasm, lightly periodic acid–Schiff (PAS)-positive in contrast to the intervening inflammatory cells. B, CD68 (KP-1) stains the cytoplasm with a paranuclear accentuation, wheras CD163, factor 13a, and fascin (not shown) would also highlight the cells.

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Prognosis and Therapy

A congenital skin form is self-limiting, which is common in children. Adults should be evaluated for polyosteoarthropathy, especially in cases with acral and face involvement.

ROSAI-DORFMAN DISEASE

Also known as *sinus histiocytosis with massive lymphadenopathy* after its most common presentation of the lymph node, Rosai-Dorfman disease is a benign condition of a unique large S100-positive histiocyte and has a variable clinical course, including solitary, multifocal, and systemic forms.

Clinical Features

The usual presentation is painless, with large, firm, usually bilateral cervical nodes associated with fever, leukocytosis, anemia, raised sedimentation rate, and a polyclonal hypergammaglobulinemia. The disease can also occur in non-nodal sites. Common extranodal sites include skin, nasal cavity and paranasal sinuses, soft tissue, orbit, bone, salivary gland, the CNS and its dural/leptomeningeal coverings. Rare involvement of thyroid gland and breast tissue has been reported but typically not bone marrow or spleen. Extranodal involvement presents most commonly as a mass, and the diagnosis comes as a surprise. Multifocal lesions, nodal and extranodal, can simulate malignancy. Most cases occur in adolescents or young adult males with only rare intrafamily clusters.

Radiologic Features

Imaging manifestations are nonspecific, and FDG-PET shows increased metabolism. These features do not distinguish Rosai-Dorfman disease from malignancy or other causes of lymphadenopathy. However, concomitant cervical lymphadenopathy with orbital or paranasal sinus mass should suggest further testing. Decreased T2-weighted signal intensity in dural-based lesions and lack of arteriovenous shunting on angiography helps to rule out meningioma. Lytic bone lesions associated with other soft-tissue involvement should be distinguished from LCH and other malignancies.

Pathologic Features

Gross Findings

The nodes are often strikingly enlarged and firm and have thick fibrous capsules matting them together. The cut surface is commonly yellow from lipid.

ROSAI-DORFMAN DISEASE—FACT SHEET

Definition
- A benign histiocyte infiltrate most common in cervical lymph nodes causing striking sinus enlargement, also occurring in extranodal sites
- Lesion identified at all sites by the unique Rosai-Dorfman histiocyte that has a variable degree of cytoplasmic emperipolesis

Incidence and Location
- Sporadic, with rare clusters of cases
- Cervical lymph nodes
- Waldeyer's ring and upper respiratory tract
- Skin and subcutaneous area, trunk and proximal limbs, orbit, bone, meninges, breast

Morbidity and Mortality
- Cosmetic problems owing to massive lymphadenopathy
- Regress without residual in most instances
- May cause serious disease at vulnerable sites such as brain

Gender, Race, and Age Distribution
- Adolescents and young adults (mean, 20 years)
- Blacks affected more commonly; males more than females for cervical lymphadenopathy
- Equal sex ratio for extranodal disease

Clinical Features
- Massive painless bilateral cervical lymphadenopathy with hyperglobulinemia and occasional autoimmune antibodies, uncommon systemic symptoms
- Space-occupying lesions at other sites, with or without cervical node involvement

Radiologic Features
- Space-occupying lesions without distinguishing features

Prognosis and Treatment
- Indolent course with slow spontaneous regression of the infiltrate and associated autoimmune phenomena, when present
- Corticosteroids and surgery for progressive disease, rarely chemotherapy for refractory or life-threatening lesions

Microscopic Findings

In the lymph nodes, the lesion involves the sinuses that are markedly distended. Often the distention and distortion are so extensive that the sinus pattern is not recognizable. In soft tissues, the lesions are commonly confluent with aggregates of lesional cells and intervening inflammatory cells that produce an alternating dark and light effect or simulate lymph node sinuses (i.e., sinusoidal pattern). The lesional Rosai-Dorfman cells are unique. The cells are large, often 75 μm or more, with a central large hypochromatic nucleus set in an abundant pale water-clear cytoplasm. Nucleoli are single, moderately large, and distinct. Mature lymphocytes,

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ROSAI-DORFMAN DISEASE—PATHOLOGIC FEATURES

Gross Findings
- Massive cervical node involvement with capsular thickening, matting of adjacent nodes, and yellow color

Microscopic Findings
- Lymph nodes and extranodal sites have zones of very large pale cells alternating with lymphocyte- and plasma cell–rich areas with sinus expansion in nodal cases
- Exceptionally large cells (>75 μm) with pale, water-clear cytoplasm and large round hypochromatic nuclei containing a single nucleolus
- Cytoplasmic emperipolesis of intact cells mostly lymphocytes, less commonly neutrophils, eosinophils
- Interspersed plasma cells prominent in most cases
- Neutrophil pseudoabscesses can be evident
- Xanthoma cells and spindle cells with collagen deposition in regressing extranodal lesions

Ultrastructural Features
- Emperipolesis, intact inflammatory cells within cytoplasm

Fine-Needle Aspiration Biopsy Findings
- There is a population of exceptionally large histiocytes that have abundant pale cytoplasm with emperipolesis. The features are distinctive, and immunocytologic confirmation is not required. S100 staining requires prior fixation because of high solubility

Immunohistochemical Features
- Large histiocytes have high $S100^+$, $fascin^+$ with $CD14^+$, $CD163^+$, $CD68^+$

Molecular Studies
- Rare reports of mutations in *MAPK* pathway genes *ARAF, NRAS,* and *KRAS*

Differential Diagnosis
- Langerhans cell histiocytosis is $CD1a^+$/$Langerin^+$
- Granulomatous disease and draining histiocytes near prosthesis lack S100/fascin
- Foci of Rosai-Dorfman reaction can be seen in lymph nodes that harbor Hodgkin disease, acquired lymphoproliferative syndrome, or LCH without other manifestations of Rosai-Dorfman disease (RDD)
- In bone lesions with high numbers of plasma cells, chronic osteomyelitis may confound the diagnosis if only small foci of RDD are represented. No clusters of S100/fascin large cells should be seen

many plasma cells, and neutrophils are often present between the histiocytes and within the cytoplasm. Emperipolesis, or the presence of intact cells within the cytoplasm, often as many as 5 to 10, is characteristic of this condition (see Fig. 19.10). Neutrophil-rich suppuration may confound the picture and ruling out infectious etiologies (i.e., *Bartonella*) may be considered. Late or involuting lesions have fewer Rosai-Dorfman cells, more xanthoma cells, and spindled fibroblasts.

Ancillary Studies

Electron Microscopy

Intact lymphocytes and phagolysosome structures are seen in the macrophages, but no Birbeck granules are present.

Molecular and Cytogenic Features

Recently, rare cases have shown missense point mutations in the *MAPK* pathway genes (i.e., *ARAF, NRAS,* and *KRAS*). Co-occurring cases of LCH and RDD have shown mutations in the LCH component only; *BRAF* mutations have not been reported in RDD.

Immunohistochemistry

The cells mark as macrophage-DC hybrids. They express strong macrophage markers, variable surface CD14, and membrane CD163, and they have fine granular cytoplasmic CD68. In addition, and most informatively, is the strong expression of dendritic cell marker, S100 and fascin in their cytoplasm, outlining the emperipolesis (Fig. 19.21). The recent classification of histiocytosis recommends evaluation of IgG4-to-IgG ratio in all RDD (expert recommendation, grade D2), although it is still uncertain whether RDD with a greater than 0.4 ratio of IgG4-to-IgG should belong in the spectrum of IgG4-related sclerosing disease or as a separate diagnostic subcategory of RDD.

Fine-Needle Aspiration Biopsy

The exceptionally large Rosai-Dorfman cells with a central round nucleus and abundant cytoplasm that contains emperipolesis of lymphocytes, plasma cells, or neutrophils are virtually diagnostic. Confirmation of the phenotype completes the diagnosis.

Differential Diagnosis

Reactive and draining nodes with sinus histiocytosis do not have classic Rosai-Dorfman features; the cells are much smaller. Langerhans cell histiocytosis involves the sinuses of lymph nodes, but the LCH cells are also smaller with nuclei that are grooved or complexly folded, unlike the large Rosai-Dorfman nucleus. Nodes draining prosthetic implants can more closely simulate RDD, usually in older patients. Rarely, intermixed foci of RDD/reaction can be seen in lymph nodes that harbor other conditions such as acquired lymphoproliferative syndrome (human immunodeficiency virus [HIV]), autoimmune lymphoproliferative syndrome (ALPS), in particular the type I with heterozygous germline mutation in *TNFRSF6*, the FAS gene (OMIM #601859), and histiocytosis-lymphadenopathy plus syndrome (e.g., Faisalabad

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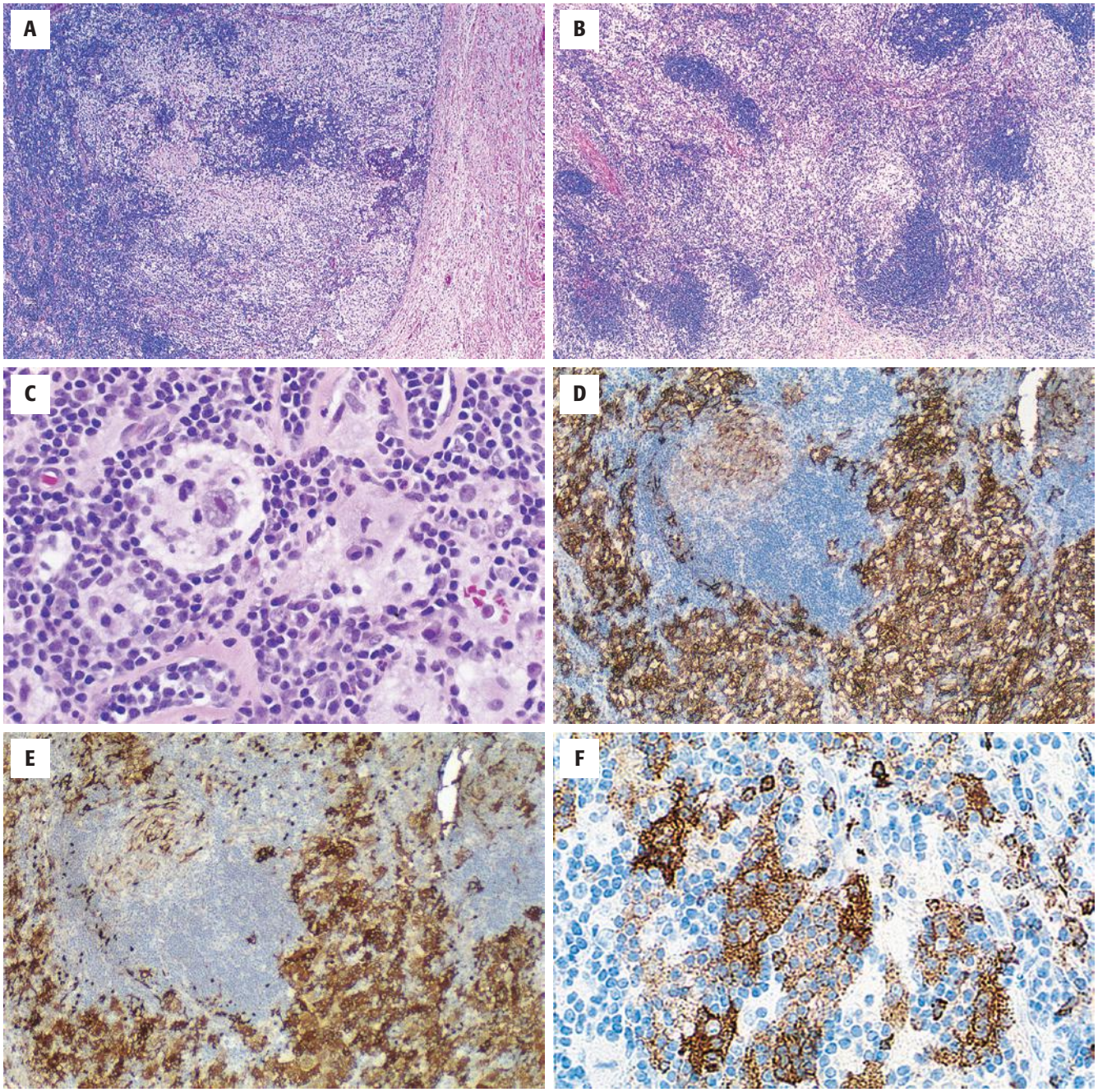


FIG. 19.21

Rosai-Dorfman disease. A and B, The thick capsule and accumulation of pale histiocytes are demonstrated although their presence in the sinuses is not obvious. C, The large cells that have clear cytoplasm that includes whole cells (emperipolesis) and the nuclei are eccentric and large and have a prominent nucleolus in a hypochromatic background. The cells have sharp membrane staining for CD14 (D) and cytoplasmic and nuclear staining for S100 (E), and large cells with their emperipolesis are well shown with immunostain for CD163 (F).

histiocytosis) with a homozygous or compound heterozygous mutation in the *SLC29A3* gene (OMIM #602782), Hodgkin disease, or Langerhans cell histiocytosis. These Rosai-Dorfman reactions contain $S100^+$ Rosai-Dorfman–type cells with emperipolesis that may partially involve lymph nodes. Late lesions that have only few Rosai-Dorfman cells can simulate inflammatory pseudotumors or IgG4-related sclerosing disease. Typically, IgG4-related disease will have a larger number of $IgG4^+$ plasma cells with a higher ratio (e.g., >0.4) of IgG4 to IgG, dense sclerosis, and elevated serum levels of IgG4.

Prognosis and Therapy

Some large nodes regress rapidly and spontaneously without treatment, whereas most regress and recur over a period of years. Soft-tissue lesions can be indolent and respond only poorly to corticosteroids, but chemotherapy has been effective. Some recommend cladribine and clofarabine for relapsed, multisystem, or non-resectable CNS disease. Mortality has been caused by disease at vulnerable sites, such as the CNS

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or upper respiratory system, or associated immunologic disorders, lymphoma, leukemia, or exceptional instances of solid tumor.

OTHER NON–LANGERHANS CELL HISTIOCYTOSES

On rare occasion, a solitary or systemic histiocytosis cannot be characterized as Langerhans cell or juvenile xanthogranuloma in type, because of failure to fulfill the phenotypic requirements. The terms *indeterminate cell lesion* or *histiocytosis* has been used in this situation, where the indeterminate cell is defined as a Langerhans-like DC that is $CD1a^+$ but lacks a Birbeck granule on ultrastructural examination. That definition is unsatisfying for a number of reasons. Langerhans cell histiocytosis can have sparse Birbeck granules, and current practice does not require electron microscopy for complete diagnosis. Immunohistochemistry shows proliferating cells that are usually positive for CD14, CD1a, CD68, and factor XIIIa. The patients are more commonly adults who have solitary or multiple skin lesions, but a more disseminated and visceral presentation in children and adults is described. The histopathology is that of a bland histiocytic lesion similar to Langerhans cell disease, although nuclear grooving and complexity may be less, and eosinophils are generally sparse. Recently, new insights into these lesions have uncovered the gene fusion *ETV3-NCOA2*. A malignant sarcomatous counterpart, indeterminate cell sarcoma, is described in addition to an association with myeloid leukemia (vide infra).

Rare Langerhans cell–like lesions—solitary, multiple, and systemic—closely resemble LCH but lack CD1a expression. These DC histiocytomas and histiocytoses have been seen in children and young adults and appear to be composed of Langerhans-type cells that are more mature than LCH cells and have lost CD1a expression, whereas HLA-DR expression on the surface is upregulated and S100/fascin expression is high and more like that of the interdigitating DCs, although they are not spindled. In support of the relationship to LCH, one case occurred at the site of a previous classic LCH. A collection of some of these cases has shown that the clinical spectrum is similar to LCH but that the CNS and its coverings are more likely to be involved, and local recurrence is more common than that seen with LCH. LCH-type treatment has been effective. A sarcomatous counterpart exists: DC sarcoma, not otherwise specified.

COMBINED LANGERHANS AND NON-LANGERHANS HISTIOCYTOSIS

"Combined" or "mixed histiocytic" disorders are not a well-defined group of disorders, with few case reports and small case series. We have largely subscribed to the notion that mixed histiocytic disorders entail two distinct histiocytic lesions present within the same lesion, in which microdissection of the two lesions could be technically achieved. This serves to distinguish the so-called secondary histiocytic hyperplasias seen in some disorders. However, as described recently with combined LCH/ECD cases, this term may also serve to include lesions occurring at different sites or at asynchronous time points within the same patient.

Langerhans Cell Histiocytosis and Juvenile Xanthogranuloma

Rare instances are described in which the two patterns are discernible in the same lesion, but more commonly clinical LCH is followed by lesions that are clinically and morphologically JXG in type. This asynchronous event needs to be distinguished from the xanthomatous (JXG-like) histiocytic reaction that accompanies regressing or treated LCH most conspicuously in the meninges or orbit.

Langerhans Cell Histiocytosis and Erdheim-Chester Disease

The revised classification proposal of a shared "L group" lesion for these histiocytic disorders may be timely as we begin to understand both LCH and ECD as inflammatory myeloid neoplasms. As previously discussed, increasing evidence has shown a number of examples of ECD that have followed LCH lesions or concurrently present either at the same site or different sites within the same patient (see Fig. 19.17, vide supra).

Langerhans Cell Histiocytosis and Rosai-Dorfman Disease

Coexistence of LCH and Rosai-Dorfman disease can occur at different sites synchronously or asynchronously and even at the same site as distinctly identifiable areas of growth.

HISTIOCYTOSIS FOLLOWING LEUKEMIA, OR FOLLICULAR LYMPHOMAS

A variety of histiocytic lesions—focal, systemic, and malignant—has been described following prior acute lymphoblastic leukemias or follicular lymphomas. We have also seen a recent case following an acute myeloid leukemia with a shared molecular signature. These histiocytic lesions may share the cytogenetic or molecular signature of the prior

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leukemia or lymphoma. Although the histiocytic lesions may fit the morphologic categories of Langerhans cell disease, juvenile or adult xanthogranuloma, and Rosai-Dorfman disease, their biological behavior is commonly more aggressive, in keeping with the primary lymphoid malignancy. Instances of histiocytic sarcoma are high grade.

HISTIOCYTIC MALIGNANCIES

In the 2016 revised classification of histiocytosis and neoplasms of the macrophage-dendritic cell lineages, the group of histiocytic malignancies, including histiocytic sarcoma, Langerhans cell sarcoma, interdigitating cell sarcoma, and indeterminate cell sarcoma, are proposed to be grouped under the old term of *malignant histiocytoses (MH), M-group* The old term was revisited as many of the primary histiocytic malignancies share features of anaplasia, increased and atypical mitoses, with variable and often overlapping expression of at least two or more histiocyte/dendritic cell markers (e.g., CD163, CD68, lysozyme, S100, CD1a, Langerin, CD4). The exclusion of lymphoma and other sarcomas/carcinomas with a broad panel of B and T lymphocyte markers, cytokeratins, epithelial membrane antigen (EMA), melanocytic, and muscle markers is imperative. For the purposes of this chapter we herein include the main recognized subtypes as histiocytic malignancies, while noting that the category of follicular dendritic sarcoma is now excluded from this group and should be classified according to its mesenchymal, non-hematopoietic origin.

HISTIOCYTIC SARCOMAS—FACT SHEET

Definition

- Histiocytic sarcomas, including the broader term of *M group: histiocytic malignancies* are a collection of hematopoietic malignancies that are cytologically malignant and are categorized by their phenotypic affiliation to the various histiocyte, macrophage, and dendritic cell markers

Incidence and Location

- Incidence is rare, with mostly single case reports
- Some follow a prior leukemia and may contain the same molecular signal
- Some are lymphoma-like and nodal; others are soft-tissue lesions

Gender, Race, and Age Distribution

- Mostly adult, although childhood examples are described

Clinical Features

- Symptoms vary according to site of presentation
- Nodal disease and systemic involvement in some is accompanied by B-type systemic effects

Radiologic Features

- No specific findings other than nodal or extranodal space-occupying lesions

Prognosis and Treatment

- Localized lesions in general may have low-grade behavior after resection, dependent on site
- Recurrences, multiple lesions, and systemic disease are relatively refractory to current chemotherapy, with high mortality

HISTIOCYTIC SARCOMA

Histiocytic sarcoma is a large-cell hematopoietic malignancy that develops as an epithelioid tumor in nodal or extranodal sites. The diagnosis is confirmed by demonstrating an immunophenotype of macrophages, DCs, or both. Some have occurred after acute lymphoblastic leukemias, lymphomas, or mediastinal germ cell tumors, the so-called secondary malignant histiocytoses (see Box 19.1). Of note, extramedullary myeloid tumors that have monocytic differentiation are not included.

CLINICAL FEATURES

Adults are primarily involved; mean age is 44 to 55 years, with rare pediatric cases described and males and females affected equally. Presentation is commonly as a mass, with the involved sites being lymph node, soft tissue, or gastrointestinal tract: obstructive features may dominate gastrointestinal presentations. Rarely, patients who have a more systemic form have been diagnosed with *malignant histiocytosis*, a historically fraught term, which has regained popularity in describing the general category of histiocytic malignancies.

PATHOLOGIC FEATURES

MICROSCOPIC FINDINGS

The microscopic appearance is dominated by large (>20 μm) epithelioid cells that have abundant eosinophilic cytoplasm. The abundance of the cytoplasm is a clue to the diagnosis, because phagocytic activity is hardly ever seen, although cytoplasmic vacuolation may be present. Nuclei are oval and variable in size with a large amphophilic nucleolus (Fig. 19.22). Binucleation is common, and anaplastic pleomorphism with giant cells is seen focally in some. Mitotic activity is variable but typically increased with atypical forms in the more anaplastic lesions. The cells may be diffuse or sinusoidal in their distribution in the lymph nodes, liver, or spleen. Lymphocytes, less commonly eosinophils, may be admixed.

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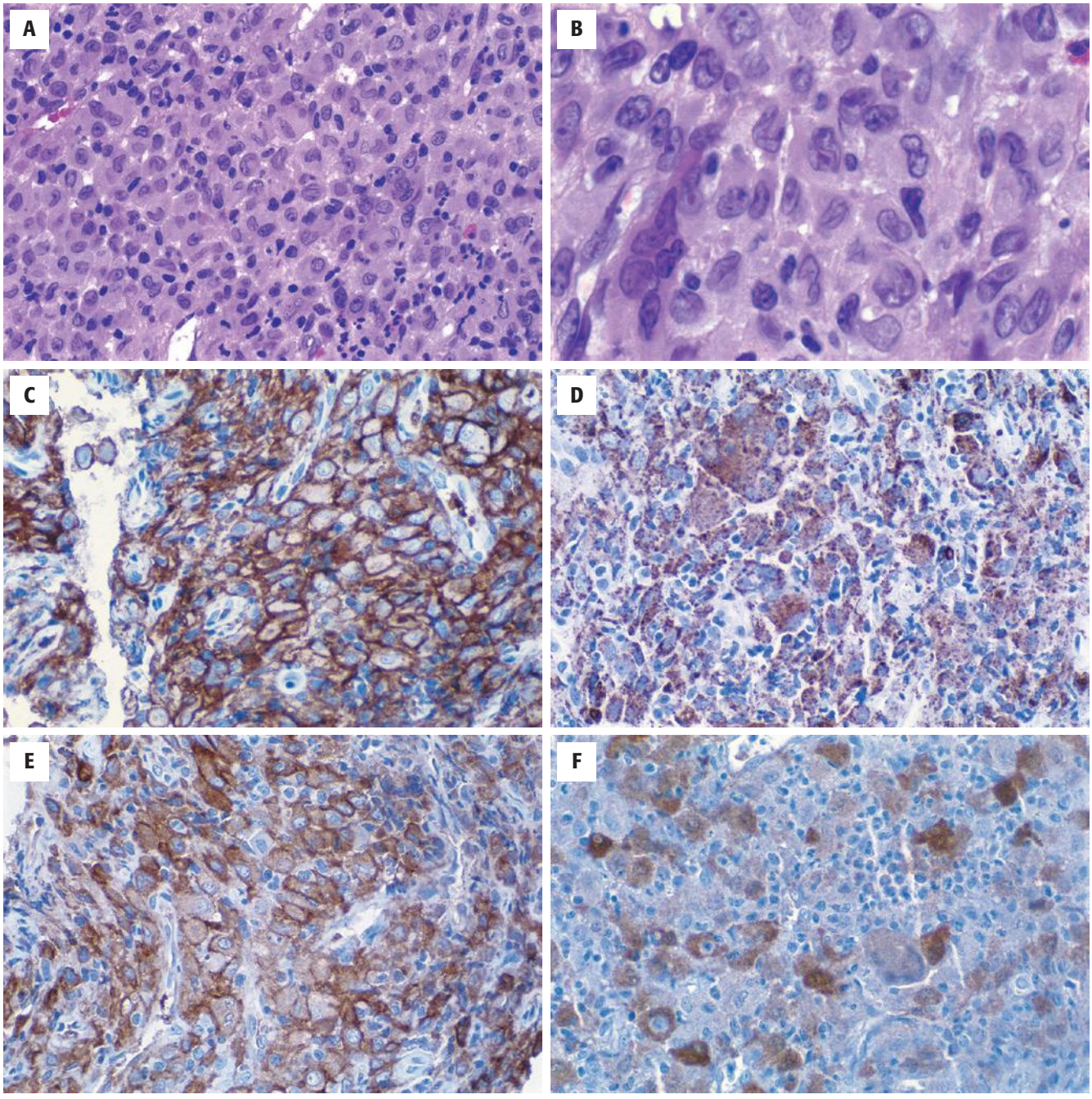


FIG. 19.22

Histiocytic sarcoma. A and B, The cell type can vary from epithelioid to cells with more complex nuclei. The phenotype is macrophage-like with CD14 (C), CD68 (D), and CD163 (E). F, S100 expression is variable but low. This lesion followed a B-cell acute lymphoblastic leukemia by 10 years.

Ancillary Studies

Molecular and Cytogenetic Features

Some cases of primary histiocytic sarcomas are now recognized as having mutations in the MEK-ERK 1/2 MAPK signaling pathway (e.g., *BRAF, HRAS, BRAF* gene fusions), in addition to chromosomal gains or losses. Study of additional cases is needed to better define recurrent mutations and their frequencies. Cases that have occurred following a prior leukemia/lymphoma generally have the same clonal markers as the prior leukemia/lymphoma.

Electron Microscopy

No cell processes or tight junctions are present, but there is a prominent Golgi and secondary lysosomes. Birbeck granules are absent.

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HISTIOCYTIC MALIGNANCIES INCLUDING HISTIOCYTIC AND DENDRITIC CELL SARCOMAS—PATHOLOGIC FEATURES

Gross Findings
- Firm nodal or soft-tissue lesions

Microscopic Findings
- A variety of appearances, generally stubby oval to spindled cells, some epithelioid
- Histiocytic sarcoma, predominantly epithelioid

Langerhans Sarcoma, Variable Nuclear Grooves
- Interspersed lymphocytes, T cells predominant in interdigitating cell lesions

Immunohistochemical Features
- Table 19.3

Molecular Studies
- Histiocytic sarcoma: *BRAF, HRAS, BRAF* gene fusion
- Rare reports of Langerhans cell, indeterminate, interdigitating cell sarcoma: *BRAF*-V600E

Ultrastructural Features
- Interdigitating DC lesions have interdigitation processes
- No Birbeck granules unless in LCS

Fine-Needle Aspiration Biopsy Findings
- Epithelioid or spindle cell lesions that have interspersed lymphocytes
- Lesional cells have characteristic phenotype

Differential Diagnosis
- The histiocytic dendritic cell lesions may show phenotypic overlap with one another (see Box 19.1 and Table 19.3)
- Histiocytic sarcoma has the differential diagnosis of epithelioid cell tumors and large cell (ALK and T cell) lymphomas, Hodgkin lymphoma, metastatic carcinoma, epithelioid sarcoma, and melanoma. Extramedullary myeloid granulocytic processes, especially monocytic leukemias, may have similar features
- The spindle cell dendritic processes of LCS, interdigitating dendritic cell sarcoma (IDS), and follicular dendritic cell sarcoma (FCS) must be distinguished from other spindle cell lesions, myoid, gastrointestinal stromal tumors, myofibroblastic, and angiomatoid fibrous histiocytoma

TABLE 19.3
Immunohistochemical Features of the Histiocytic Malignancies

	LCA	CD14	CD68	CD163	Lysozyme	S100	CD1a	Langerin	Fascin	CD21	CD35	Clusterin	FXIIIa
HS	+	++	++	++	++	±	–	–	–	–	–	N/D	–
JXGS	–	++	++	++	–	–	–	–	+	–	–	–	+
LCS	–	–	±	–	–	+	++	+	–	–	–	–	–
Indeterminate cell	–	–	±	–	–	++	++	–	–	–	–	–	–
IDC	–	–	±	–	–	++	–	–	++	–	–	±	–
FCT[a]	–	–	±	–	–	–	–	–	±	++	++	++	–

[a]FCT included for historical purposes but no longer considered histiocytic/dendritic cell neoplasia.
FCT, Follicular cell tumors; *HS,* histiocytic sarcoma; *IDC,* interdigitating cell sarcoma; *JXGS,* juvenile xanthogranuloma-like histiocytic sarcoma; *LCS,* Langerhans cell sarcoma.

Immunohistochemistry

The immunophenotypic pattern is variable; the cells generally stain for LCA and CD45RO. Some have a more macrophage-like pattern with expression of CD14, CD68, CD163, and variable Golgi/paranuclear lysozyme expression. Typical cases are CD1a/Langerin negative. Others are more dendritic-like with S100, fascin, or even Langerin in a few. Both B- and T-cell–specific markers are absent, and CD30 and ALK are not expressed. Cases with JXG phenotype, including variable factor 13a and fascin, are described (vide infra).

Differential Diagnosis

The differential diagnosis is wide—generally that of other large-cell lymphomas with epithelioid features; T-cell, B-cell, or even granulocytic/monocytic sarcomas; Hodgkin lymphoma;

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metastatic carcinomas; epithelioid sarcoma; and melanoma. Monocytic and monoblastic leukemias must be excluded by bone marrow examination.

Prognosis and Therapy

For patients with localized disease, the outcome following tumor removal and therapy may be good. The majority of patients have high-stage disease at presentation, and the biologic behavior is that of a high-grade sarcoma with aggressive clinical course, poor response to therapy, and high mortality (60% to 80%) from progressive disease. *BRAF*-mutated cases may benefit from targeted therapy such as with vemurafenib.

Histiocytic Sarcoma With Juvenile Xanthogranuloma Phenotype

A histiocytic lesion with sarcomatous cytologic features and a rapidly malignant clinical course has been seen. Generalizations about the clinical presentation are not warranted because of the rarity; however, the constituent atypical histiocytes have the JXG family appearance and phenotype of CD14, CD163, CD68, FXIIIa, and fascin, with negative S100 (Fig. 19.23).

Langerhans Cell Sarcoma

Langerhans cell sarcoma is a high-grade malignant proliferation of dendritic histiocytes that have an immature Langerhans cell phenotype. Although one case evolved from a prior Langerhans cell histiocytosis, almost all arise de novo in adults. The tumor develops as soft-tissue masses, nodal tumors, or as multiorgan involvement with spleen, liver, lung, and bone marrow disease. Most cases are multifocal, high-stage disease, fewer are primarily nodal. The mean age is 46 years (range, 8 to 81 years) with an equal sex ratio.

Pathologic Features

When lymph nodes are involved, the pattern is that of sinus spread but may be obscured by diffuse effacement. The constituent cells are large and oval to spindled, with malignant to anaplastic cytologic features, variation in nuclear size and shape, and atypical mitoses. The nuclei may be grooved or folded, like the typical complex membrane foldings of the LCH cells, or more anaplastic. Mitoses including atypical forms are common. The cytoplasm is generous and eosinophilic. Interspersed eosinophils are sparse.

Ancillary Studies

Molecular and Cytogenetic Features

Like LCH, activating kinase mutations in *BRAF*-V600E and other MAP kinase pathway mutations are described in case report findings, given the rarity of disease.

Electron Microscopy

Birbeck granules are present, but electron microscopy has largely been supplanted by the use of Langerin immunohistochemistry.

Immunohistochemistry

Immunohistochemistry reveals the same phenotype as the LCH cell (i.e., $S100^+$, $CD1a^+$, $Langerin^+$), although expression may be more variable (Fig. 19.24). Ultrastructural confirmation of a Birbeck granule is usual. CD68 is present in small amounts in a paranuclear distribution. Lysozyme is not present, and follicular DC markers, CD21, and CD35 are absent. CD56 expression is said to identify a more refractory clinical subtype but has not been further substantiated. Rarely, CD30 may be expressed.

Differential Diagnosis

Distinction from LCH is based on the cytologic pleomorphism and, in most instances, the anaplasia and atypical mitoses. The differential diagnosis includes LCH and other $CD1a^+$ infiltrates, such as some acute myeloid leukemias, and rarely $CD1a^+$ T-lymphoblastic leukemias and lymphomas. Indeterminate cell histiocytosis (and sarcoma) refers to a related condition that is $CD1a^+$ but lacks the Birbeck granule or Langerin expression. Because LCS is variable in its phenotypic expression, there may not be a clear dividing line between indeterminate cell histiocytosis (and sarcoma) and LCS. The phenotype distinguishes LCS from other spindle cell sarcomas, most notably interdigitating DC tumors. However, the potential always exists for more extreme phenotypic ambiguity in which the divide between an HS with dendritic cell expression (e.g., variable CD163, CD68, light LCA) and LCS with an incomplete phenotype (e.g., variable patchy S100, Langerin, negative CD1a) cannot be made. It is plausible that in these cases with mixed/heterogeneous lineage that the old term *malignant histiocytosis* or rather *histiocytic malignancy of incomplete dendritic cell phenotype* may be most appropriate.

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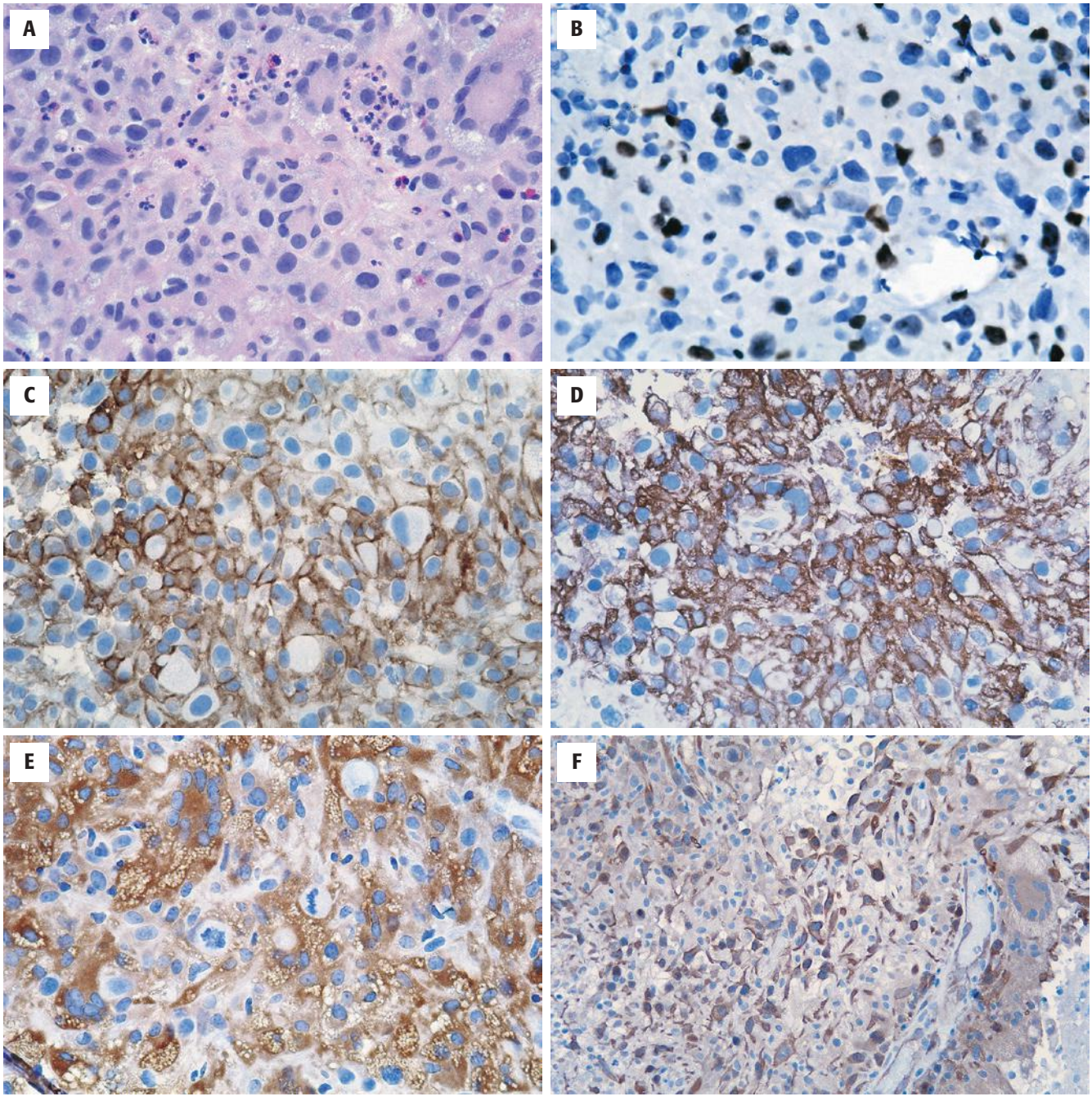


FIG. 19.23

Histiocytic sarcoma, juvenile xanthogranuloma phenotype. Adult female with brain lesions. A, The histologic appearance is that of juvenile xanthogranuloma with a Touton cell, but there is pleomorphism and tumor necrosis. B, Cell cycle is vigorous, Ki-67 immunostain. The phenotype is that of the juvenile xanthogranuloma family, CD14 (C), CD163 (D), factor XIIIa (E), fascin (F), CD68 (not shown), and no S100 (not shown).

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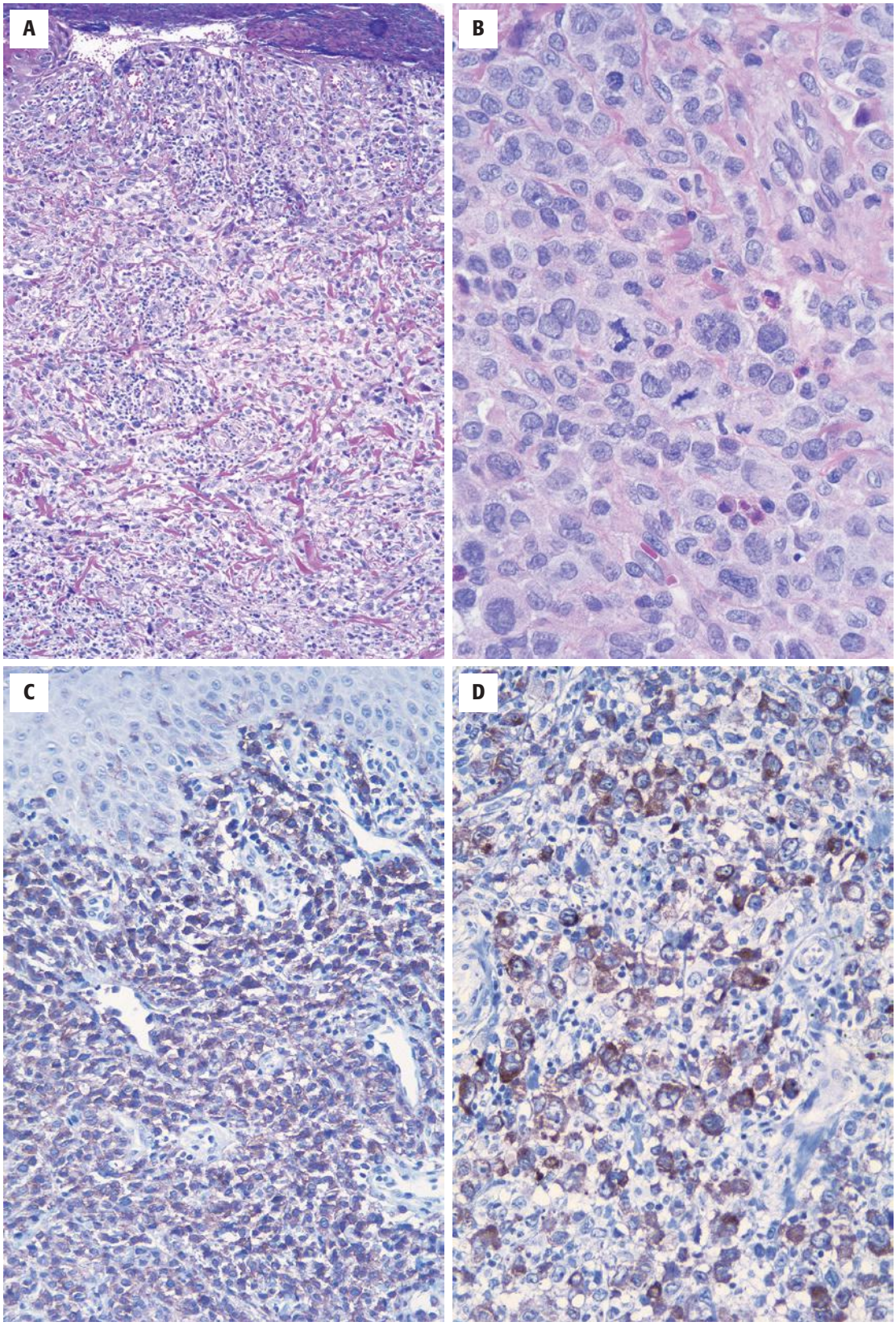


FIG. 19.24

Langerhans cell sarcoma. A, The lesions have more nuclear pleomorphism than would be expected from LCH. B, Atypical mitosis is present. The Langerhans phenotype requires (C) CD1a and (D) Langerin expression.

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Prognosis and Therapy

Langerhans cell sarcoma is aggressive, with greater than 50% mortality from progressive disease. *BRAF* mutated cases may benefit from targeted therapy such as with vemurafenib.

Indeterminate Cell Sarcoma

Clinical Features

Indeterminate cell sarcoma is rare, primarily affecting older adults. Most are either cutaneous, soft tissue, or nodal in presentation. Secondary indeterminate cell lesions can follow prior lymphomas or leukemia and can share the genetic signature of the primary tumor, similar to the other described histiocytic malignancies.

Pathologic Features

Microscopic Findings

Indeterminate "dendritic" cell sarcoma is a high-grade lesion with similarities to LCS but is defined by its lack of Langerin and the ultrastructural Birbeck granule despite its expression of $S100^+/CD1a^+$. The lesions generally lack the convoluted nuclear morphology seen in LCS. The biological spectrum is wide, from low-grade and localized lesions to more anaplastic high-grade "sarcoma."

Ancillary Studies

Molecular

BRAF mutations have been described, but the *ETV3-NCOA2* translocation described in indeterminate cell histiocytic lesions has not yet been described in indeterminate cell sarcoma.

Electron Microscopy

Although few studies have specifically addressed, it is presumed that with the lack of Langerin staining Birbeck granules would be absent in such cases.

Immunohistochemistry

Like in LCS, the defining feature is that of dendritic cell phenotype lacking Langerin expression with $S100^+/CD1a^+$. The expression of CD68 may be that of paranuclear dot/Golgi expression, which is noted in dendritic cells.

Differential Diagnosis

The differential diagnosis includes LCS that is, by definition $CD207^+$. However, LCS can progressively lose is markers, including CD207, and mimic indeterminate dendritic cell sarcoma. Other $CD1a^+/CD207(-)$ high-grade infiltrates include some myelomonocytic and rare T-cell leukemias.

Prognosis and Therapy

Although indeterminate dendritic cell sarcoma has morphologic similarity to LCS, biological outcome in reported cases has varied, with suggestion of a more aggressive tumor in some.

Interdigitating Dendritic Cell Sarcoma

Interdigitating cell sarcoma is a malignant proliferation of cells that have a phenotype of the mature paracortical interdigitating DC of the lymph node. A close relationship between spindle cell melanoma may exist, which has called into question its unique classification, although ultrastructural differences differentiate.

Clinical Features

The tumors develop as masses, most commonly involving lymph nodes, but extranodal sites such as the nasopharynx, intestine, retroperitoneum, mesentery, skin, and testis have been described. Although commonly asymptomatic, B symptoms and signs including diffuse lymphadenopathy occur in patients with high-stage disease. The median age is 58 years (range, 2 to 88 years) with sex parity.

Pathologic Features

Gross Findings

The tumors have a lobular pattern and are firm as befitting spindle cell lesions, with gross necrosis being unusual.

Microscopic Findings

When lymph nodes are partially involved, the lesions may appear to be paracortical, a helpful distinction from the follicular lesions. Like the other DC sarcomas, the interdigitating cell sarcoma is cytologically malignant, but not anaplastic,

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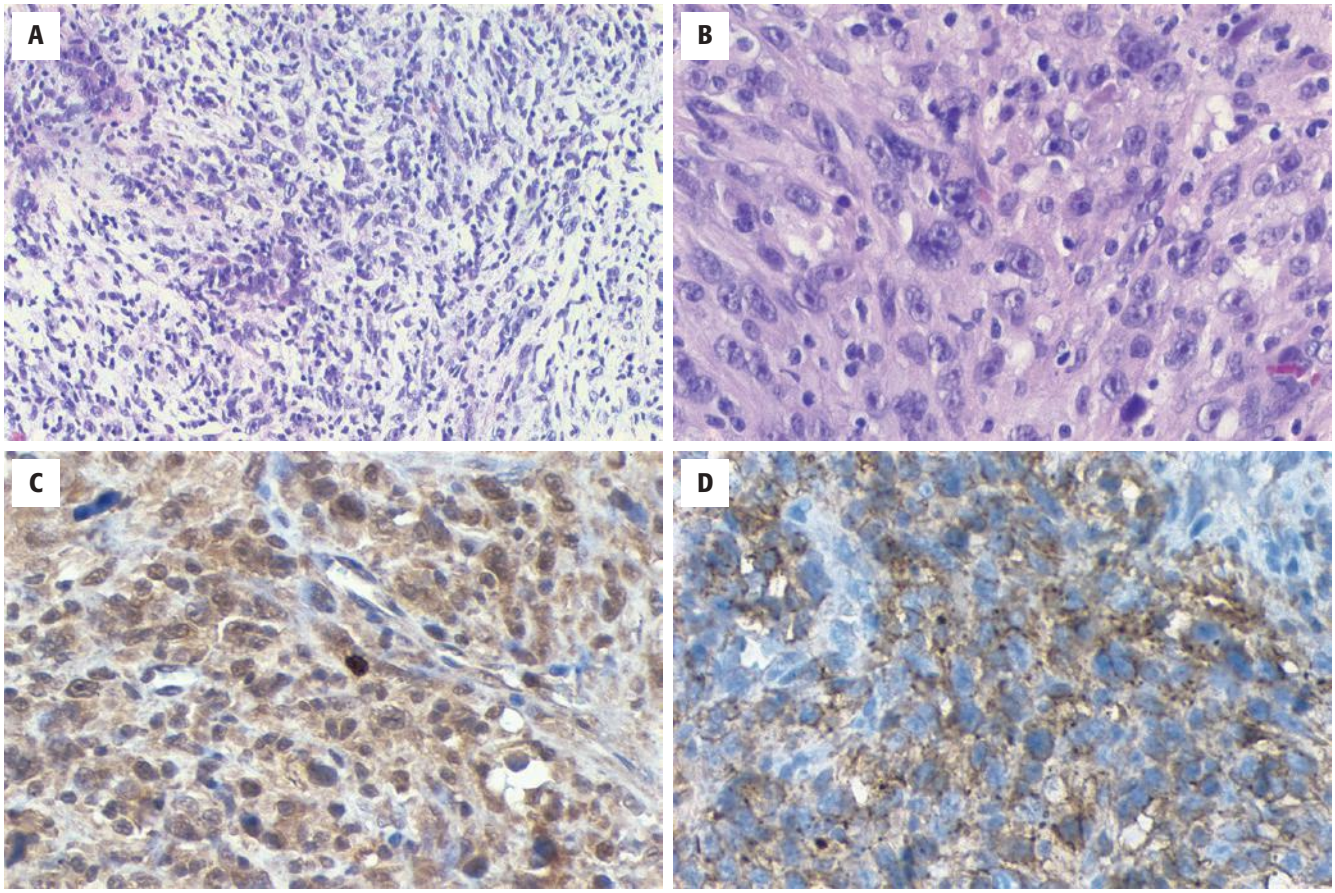


FIG. 19.25

Interdigitating cell sarcoma. A and B, The lesions are generally spindled with high-grade nuclear features. The phenotype is that of mature dendritic cells with high (C) S100 expression and (D) human leukocyte antigen HLA class II.

and has the general appearance of a spindle-cell sarcoma of medium-sized cells (>20 μm) that forms fascicles and whorls. Round and oval cell areas may be present and can predominate (Fig. 19.25). Nuclei are oval to elongated and chromatic with finely aggregated chromatin and a distinct small nucleolus. Rarely, the nuclei can be folded and simulate Langerhans cell nuclei. Small T cells commonly abound between the spindle cells.

Ancillary Studies

Molecular

BRAF mutations have been described in rare cases of interdigitating cell sarcoma. Rare cases of interdigitating cell sarcoma associated with B-cell lymphomas (e.g., follicular lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma) either following or concomitant share clonal immunoglobulin gene rearrangements with the lymphoma and may also display a variety of cytogenetic abnormalities including deletion, aneusomy, and duplications of chromosome 17p.

Ultrastructural Features

Spindled lesions have long, complex interdigitating cytoplasmic extensions, but the features of Langerhans cells (Birbeck granule), follicular DCs (true desmosomes), basal lamin, or melanosomes are not demonstrable.

Immunohistochemistry

The immunohistologic profile is that of a mature DC, although there are gaps in their characterization. CD45 is negative (or weak), although CD45RB is expressed. The spindle cells express vimentin, high S100, high HLA class II, and high fascin (see Fig. 19.25). CD30 and mature DC markers are absent. Small amounts of CD68 have been reported in some, but other macrophage markers (CD163) are absent, and neither Langerhans cell (CD1a, Langerin) nor follicular DC markers (CD21, CD35) are described. Clusterin is identified only at low levels. Proliferative activity (assessed using

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the MIB-1 antibody) is generally modest (~10%), and p53 expression has been mentioned as a correlate of poor outcome.

Differential Diagnosis

The differential diagnosis is wide and covers the gamut of spindle cell sarcomas, the other DC sarcomas, and the fibrohistiocytic lesions. Muscle tumors have a spectrum of myoid markers. Inflammatory pseudotumors are recognized by their myofibroblastic qualities and occasional ALK expression. Melanomas (also $S100^{+}$) have HMB45, SOX10, and tyrosinase. The nodal site, paracortical involvement, and phenotype are distinctive.

Prognosis and Therapy

The interdigitating cell sarcoma appears to be aggressive, with remissions and local recurrences but with a 50% mortality rate. Localized disease that is resected, however, may have a much better outcome.

Follicular Dendritic Cell Tumors and Sarcomas

The follicular dendritic cell (FDC) is reputed to be of mesenchymal, non-hematopoietic origin, and follicular dendritic cell tumors are not included in the review of histiocytic lesions or considered in the revised classification of histiocytoses and neoplasms of macrophage-dendritic cell lineages. A brief overview of the lesion is included herein for historical purposes and completeness.

Clinical Features

Follicular DC tumors, including sarcoma, are spindle cell lesions that develop as a large, slow-growing mass in nodal or extranodal sites. Adults are primarily affected, with no sex predilection, except for the inflammatory pseudotumor variant that has a marked female preponderance. There are associations with hyaline-vascular Castleman follicular hyperplasia and, rarely, with EBV. Most tumors arise in cervical lymph nodes, and mediastinal and retroperitoneal involvement is also noted. Extranodal sites include skin and soft tissue, tonsil, gastrointestinal tract, liver, and spleen. The lung is the most usual metastatic site; lymph nodes and liver are involved less commonly. Systemic symptoms are more common in the inflammatory pseudotumor variant.

Pathologic Features

Gross Findings

Tumors are solid and firm, with smaller lesions being seen in lymph nodes and larger lesions in visceral organs or soft tissue. The borders are smooth and well demarcated and push into the surroundings.

Microscopic Findings

In lymph nodes the growth pattern can be serpiginous, leaving a residual node between the nodules. The cells are spindle shaped, and the lesions are commonly highly cellular with a tight, whorling, meningioma-like pattern. The cell shape varies from elongated spindle cells to plump or ovoid, with moderate amounts of brightly eosinophilic cytoplasm and indistinct cell borders. Nuclei are usually single with occasional binucleated or multinucleated cells. The nuclear outline is oval, and the degree of pleomorphism can vary, although uniformly oval nuclei are the rule. The nuclear membrane is sharp, and the nuclei are chromatic with fine to coarsely granular chromatin and small distinct nucleoli. High-grade lesions have prominent nuclear size variability with frequent and atypical mitoses greater than 30 per 10 high-power fields. These lesions also have necrosis. Lymphocytes are mostly B cells, typically interspersed, although sometimes are in broad sheets or aggregated around blood vessels. The inflammatory pseudotumor-like variant of follicular DC sarcomas involves the liver and spleen predominantly. It has spindle cells dispersed in a background of lymphocytes and plasma cells with foci of necrosis and hemorrhage.

Ancillary Studies

Molecular

Recent work has shown *BRAF*-V600E mutations in FDC sarcomas, including in a subset of inflammatory pseudotumor-like FDC sarcomas. The impact of this mutation on prognosis and pathogenesis is not known. The inflammatory variant of follicular DC tumor has nuclear EBV RNA (EBER in situ hybridization positive), and Southern blot studies reveal the presence of virus in monoclonal episomal form.

Ultrastructural Features

The spindle cells have cytoplasmic projections that interdigitate and display typical desmosomes. Organelles, including lysosomes, are sparse, and no Birbeck granules are present.

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Immunohistochemistry

The tumors have a unique and consistent phenotypic profile that includes the follicular DC markers CD21 and CD35. CD23, CD68, fascin, and clusterin are also expressed, but CD1a, S100, actin, desmin, and cytokeratin are not. EMA has been reported to be present in a variable amount in most cases.

Fine-Needle Aspiration Biopsy

Spindle to oval cell lesions have interspersed lymphoid cells. The phenotypic confirmation is required.

Differential Diagnosis

The differential diagnosis includes the spindle cell lesions most appropriate to the site of the presentation. In the lymph node, distinction from other DC lesions, such as the interdigitating cell sarcoma, nodular sclerosing Hodgkin lymphoma, and fibroblastic reticular cell tumor, is required. In soft tissues, liver, and spleen, the inflammatory myofibroblastic tumors, angiomatoid fibrous histiocytoma, and meningioma must be excluded. In the gastrointestinal tract, gastrointestinal stromal tumors and smooth muscle tumors are similar, as are the spindle cell posttransplant lesions and the mycobacterial spindle cell pseudotumor.

Prognosis and Therapy

The biological behavior is generally indolent and low grade, with localized lesions being treated by excision, but late local recurrence is seen in 50% of cases. Eventually, distant metastases can occur in 25% of patients, and less than 20% eventually die of their disease. Large lesions and those that are organ based and that have cytologic anaplasia and necrosis may have a more fulminant course.

Dendritic Cell Sarcoma, Not Otherwise Specified

The designation *dendritic cell sarcoma, not otherwise specified,* is reserved for the rare lesions that do not fit easily into one of the defined DC categories—Langerhans cell, interdigitating cell, or follicular cell—as well as the juvenile xanthogranuloma phenotype of HS. Because there are occasional lesions with hybrid features that cross rigid and rather arbitrary boundaries, they too could occupy this category.

Fibroblastic Reticular Cell Sarcoma

The provenance of this lesion is undetermined, likely of mesenchymal origin, but included here because of the histopathologic resemblance to the nodal DC lesions. The reticular cell forms a complex network of channels in the lymph node paracortex. Lesions have been described in adolescents and adults, and the key to their phenotypic differentiation from the DC lesions is their consistent lack of the Langerhans, interdigitating, and follicular phenotypes, but they strongly express cytokeratins and vimentin, with variable, actins, desmin, and CD68.

CONCLUSION

Large international registries sponsored by the Histiocyte Society, including the International Rare Histiocytic Disorders Registry (IRHDR) (NCT02285582, https://clinicaltrials.gov/ct2/show/NCT02285582) and LCH-IV (NCT02205762, https://clinicaltrials.gov/ct2/show/NCT02205762?term=lch&rank=1), are positioned to collect epidemiologic data on disease presentation, treatments, and outcomes of patients with non-LCH and LCH histiocytic disorders, respectively. It is only through the dedicated international collaboration of pathologists, clinicians, and scientists that we can continue to make sustained progress in the pathogenesis and best-practice treatment regimens for these rare diseases.

The understanding of histiocytic disorders and neoplasms of the macrophage-dendritic cell lineages can be challenging, if not a minefield at times, given their varied and sometimes overlapping features with one another, much akin to their functional counterparts of the mononuclear phagocytic system. Whereas the discovery of molecular aberrations is helping to further understand the pathogenesis of these disorders, including refining the classification of LCH and ECD as "inflammatory myeloid neoplasms," the "molecular microscope" has only exposed the tip of the iceberg. The coming years will no doubt shed more light into the cellular origins of this enigmatic group of diseases. The latest attempt to revise the classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages has proposed grouping this diverse group of over 100 clinical entities into five main groups (see Box 19.1) based on clinical, histologic, and molecular relevance. At the writing of this chapter, the 2016 WHO classification for histiocytosis and neoplasms of the macrophage-dendritic cell lineages has only recently recognized ECD as a separate category. The clinical/pathologic relevance of this revised classification will ultimately determine if this new grouping is further adopted into practice as a framework for further study.

The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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SUGGESTED READING

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Mastocytosis

■ Dong Chen, MD, PhD ■ Tracy I. George, MD

■ INTRODUCTION

Mast cells (MCs) were initially recognized in 1878 by Paul Ehrlich, a Nobel laureate in immunology. They are unique inflammatory mediator cells that contain distinctive metachromatic and electron-dense cytoplasmic granules. MCs derive from bone marrow pluripotent hematopoietic progenitors. The committed bone marrow MC progenitors are first released into the bloodstream, then they transmigrate and subsequently mature in various organs under the influence of a complex network of cytokines and growth factors. The central piece of this network is activation of the tyrosine kinase receptor, *KIT*, by its ligand, stem cell factor (SCF).

Phenotypically, the MC progenitors express CD13, CD34, CD38, and CD117. Once committed, MCs downregulate CD34 (and several other receptors), but maintain strong CD117 expression. Under normal physiologic conditions, MCs and their precursors do not express CD2, CD25, CD30, CD35, or CD123. MC granules contain numerous mediators, such as tumor necrosis factor-α, histamine, acid hydrolases, cathepsin G, and carboxypeptidase. On activation by numerous stimuli in either an immunoglobulin E receptor–dependent or an independent manner, MCs instantaneously release these mediators and generate lipid-derived substances that induce abrupt allergic responses in skin, gastrointestinal, cardiovascular, respiratory, and neurologic systems.

Mastocytosis, also known as *mast cell disease*, denotes a heterogeneous group of disorders characterized by abnormal growth and accumulation of morphologically and immunophenotypically aberrant MCs in skin (cutaneous mastocytosis [CM]) or in one or more extracutaneous systems (systemic mastocytosis [SM]). Childhood CM frequently regresses spontaneously, whereas virtually all adult-onset mastocytosis skin lesions represent SM. Most mastocytosis, especially SM, are clonal disorders. The aberrant MC proliferation and release of MC granular contents accounts for the unique clinicopathologic features of mastocytosis. Over the past several years, various proposals for mastocytosis classification have been published. The first World Health Organization (WHO) classification system was established in 2001. Later, it was slightly modified in 2008 and 2016. We focus this chapter on the 2016 WHO classification system and other provisional or uncommon entities.

Clinical Features

Cutaneous mastocytosis is more frequent in children than SM, and 80% of children with CM have disease manifestation by 6 months of age. Conversely, virtually all cases of SM occur in adults after age 30 years, and its occurrence peaks between the ages of 50 and 80 years. Depending on the pattern of referral, CM presenting as urticaria pigmentosa (UP) is associated with 50% to 100% of patients with SM. No significant sex predilection has been reported for either CM or SM.

Based on disease burden and severity, clinical manifestations of mastocytosis are organized into four major categories: skin manifestations (e.g., pruritus, urticaria, dermatographism), mediator release syndromes (e.g., abdominal pain, diarrhea, nausea and vomiting, hypertension, headache, flushing, syncope, hypotension, tachycardia, respiratory symptoms), constitutional symptoms (e.g., fatigue, weight loss, fever, sweats), and organ dysfunction from tissue infiltration by neoplastic MCs.

Cutaneous manifestations, including flushing, pruritus, urticaria, and dermatographism due to local release of MC mediators, are the most common symptoms in mastocytosis. Structural lesions are the most common form, represented as red-brown macules, papules, and plaques of UP/maculopapular CM (UP/MPCM) (Fig. 20.1). The lesions of UP/MPCM tend to cluster on the upper arms, shoulders, trunk, and thighs while sparing the palms, soles, face, and scalp. Telangiectases, petechiae, or ecchymoses may occur in the lesions or in adjacent skin. Other rarer cutaneous manifestations, including mastocytoma and diffuse CM, are found in children. Lesions of mastocytoma (solitary or numerous) are red-brown nodules or plaques, up to several centimeters in diameter. Diffuse CM may appear as grossly normal skin or as red-brown, thickened, edematous skin with an orange-peel texture. The cutaneous lesions of all forms may induce urticaria when stroked (Darier sign). Blistering

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FIG. 20.1

Cutaneous mastocytosis. A, The most common cutaneous lesions in adults with mastocytosis (i.e., urticaria pigmentosa) are red-brown macules in large numbers with highest density on the trunk. B, In children, the lesions tend to be papular and fewer in number.

or bullous formation, an exaggerated Darier sign, is frequently observed in children.

Mediator release symptoms (MRS) caused by MC degranulation and the release of biologic amines (e.g., histamine), proteolytic enzymes (e.g., tryptase), proteoglycans (e.g., heparin), prostaglandin D_2, and leukotriene C_4 include recurrent syncope, hypotensive shock, severe bone pain, headache, lightheadedness, anaphylaxis, flushing, pruritus, urticaria, and gastrointestinal (GI) symptoms. Identification of the characteristic skin lesions, as well as associated MRS, is the key to early recognition of mastocytosis. Importantly, an initial manifestation of SM may masquerade as GI, cardiovascular, respiratory, skeletal, or neuropsychiatric symptoms, with or without skin involvement.

GI symptoms due to either MC mediators or direct infiltration are frequent manifestations in mastocytosis. The common symptoms are abdominal pain, diarrhea, nausea, and vomiting. Abdominal pain may manifest as either typical dyspeptic pain or nondyspeptic discomfort. The former actually may be associated with peptic disease. Nondyspeptic pain occurs often in association either with consumption of alcohol or certain foods, or with stress. Involvement of the small intestine beyond the duodenum may cause lower abdominal pain, diarrhea, and steatorrhea secondary to malabsorption.

Cardiovascular symptoms include syncope and episodic vascular collapse. Episode severity and frequency vary greatly; fatalities may occur in patients with severe and prolonged hypotension. They sometimes are preceded by lightheadedness or palpitations or are precipitated by exposure to specific medications, sudden temperature alterations, friction (rubbing of skin), alcohol consumption, or stress.

Respiratory manifestations, caused by MC mediators, include wheezing, dyspnea, and rhinitis. Occasionally, they can be part of anaphylactic shock and have a significant impact on the patient's morbidity and mortality. Skeletal symptoms are related to bone involvement and include bone pain that usually affects the long bones, fractures, and severe osteoporosis with deforming kyphoscoliosis. Neuropsychiatric symptoms include various types of headaches, including a typical mild frontal, dull, non-pounding type, a vascular variant with migraine characteristics, and headache associated with rhinorrhea, pruritus, and lacrimation suggestive of a histaminergic variety. Other

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neuropsychiatric symptoms include decreased attention span, difficulty in concentration, forgetfulness, irritability, depression, poor motivation, confusion, anger, anxiety, lethargy, and somnolence.

Radiologic Features

Patients with SM may have distinct skeletal or abdominal radiologic abnormalities. Diffuse or, much less frequent, circumscribed bone lesions, including osteoporosis, osteosclerosis, or both, occur in about 70% of cases (Fig. 20.2). Scintigraphic bone scans are generally more sensitive than routine radiographs in detecting SM-related bone abnormalities. The diffuse lesions often are poorly demarcated with various degrees of osteoblastic and osteolytic changes. Widely spread diffuse bone lesions usually accompany an SM progression. The circumscribed lesions radiologically may resemble metastatic carcinoma, multiple myeloma, or Paget disease.

The frequency of abnormal radiographic findings in the GI tract varies from 10% to 38% in the various reported series of SM. The most frequent radiographic findings in the



FIG. 20.2

Systemic mastocytosis. Radiologic features of shoulder and upper extremity show mixed osteoblastic and osteolytic lesions.

CUTANEOUS AND SYSTEMIC MASTOCYTOSIS—FACT SHEET

Definition

- Accumulation of clonal abnormal mast cells in skin and/or any extracutaneous organ(s)

Incidence and Location

- Rare, estimated annual incidence, 6.6 per 1 million persons
- Predominantly involves the skin, bone marrow, liver, spleen, lymph nodes, and gastrointestinal tract

Sex, Race, and Age Distribution

- No sex predilection in cutaneous mastocytosis
- Male-to-female ratio in systemic mastocytosis is 1 : 1.3
- More frequently reported in Caucasian patients
- CM is most common in children, with 80% showing lesions by 6 months of age
- SM is most common in adults >30 years of age

Clinical Features

- Skin: pruritus, urticaria, dermatographism, pigmentation
- Constitutional: fatigue, weight loss, fever, sweats
- Skeletal: bone pain, arthralgia, fractures
- Mediator-release symptoms
  - Abdominal pain, diarrhea, nausea and vomiting
  - Headache, hypertension, flushing
  - Syncope, hypotension, tachycardia

Radiologic Features

- Bone: osteoporotic and osteosclerotic changes
- Gastrointestinal: peptic ulcer, abnormal mucosal patterns, motility disturbances
- Hepatosplenomegaly
- Possible retroperitoneal lymphadenopathy

Morbidity and Mortality Rates

- All mastocytosis types may have disabling symptoms related to mast cell mediator release
- Systemic mastocytosis with associated hematologic neoplasm: clinical course determined by the associated hematologic disorder
- Advanced systemic mastocytosis: progressive course with organ dysfunction
- Mast cell leukemia and myelomastocytic leukemia: most aggressive, with survival of weeks or months

Prognosis and Therapy

- Pediatric cutaneous mastocytosis mostly regresses spontaneously
- Prognosis of systemic mastocytosis is directly correlated with 2016 WHO clinicopathologic classification
- Mediator release symptoms in cutaneous mastocytosis and indolent systemic mastocytosis can be alleviated by mediator-targeting drugs
- Advanced systemic mastocytosis (mast cell leukemia, aggressive systemic mastocytosis, systemic mastocytosis with an associated hematologic neoplasm) requires cytoreductive therapy
- Most systemic mastocytosis patients have the *D816V* KIT mutation and are resistant to imatinib
- Alternative tyrosine kinase inhibitors show promise in patients with advanced systemic mastocytosis

WHO, World Health Organization.

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GI tract are caused by peptic ulcer disease, followed by abnormal mucosal patterns and motility disturbances. The mucosal abnormalities reported in patients with SM include gastric rugal hypertrophy, coarsening or loss of mucosal folds, patchy mucosal lesions, diffuse mucosal thickening, nodular lesions, and single or multiple polyps. Motility disturbances are usually found in patients who have diarrhea. However, both rapid and delayed GI transit times have been reported. Liver and spleen scans in patients with SM usually show hepatosplenomegaly with an abnormal uptake pattern consistent with diffuse parenchymal disease.

Pathologic Features

Histology of Normal Mast Cells and Their Precursors

Normal MC progenitors undergo differentiation and sequentially acquire distinct morphologic, cytochemical and immunophenotypic properties, and metachromatic granules.

Cutaneous Mastocytosis

The diagnosis of CM requires a typical clinical presentation, histologic evidence of skin infiltration by morphologically abnormal MCs, and the absence of SM.

Gross and Histologic Findings in Cutaneous Mastocytosis

CM includes three distinct clinicohistopathologic entities: UP/MPCM (see Fig. 20.1), diffuse CM, and mastocytoma of the skin. The histopathologic feature of all CM lesions is an infiltrate by MCs (Fig. 20.3). Only partial correlation exists between the histologic patterns and the clinical appearance of lesions. The diagnosis of CM requires the demonstration of typical clinical findings and histologic evidence of cutaneous infiltration by MCs.

Urticaria Pigmentosa/Maculopapular Cutaneous Mastocytosis

Urticaria pigmentosa is the most frequent form of CM (see Fig. 20.1A). In children, the lesions tend to be papular (see

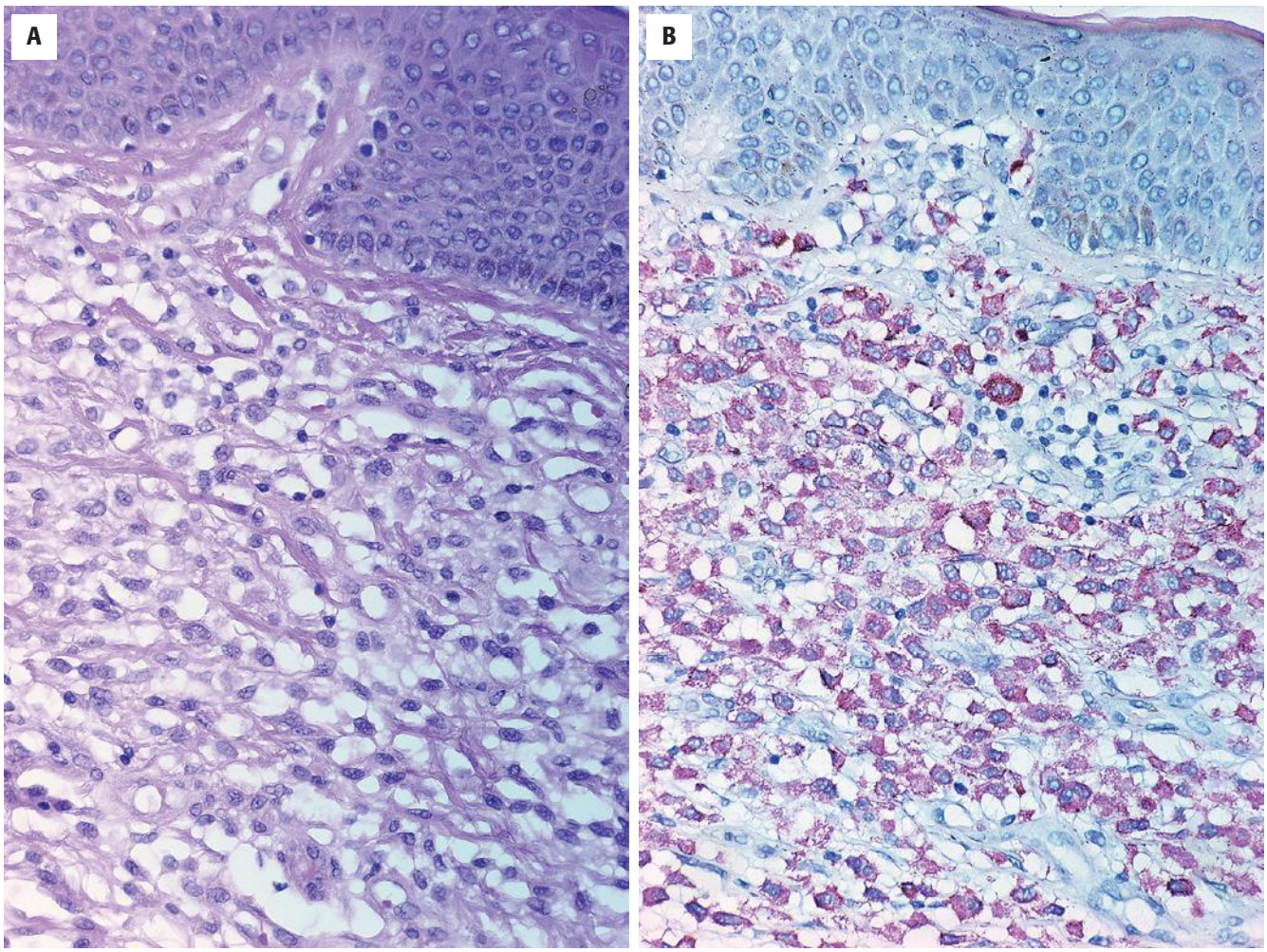


FIG. 20.3

Histologic and histochemical features of cutaneous mastocytosis. A, Section of skin biopsy specimen shows sheets and aggregates of elongated mast cells involving the upper dermis and papillary dermis (H&E-stained, original magnification ×240). B, Histochemical stain of chloroacetate esterase shows positive staining in all atypical mast cells (original magnification ×240).

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Fig. 20.1B) and are characterized by aggregates of elongated MCs, which typically fill the papillary dermis and extend as sheets and aggregates into the reticular dermis, often following the vasculature (see Fig. 20.3). In adults, the lesions tend to be macular in large numbers, more darkly pigmented, and sometimes associated with telangiectasia (see Fig. 20.1A). Telangiectasia macularis eruptiva perstans is characterized by a small number of larger lesions (2–6 mm), which are lightly pigmented telangiectatic macules with minimal or no increase in the number of MCs. Adult UP-like lesions tend to have fewer MCs than the lesions in children and often represent skin lesions found in indolent SM.

Diffuse Cutaneous Mastocytosis

This lesion is less frequent than UP/MPCM and occurs in children exclusively. Patients usually present with erythroderma involving almost the entire skin. The skin may be simply red or it may have a red to yellow color. Diffuse edema and thickening with a doughy consistency may be observed. Histologically, skin biopsy usually shows a band-like infiltrate of MCs in the papillary and upper reticular dermis. Occasionally, MCs may extend into the deep dermis in heavily infiltrated skin lesions.

Mastocytoma(s) of the Skin

Mastocytoma, a rare variant of CM that usually occurs in infants, presents as nodules or plaques larger than 1.0 cm in diameter. Usually, they are located on the extremities, but they also may occur on the face, scalp, or trunk or in any combination of these locations. Histologically, sheets of MCs with abundant cytoplasm fill the papillary and reticular dermis and may extend into the deep dermis and subcutaneous tissues.

Systemic Mastocytosis

SM is defined by involvement of at least one extracutaneous organ with or without evident CM. It is further classified into five subcategories (Box 20.1): indolent SM (ISM), smoldering SM (SSM), aggressive SM (ASM), SM with associated hematologic neoplasm (SM-AHN), and MC leukemia (MCL). In order to differentiate SM from reactive mast cell hyperplasia, the previous (2001 and 2008) and current (2016) WHO classification schemes use one major criterion and four minor criteria to distinguish the SM from a reactive MC hyperplasia (Box 20.2). The major criterion is the evident, multifocal, dense infiltrates of MCs (≥15 MCs in aggregates) in sections of bone marrow or other extracutaneous tissues or organs. Sometimes, the major criterion may not be fulfilled owing to sampling issues or the mast cell clusters may contain smaller numbers of MCs. The four minor criteria are intended to establish an aberrant clonal MC process: (1) more than 25% of MCs in biopsy sections or on bone marrow aspirate smear are spindle shaped or have other atypical or immature morphologic features; (2) detection of an activating point mutation at codon 816 of *KIT* in bone marrow, blood, or other extracutaneous organ; (3) aberrant surface MC expression of CD25, with or without CD2; and (4) serum total tryptase level persistently greater than 20 ng/mL. It is important to remember that the fourth minor criterion is only valid when there is no evidence of non-MC clonal myeloid disorder. The diagnosis of SM requires either one major criterion and one minor criterion, or three minor criteria.

Box 20.1 2016 World Health Organization (WHO) Classification and Related Clinical Findings of Mastocytosis

Classification

Cutaneous mastocytosis
- Absent systemic mastocytosis

Systemic mastocytosis (SM)

Indolent systemic mastocytosis (ISM)
- Skin lesions are frequently present. No "C" findings or SM-AHN; low mast cell burden (≤30% bone marrow infiltration by mast cells). As a subclassification of ISM, **isolated bone marrow mastocytosis** lacks skin lesions.

Smoldering systemic mastocytosis
- No "C" findings but at least 2 "B" findings.

Systematic mastocytosis with associated hematologic neoplasm (SM-AHN)
- Clonal non–mast cell lineage disorders include myeloproliferative neoplasm (MPN), MDS/MPN, MDS, AML, or other hematologic neoplasm that meets the WHO classification.

Aggressive systemic mastocytosis (ASM)
- No evidence of MCL. At least one "C" finding. Usually fewer skin lesions.

Mast cell leukemia (MCL)
- Bone marrow aspirate smears show ≥20% mast cells. Usually no skin lesions.

Mast cell sarcoma (MCS)
- Unifocal tumor with anaplastic mast cells in destructive growth pattern. No evidence of SM.

Related Clinical Findings

"B" findings
- Bone marrow biopsy showing >30% infiltration by mast cells and/or serum total tryptase level >200 ng/mL.
- Normal or borderline normal blood counts with features of myelodysplasia or myeloproliferation in non–mast cell lineage(s) that are insufficient for a definitive diagnosis of AHN.
- Hepatomegaly without impairment of liver function; and/or splenomegaly without hypersplenism; and/or lymphadenopathy.

"C" findings
- Cytopenias (ANC <1.0 × 10^9/L, Hgb <10 g/dL, or platelet count <100 × 10^9/L).
- Palpable hepatomegaly with impairment of liver function, ascites, and/or portal hypertension.
- Skeletal involvement with large osteolytic lesions and/or pathologic fractures.
- Palpable splenomegaly with hypersplenism.
- Malabsorption with weight loss due to GI mast cell infiltration.

From Horny H-P, Akin C, Arber DA, et al: *World Health Organization classification of tumors of haematopoiesis and lymphoid tissues,* ed 4, Lyon, France, 2016, IARC Press.

Microscopic Findings in Systemic Mastocytosis: Bone Marrow Aspiration and Biopsy

Morphologic examination of bone marrow aspirate and biopsy specimen is a critical step in the workup of SM. It is

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Box 20.2 Criteria for Cutaneous Mastocytosis (CM) and Systemic Mastocytosis (SM)

Cutaneous Mastocytosis

Skin lesions show the typical clinical findings of urticaria pigmentosa/maculopapular CM, diffuse cutaneous mastocytosis, or solitary mastocytoma and typical histologic infiltrates of mast cells in a multifocal or diffuse pattern in an adequate skin biopsy specimen. In addition, a diagnostic prerequisite for the diagnosis of CM is the absence of features/criteria sufficient to establish a diagnosis of SM.

Systemic Mastocytosis

The diagnosis of SM may be made when 1 major criterion and 1 minor criterion are present or when 3 minor criteria are fulfilled.

A. Major Criterion

Multifocal, dense infiltrates of mast cells (≥15 mast cells in aggregates) in bone marrow and/or other extracutaneous organ(s).

B. Minor Criteria

1. In biopsy sections of bone marrow or other extracutaneous organs, >25% mast cells in the infiltrate are spindle shaped or have atypical morphologic characteristics. Alternatively, of all mast cells in a bone marrow aspirate smear, >25% are immature or atypical.
2. Detection of an activating point mutation at codon 816 of *KIT* in bone marrow, blood, or other extracutaneous organ.
3. Mast cells in bone marrow, peripheral blood, or other extracutaneous organ(s) express CD25 with or without CD2 in addition to normal mastocytosis markers.
4. Serum total tryptase level are persistently >20 ng/mL (unless an associated clonal myeloid disorder is present, in which case this parameter is not valid).

From Horny H-P, Akin C, Arber DA, et al: *World Health Organization classification of tumors of haematopoiesis and lymphoid tissues,* ed 4, Lyon, France, 2016, IARC Press.

TABLE 20.1

Cytochemical and Immunohistochemical Characteristics of Hematopoietic Cells With Prominent Cytoplasmic Granules and/or Positive for Tryptase

Stain	Mast Cells	Basophils	Tryptase-Positive Myeloblasts
Cytochemical			
Peroxidase	–	–	++
Toluidine blue	+++	++	±
Chloroacetate esterase	+++	–	+
Aminocaproate esterase	++	–	±
Immunohistochemical			
Tryptase	+++	±	+
CD117 (c-kit)	+++	–	–
CD34	–	–	±
CD25	++/–[a]	–	±

[a]Positive in abnormal clonal mast cells, but negative in normal or reactive mast cells and well-differentiated systemic mastocytosis.
+++, Strongly positive; *++*, moderately positive; *+*, weakly positive; *±*, either positive or negative; –, negative.

important to keep in mind that MC lesions commonly consist of patchy clusters of spindle-shaped MCs with associated reticulin fibrosis. Therefore, when evaluating a bone marrow aspirate smear, it is essential to pay close attention to the thick, inadequately spread marrow particles and the poorly stained areas where the abnormal MC clusters commonly present. Careful examination of adequately spread and stained areas surrounding these thick marrow particles can identify individual atypical MCs commonly intermixed with eosinophils. Since MC aggregates are usually associated with focal fibrosis, cytologic atypical MCs may not be readily visible on a bone marrow aspirate smear. Therefore, in general, bone marrow aspirate smear is less sensitive than bone marrow biopsy sections.

In contrast to normal MCs with abundant cytoplasmic granules and centrally located round nuclei (Fig. 20.4A and B), neoplastic MCs in SM are cytologically atypical, which can be categorized into four types. Type I atypical MCs have excessive cytoplasmic extensions, elongation of nuclei, and hypogranular cytoplasm (Fig. 20.4C and D). These atypical features are typically seen in ISM. However, the variation is wide in the degree of cytoplasmic extensions, nuclear elongation, and cytoplasmic hypogranulation among cases or even within the same case (Fig. 20.5A–C). In rare cases, the cytologic features of individual bone marrow MCs may be indistinguishable from normal mature MCs but show an excessive number of MCs that are often present in sheets (Fig. 20.5D). Such cases recently have been described as a potential new variant, well-differentiated SM (WDSM). MCs in WDSM need to be distinguished from other tryptase-positive round cell infiltrates, including tryptase-positive blasts in acute myeloid leukemia or neoplastic basophils in acute basophilic leukemia and chronic myeloid leukemia, through cytochemical or immunophenotyping studies (Table 20.1). Type II atypical MCs are promastocytes exhibiting bilobed or multilobed nuclei that are more often seen in ASM and MCL. Last, metachromatic blasts with immature chromatin and a few scattered metachromatic granules can also be seen (Fig. 20.6). These MCs are more common in MCL and myelomastocytic leukemia (MML); MML is discussed later in this chapter.

Apart from MCs, all other lineages of the bone marrow should be examined to identify concomitant myelodysplastic syndrome (MDS), myeloproliferative neoplasm (MPN), MDS/MPN, or acute myeloid leukemia. The associated hematologic neoplasm (AHN) found with SM is usually of myeloid origin, but concomitant lymphomas and plasma cell neoplasms have also been described. In such cases, the diagnosis of SM-AHN may be established when diagnostic criteria of both SM and the non-MC clonal hematologic malignancy are fulfilled.

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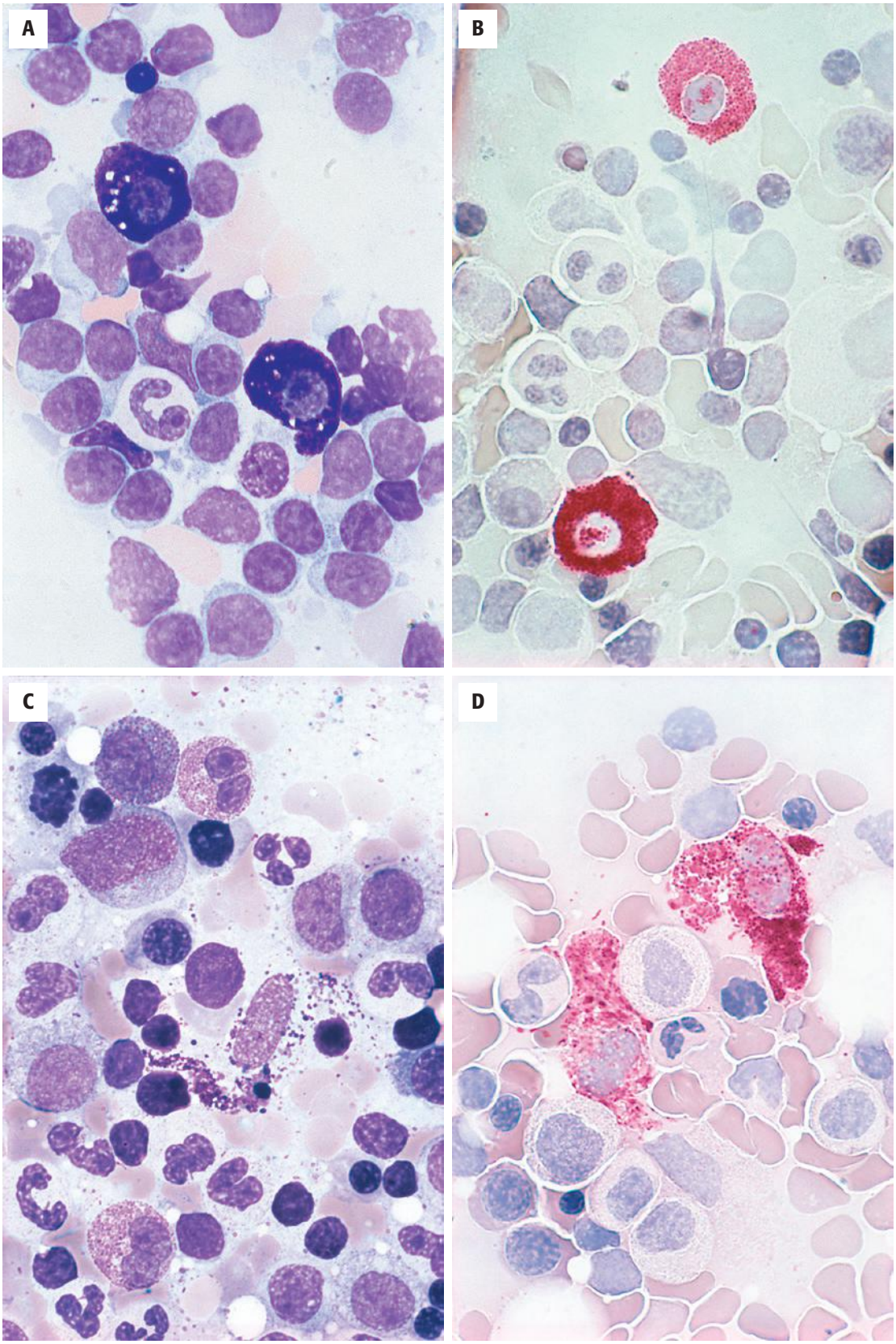


FIG. 20.4

Microscopic features and cytochemical characteristics of normal and atypical mast cells. A, Normal or reactive mast cells are characterized by a centrally located round nucleus and abundant basophilic granules packed in the well-defined cytoplasm (Wright-Giemsa stain, original magnification ×1000). B, Bone marrow smear showing strong aminocaproate esterase staining in normal mast cells and negative staining in all other hematopoietic cells (original magnification ×1000). C, Bone marrow aspirate smear from a patient with systemic mastocytosis (SM) showing the cytologic characteristics of atypical mast cells in SM, including excessive cytoplasmic extensions, elongation of nucleus, and hypogranular cytoplasm (Wright-Giemsa stain, original magnification ×1000). D, Atypical mast cells in SM also show positive aminocaproate esterase staining (original magnification ×1000).

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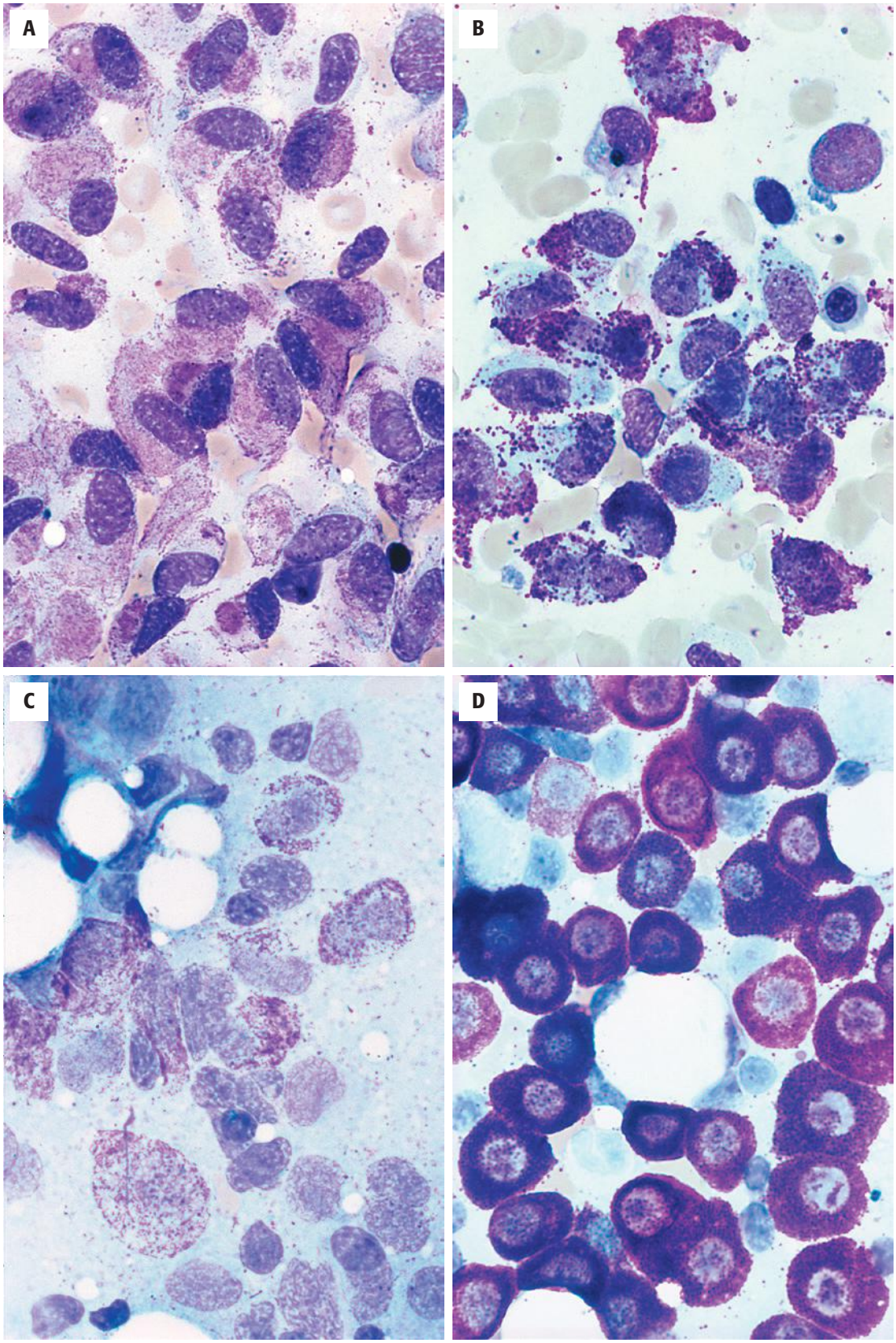


FIG. 20.5

Cytologic variation in systemic mastocytosis. A–C, Wide variations exist in the degree of cytoplasmic extension, nuclear elongation, and cytoplasmic hypogranulation (Wright-Giemsa stain, original magnification ×1000). D, In rare cases, individual mast cells are indistinguishable cytologically from normal mast cells but have excessive cell numbers in sheets (Wright-Giemsa stain, original magnification ×1000).

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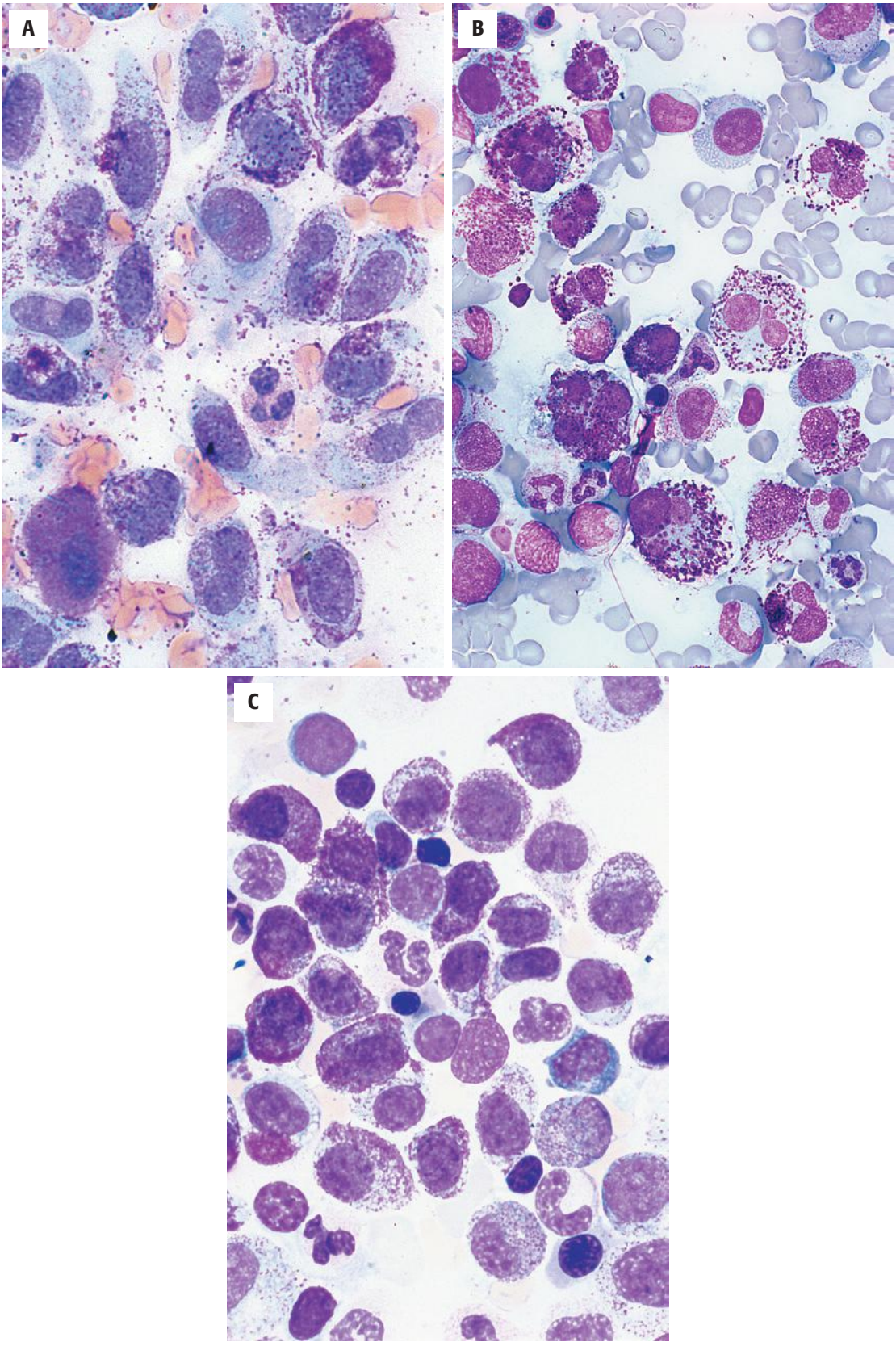


FIG. 20.6

Cytologic features of aggressive systemic mastocytosis (ASM) and mast cell leukemia (MCL). In ASM (A, B) and MCL (C), atypical mast cells, including promastocytes with bilobed or multilobated nuclei and metachromatic blasts, may be seen in bone marrow aspirate smears (Wright-Giemsa stain, original magnification ×1000).

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Histology of Systemic Mastocytosis–Bone Marrow

In most SM cases, multifocal, sharply demarcated aggregates of MCs are found in paratrabecular or perivascular locations in bone marrow biopsy specimens (Fig. 20.7A and B). In specimens with lymphoid aggregates, irregular clusters of MCs may present at the periphery of the lymphoid aggregates (Fig. 20.7C). The focal lesions are composed of varying proportions of MCs, lymphocytes, eosinophils, and fibroblasts. Marked fibrosis and thickening of the adjacent bony trabeculae are frequent (see Fig. 20.7A). In sections stained with hematoxylin-eosin (H&E), atypical MCs with elongated nuclei and clear cytoplasm may resemble fibroblasts or histiocytes. Thus, the focal lesions of mastocytosis historically have been historically described as *fibrohistiocytic lesions with eosinophilia*. The degree of eosinophilia may vary from a few eosinophils at the margins of the lesion to extremely intense eosinophilia overshadowing the actual MC infiltrates (Fig. 20.8). In addition, the degree of bone marrow involvement by mastocytosis may vary from minimal involvement showing small clusters of MCs around the lymphoid aggregates to diffuse replacement of the bone marrow in advanced-stage SM or MCL (Fig. 20.9C and Fig. 20.10A). The WHO major criterion defines MC aggregates as an aggregate that contains at least 15 MCs. In general, whenever a tight cluster of spindle-shaped MCs is observed, regardless of the number of MCs it may contain, thorough examination of the tissue is warranted to identify possible large and well-defined clusters of MCs. In a small subset of cases, the MC aggregates may be small or subtle, and immunohistochemical stains (IHC) with antibodies to tryptase or CD117 may be employed to increase the sensitivity of detecting small MC clusters (see Fig. 20.16). In addition, IHC is also useful to visualize the singly distributed MCs in bone marrow interstitium, so that the first minor criterion can also be assessed.

WHO Classification of Systemic Mastocytosis Variants and Mast Cell Sarcoma

Six SM variants are recognized by the 2016 WHO classification scheme (see Box 20.1).

- ***Indolent Systemic Mastocytosis.*** Typical ISM is characterized by modest (<30%) bone marrow involvement with multifocal dense infiltrates of MCs in a background of normocellular marrow with normal hematopoiesis, an indolent clinical course, and the presence of maculopapular skin lesion in most cases (Fig. 20.9A). However, isolated bone marrow mastocytosis with associated MRS also exist; these patients generally have similar clinical courses as those of patients with usual ISM. Isolated bone marrow mastocytosis is a subvariant of ISM. Some cases of ISM may progress to the next category discussed, smoldering SM (SSM).
- ***Smoldering Systemic Mastocytosis***. A combination of at least two "B" findings (see Box 20.1) without "C" findings is indicative of *smoldering SM*.
- ***Systemic Mastocytosis with an Associated Hematologic Neoplasm.*** It is estimated that up to 30% of patients with SM will have an associated clonal non-MC hematologic neoplasm, with this typically being a myeloid neoplasm (Fig. 20.9B). When diagnosing the AHN, the WHO classification should be used. The most frequent AHN is an MPN (40% to 50%), followed in frequency by chronic myelomonocytic leukemia (20% to 30%), MDS (10% to 20%), and acute myeloid leukemia (AML, 1% to 5%). In particular, AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* has been reported to have an association with MC disease, up to 12.5% in one study. Some authors have described the presence of mastocytosis in such patients with AML as "occult," as cytoreductive chemotherapy reveals the MC disease as the myeloblasts are eliminated. The presence of *KIT* mutations in both neoplastic MCs and non-MC neoplastic myeloid cells supports the assumption that a common neoplastic precursor gives rise to both MC and non-MC components of SM-AHN. Such findings have not been reported in SM associated with plasma cell myeloma or lymphoma, suggesting that lymphoma or myeloma may be merely a collision lesion. Interestingly, a recent study showed that as high as 30% to 40% of SM-AHN cases had associated eosinophilia, which should be distinguished from platelet-derived growth factor receptor α(*PDGFRA*) translocation–associated myeloid neoplasms through molecular studies; the latter neoplasms can also have loose proliferations of CD25-positive MCs.
- ***Aggressive Systemic Mastocytosis.*** ASM is characterized by a progressive infiltration of various organs by neoplastic MCs with consequent impairment of organ function. To have a diagnosis of ASM, the patient should have at least one "C" finding (see Box 20.1). Histologic evaluation of the bone marrow in ASM usually shows a variable degree of mixed dense, focal, and diffuse MC infiltration (Fig. 20.9C and D). A subtype of ASM, previously recognized in the WHO 2008 classification, is lymphadenopathic mastocytosis with eosinophilia, where patients present with progressive lymphadenopathy and eosinophilia of the blood. Patients are typical of other patients with ASM in that hepatosplenomegaly and extensive bony involvement are present.
- ***Mast Cell Leukemia.*** MCL is rare. It is characterized by leukemic bone marrow infiltration by neoplastic MCs, often immature MCs. It has a rapidly progressive course similar to other types of acute leukemias. Patients may present with primary or secondary MCL, the latter developing after CM or other types of SMs. Most patients have an acute presentation, but rare patients with chronic MCL have been described. The diagnosis requires that the cytologically atypical MCs are more than 20% of bone marrow nucleated cells on bone marrow aspirate smear. The bone marrow typically shows extensive replacement of normal bone marrow by either diffuse or interstitial infiltrates of immature or cytologically atypical MCs, or both. In sections stained with H&E,

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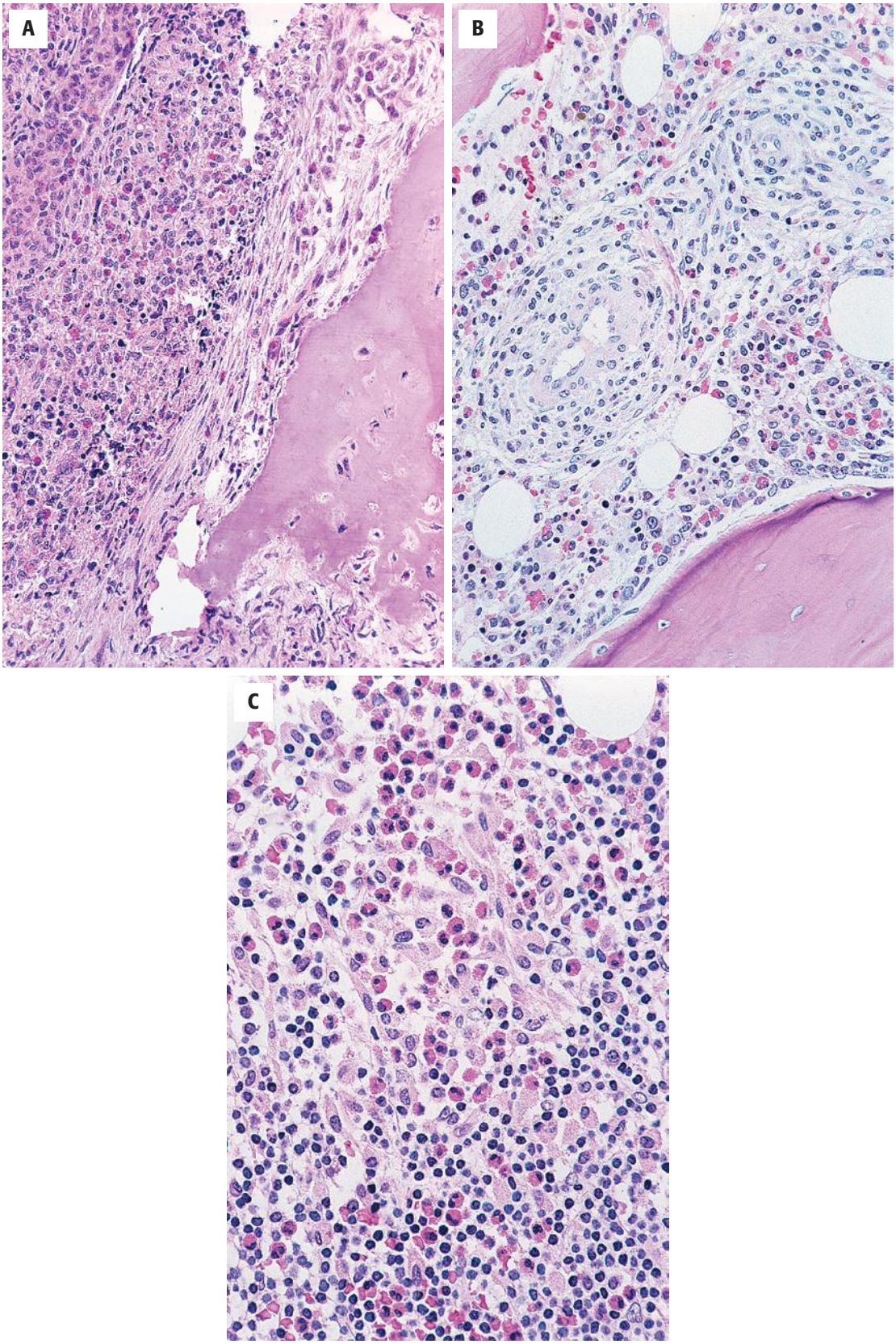


FIG. 20.7

Histologic features of bone marrow biopsy in systemic mastocytosis. Osteoblastic changes and thickening of bony trabecular (A) may be associated with paratrabecular mast cell infiltrates (H&E stain, original magnification ×240). Multifocal aggregates of atypical mast cells are found in paratrabecular (A) or perivascular (B) locations (H&E stain, original magnification ×240). C, In specimens with lymphoid aggregates, irregular clusters of mast cells are seen at the periphery of the lymphoid aggregate (H&E stain, original magnification ×400). The mast cells have abundant clear cytoplasm, a distinct cytoplasmic border, and oval or reniform nuclei with a delicate chromatin network. Eosinophils are frequently within and particularly at the margin of mast cell lesions.

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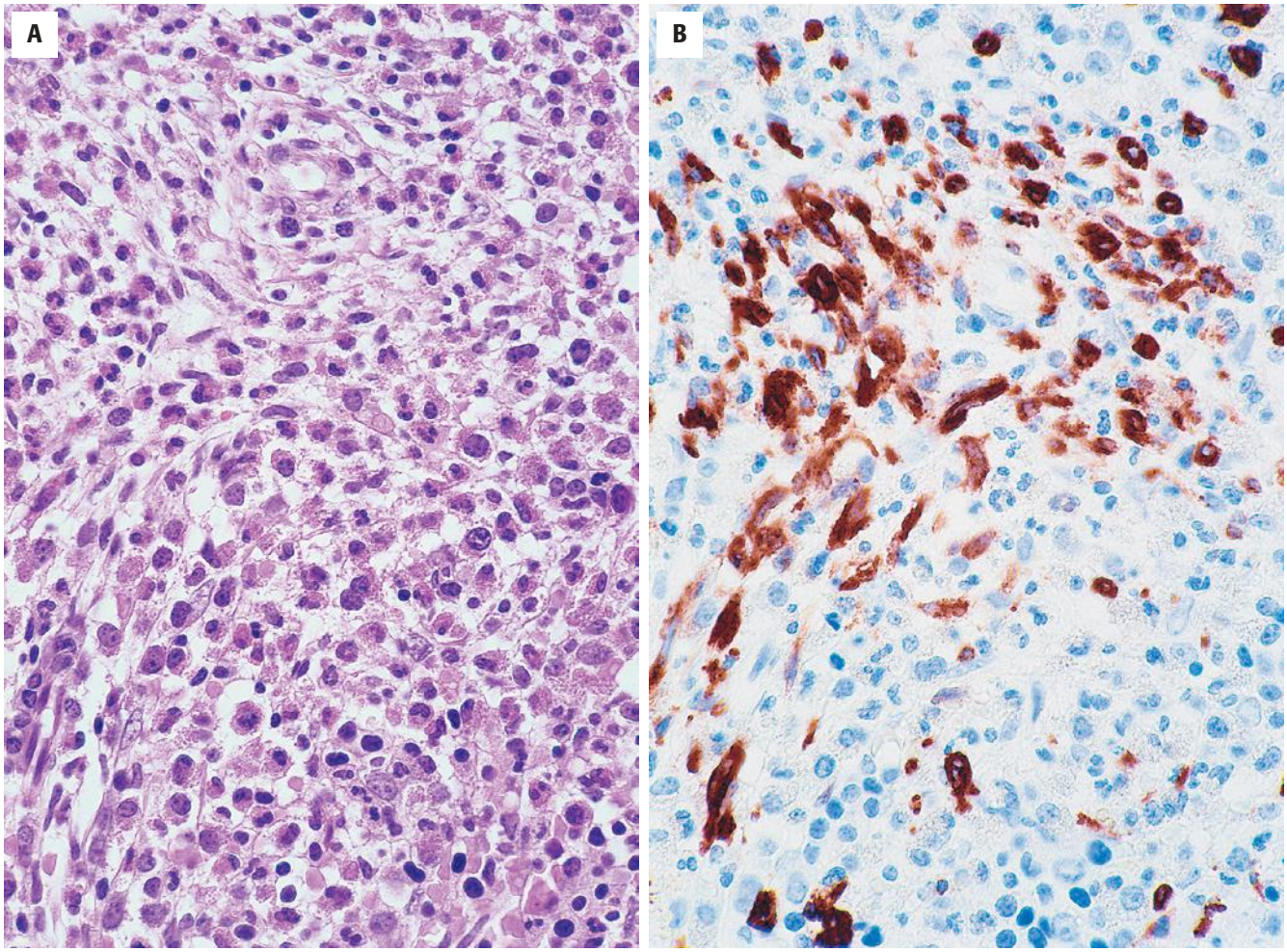


FIG. 20.8

Histologic and immunohistochemical features of systemic mastocytosis with eosinophilia. In some cases, extremely intense eosinophilia may overshadow the actual mast cell infiltrates (A) and require mast cell–specific tryptase immunostain to highlight the atypical mast cell infiltrates, usually in loose aggregates (B) (original magnification ×400).

the bone marrow is usually infiltrated by sheets of atypical MCs with abundant clear to granular cytoplasm and round to oval nuclei (Fig. 20.10A and B). The pattern of infiltration and the morphologic features of the cells may resemble those of hairy cell leukemia. Type II immature atypical MCs are frequent. MCL is further subclassified as aleukemic MCL when the circulation of MCs in the peripheral blood is less than 10% or leukemic with greater than 10% circulating MCs.

- ***Mast Cell Sarcoma.*** MCS is defined by a local destructive growth of cytologically anaplastic MCs without systemic involvement at presentation. The neoplastic cells are usually immature with a high nuclear-to-cytoplasmic ratio, prominent nucleoli, and a hypogranulated cytoplasm (Fig. 20.10C and D).

Other Variants of Mast Cell Disease

- ***Myelomastocytic Leukemia.*** MML is a rare neoplasm involving two distinct but related cell populations, myeloblasts and MCs. Athough not currently recognized by the WHO classification, MML is considered a part of the spectrum of advanced mastocytic disorders. By definition, an increase in myeloblasts (>5%) and MCs (>10%) in blood or bone marrow is present, but criteria are not met for SM. Most patients have an advanced myeloid neoplasm (high-grade MDS or AML) with dysplastic features and elevated numbers of immature MCs (e.g., metachromatic blasts). MCs are present in the bone marrow in an interstitial and diffuse pattern without focal dense aggregates, lack expression of CD2 or CD25, and do not have the D816V *KIT* mutation. Cytogenetic karyotype typically shows a complex karyotype, and prognosis is poor.
- ***Well-Differentiated SM.*** WDSM is a rare subtype of SM first described by Cem Akin and colleagues in 2004. This subtype of mastocytosis contains well-differentiated MCs that are round and well granulated and resemble normal mature tissue MCs. Importantly, this subtype of SM lacks CD25 and CD2 expression and exon 17 *KIT* mutations but is responsive to imatinib. Most patients with WDSM show a female predominance with childhood onset of disease, and a high percentage of familial disease is present

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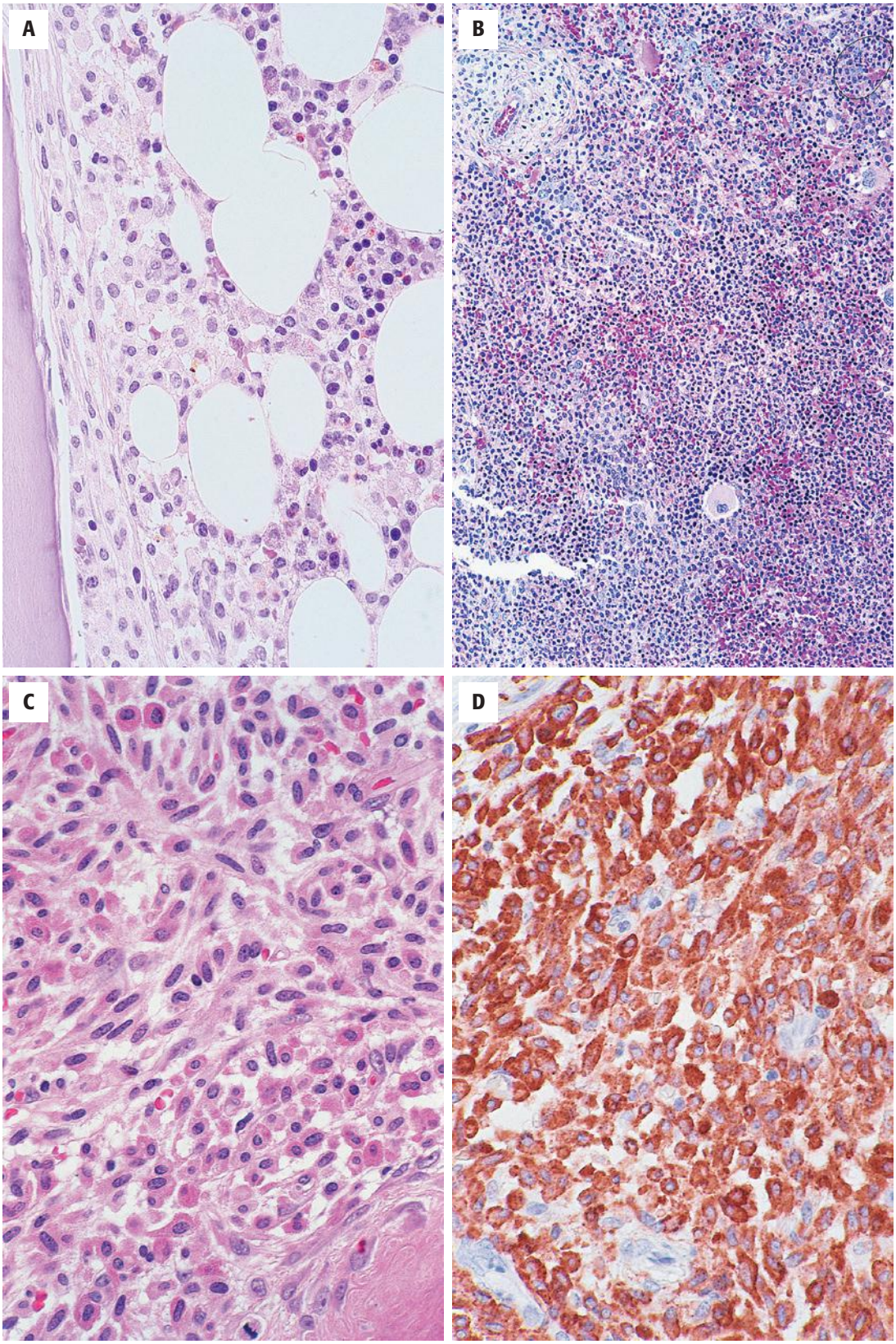


FIG. 20.9

Histologic and immunohistochemical features of subtypes of systemic mastocytosis. A, Indolent systemic mastocytosis showing paratrabecular atypical mast cell infiltrates in a background of normocellular marrow with normal hematopoiesis (H&E stain, original magnification ×400). B, Systemic mastocytosis with associated hematologic neoplasm showing a perivascular atypical mast cell aggregate (left upper corner of field) in a background of markedly hypercellular marrow due to associated hematologic disease (H&E stain, original magnification ×96). C, Aggressive systemic mastocytosis showing extensive bone marrow involvement by spindle-shaped atypical mast cells with elongated nuclei and few scattered eosinophils (H&E stain, original magnification ×400). D, Immunohistochemical tryptase staining showing strong staining of all atypical mast cells (original magnification ×400).

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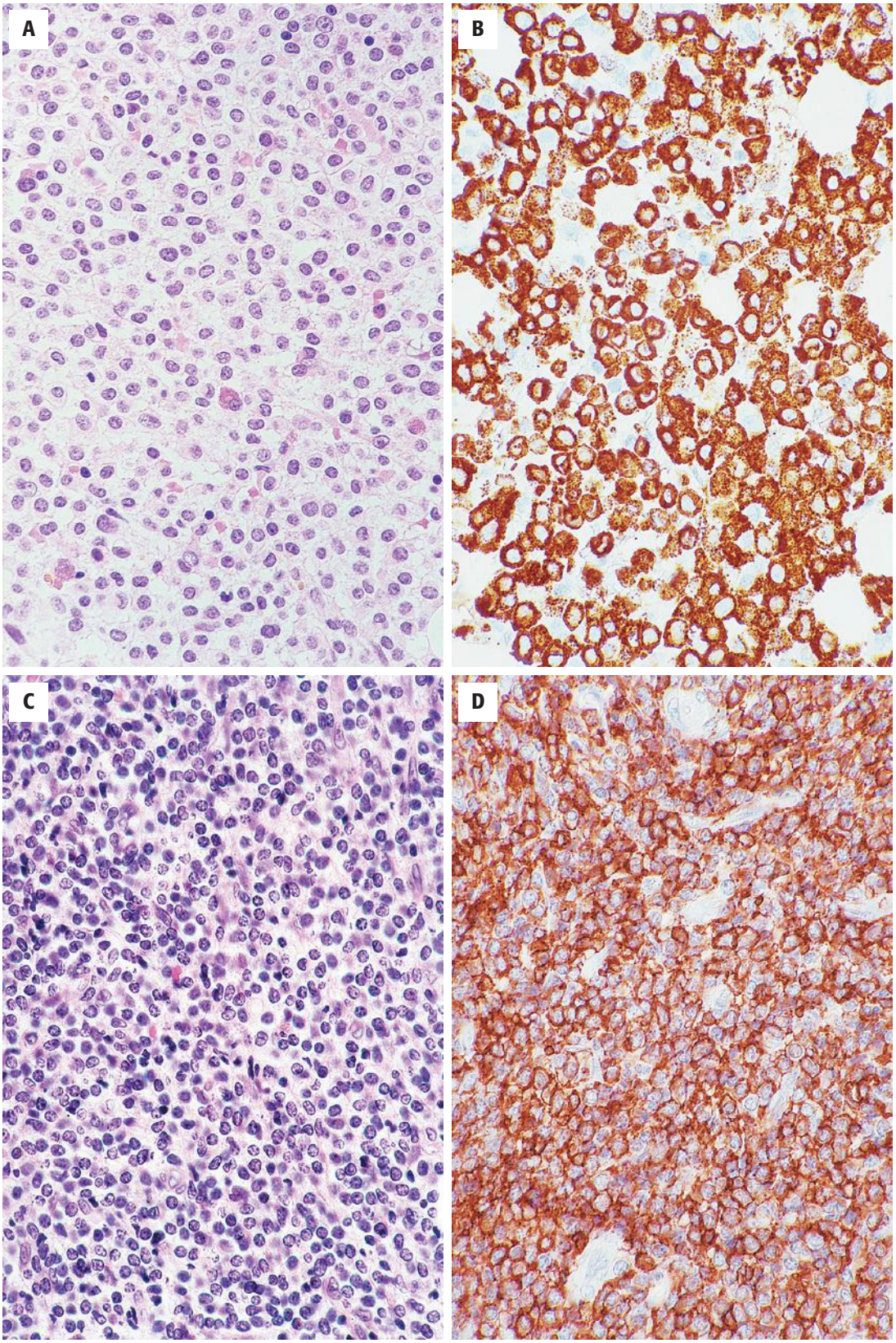


FIG. 20.10

Histologic and immunohistochemical features of mast cell leukemia (MCL) and mast cell sarcoma (MCS). A, MCL showing extensive interstitial infiltration of bone marrow by immature mast cells with morphologic features of immature cells with abundant clear cytoplasm and round to oval nuclei (H&E, original magnification ×400). B, Strong tryptase immunostaining of leukemic cells confirmed the mast cell lineage (original magnification ×400). C, MCS that clinically presented as a local destructive tumor (H&E, original magnification ×400). The neoplastic cells are immature with a high nuclear-to-cytoplasmic ratio, fine nuclear chromatin, and clear cytoplasm. The cells are negative for most immunohistochemical markers for hematolymphoid cells, except CD43 (D; original magnification ×400) and tryptase.

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(~40%). Skin involvement is common and predominantly MPCM skin lesions. Approximately 30% of patients will have *KIT* mutations, which can include D816V, K509I, N819Y, and I817V. Immunophenotype shows expression of tryptase, CD117, carboxypeptidase A (CPA), CD30 with diminished to absent expression of CD2 and CD25. Given that three of the four minor criteria for the WHO diagnosis of SM are lacking in this subtype, alternative criteria have been proposed for WDSM.

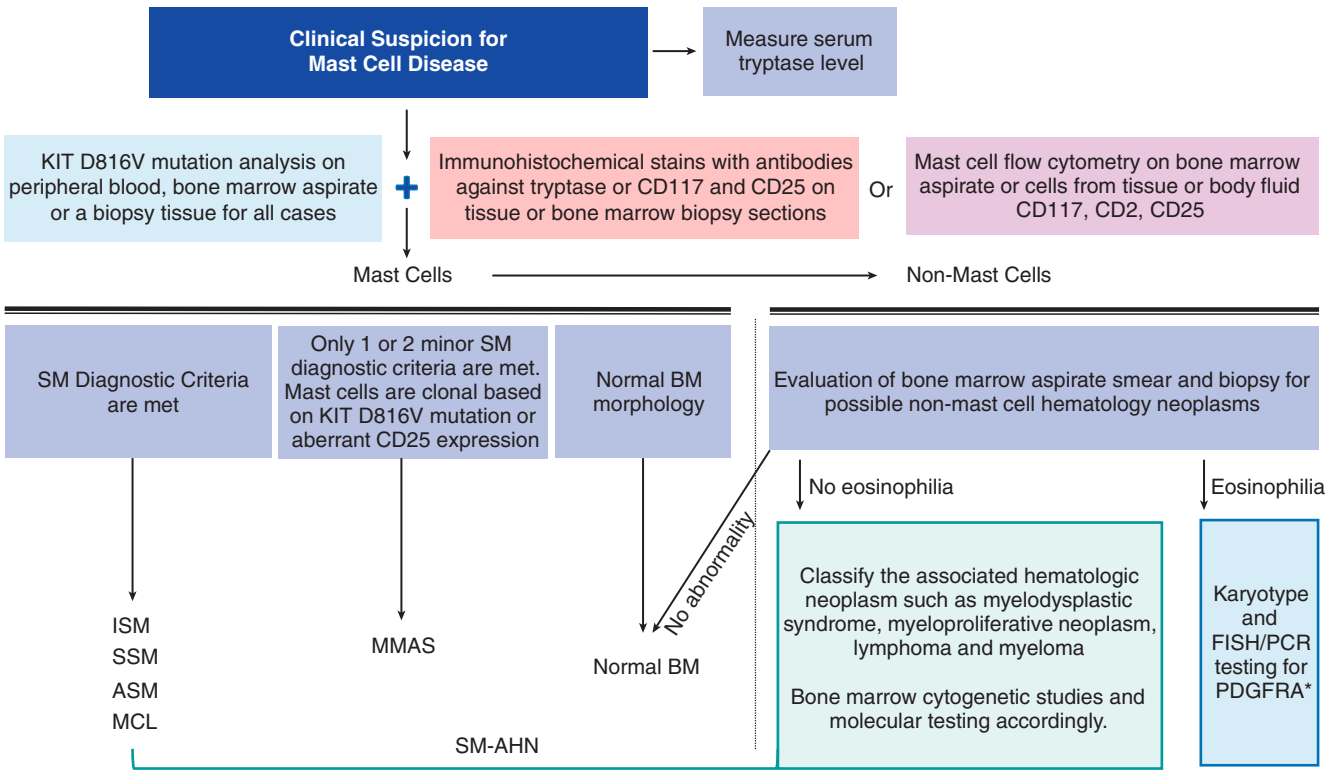
- ***Monoclonal MC Activation Syndrome***. MMAS (or primary mast cell activation syndrome) describes patients with recurrent anaphylaxis of unknown etiology without skin lesions with evidence of MC clonality—that has a *KIT* mutation or CD25-positive MCs—but not meeting criteria for SM. Importantly, a normal serum tryptase level does not exclude SM or MMAS. MMAS may have a precursor form of SM but have the same risk of severe anaphylaxis. Thus, patients with MMAS typically receive the same treatments as those patients with SM and anaphylaxis. It is recommended that patients with unexplained anaphylaxis be worked up for MC disease.
- ***Extracutaneous Mastocytoma***. Mastocytoma is a localized unifocal growth of tissue MCs without systemic involvement. In contrast to mastocytoma of skin, extracutaneous mastocytomas, primarily reported in lung, are exceedingly rare. In contrast to MCS, mastocytomas do not show a destructive growth pattern; and MCs in mastocytoma show a low-grade cytology. Extracutaneous mastocytoma was previously a part of the WHO 2008 classification but has since been removed from the classification given its extremely rare nature.

WDSM Criteria (One Major and One Minor or Three Minor Criteria)

Major criterion
- Dense aggregates in the bone marrow

Minor criteria
- Expression of CD30, overexpression of CPA and/or tryptase
- *KIT* mutation or a clonal human androgen receptor (HUMARA) pattern
- Association of female sex with either pediatric disease onset or familial aggregation in adults
- Clustering of BM mast cells outside of particles where a cluster is two or more mast cells

In light of the somewhat complicated clinicopathologic diagnostic criteria and existence of various SM subtypes, a systematic approach as illustrated in Fig. 20.11 will help to facilitate the effective workup for accurately diagnosing SM.



*Confirmatory testing for PDGFRA, FGFR1, PCM1-JAK2 as needed if karyotype suggests these abnormalities

FIG. 20.11

Systematic approach to workup and diagnosis of a mast cell disorder. ASM, aggressive systemic mastocytosis; CM, cutaneous mastocytosis; ISM, indolent systemic mastocytosis; MC, mast cell; MCL, mast cell leukemia; MMUS, monoclonal mast cells with undetermined significance; MMAS, monoclonal mast cell activation syndrome; SM, systemic mastocytosis; SM-AHN, systemic mastocytosis with an associated hematologic neoplasm; SSM, smoldering systemic mastocytosis.

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Histology of Systemic Mastocytosis in Gastrointestinal Tract

Endoscopic mucosal biopsy specimens from the upper or lower GI tract of patients with mastocytosis usually show increased numbers of plasma cells and eosinophils and, in some cases, increased numbers of MCs in the lamina propria and the submucosa (Fig. 20.15). Extensive submucosal infiltration by MCs has been documented in a cholecystectomy specimen showing contracted gallbladder with thickened walls.

Histology of Systemic Mastocytosis in Liver

Most liver biopsies from patients with SM show some degree of fibrosis and chronic inflammatory cellular infiltration with plasma cells, lymphocytes, eosinophils, and mononuclear fibroblast-like cells (including MCs) in the portal area (Fig. 20.12). The predominant infiltrate appears to be mononuclear cells. However, differentiating MCs from other mononuclear cells is usually difficult on H&E-stained sections. The presence of eosinophils, fibrosis, and oval or elongated nuclei of the mononuclear cells in portal areas may suggest MCs and require cytochemical or IHC for confirmation. In cases of MCL, both portal and sinusoidal MC infiltrates may be present.

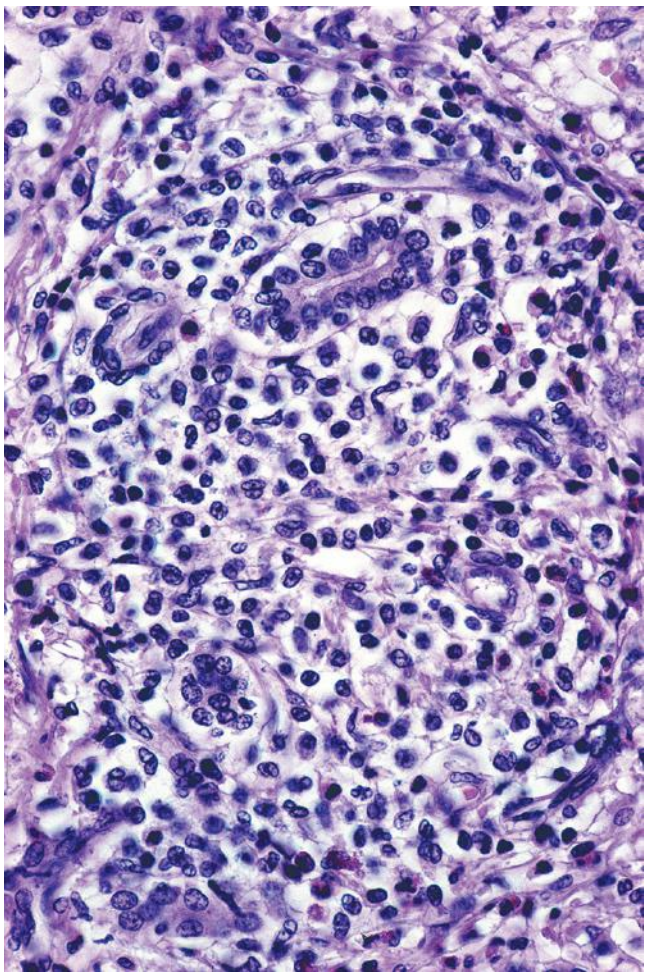


FIG. 20.12

Histologic features of the liver in systemic mastocytosis. Sections of liver biopsy show intense portal infiltration by atypical mast cells intermixed with a few eosinophils (H&E, original magnification ×400).

Histology of Systemic Mastocytosis in Lymph node

Central lymph nodes (abdominal or thoracic) frequently show definitive involvement by SM. The abnormal MC infiltrates may be observed in any compartment of the lymph node. Common features of lymph node involvement are mixed MCs and eosinophil infiltration in the perifollicular and paracortical areas with associated increased vascularity or fibrosis, or both (Fig. 20.13A and B). Occasionally, dense eosinophils (eosinophilic abscess) may mask the MCs. Also in rare cases, MC aggregates partially or completely replace lymphoid follicles, showing features that resemble follicular lymphoma (Fig. 20.13C). Intense MC infiltration in the medullary cords of the lymph node may occur in rare cases. Selective intense sinusoidal MC infiltration with dilatation of medullary sinuses resembling sinus histiocytosis may be seen in cases of MCL (Fig. 20.13D).

Histology of Systemic Mastocytosis in Spleen

Mastocytosis infiltrates may involve all compartments of the spleen, including the fibrous trabeculae and the white and red pulp. The most frequent location of MC infiltrates is adjacent to and within the fibrous trabeculae (Fig. 20.14A). The MCs may be found in small clusters, aggregates, sheets, or perivascular distribution. Eosinophils, fibrosis, and plasma cells are frequently observed in the areas of MC infiltration. White pulp infiltrates are often manifested by small clusters of MCs in the marginal zone of the lymphoid follicles (Fig. 20.14B). Diffuse red pulp MC infiltration is seen only in the spleen of patients with MCL (Fig. 20.14C). In addition, extramedullary hematopoiesis, focal sinusoidal dilatation, and increased numbers of myeloid elements may occur in the red pulp.

Ancillary Studies

Over the years, numerous cell type–specific cytochemical and immunochemical markers have been developed for the identification of normal and abnormal MCs. Subsequently, the diagnostic accuracy of mastocytosis has been vastly improved.

Cytochemical and Histochemical Stains

Cytochemical and histochemical stains of bone marrow aspirate smears or biopsies are easy and helpful tools for the diagnosis of mastocytosis (see Table 20.1). Wright-Giemsa stain can highlight the basophilic granules of MCs (see Fig. 20.4A and C) and the accompanying eosinophils. MC granules can be visualized readily with toluidine blue stain owing to

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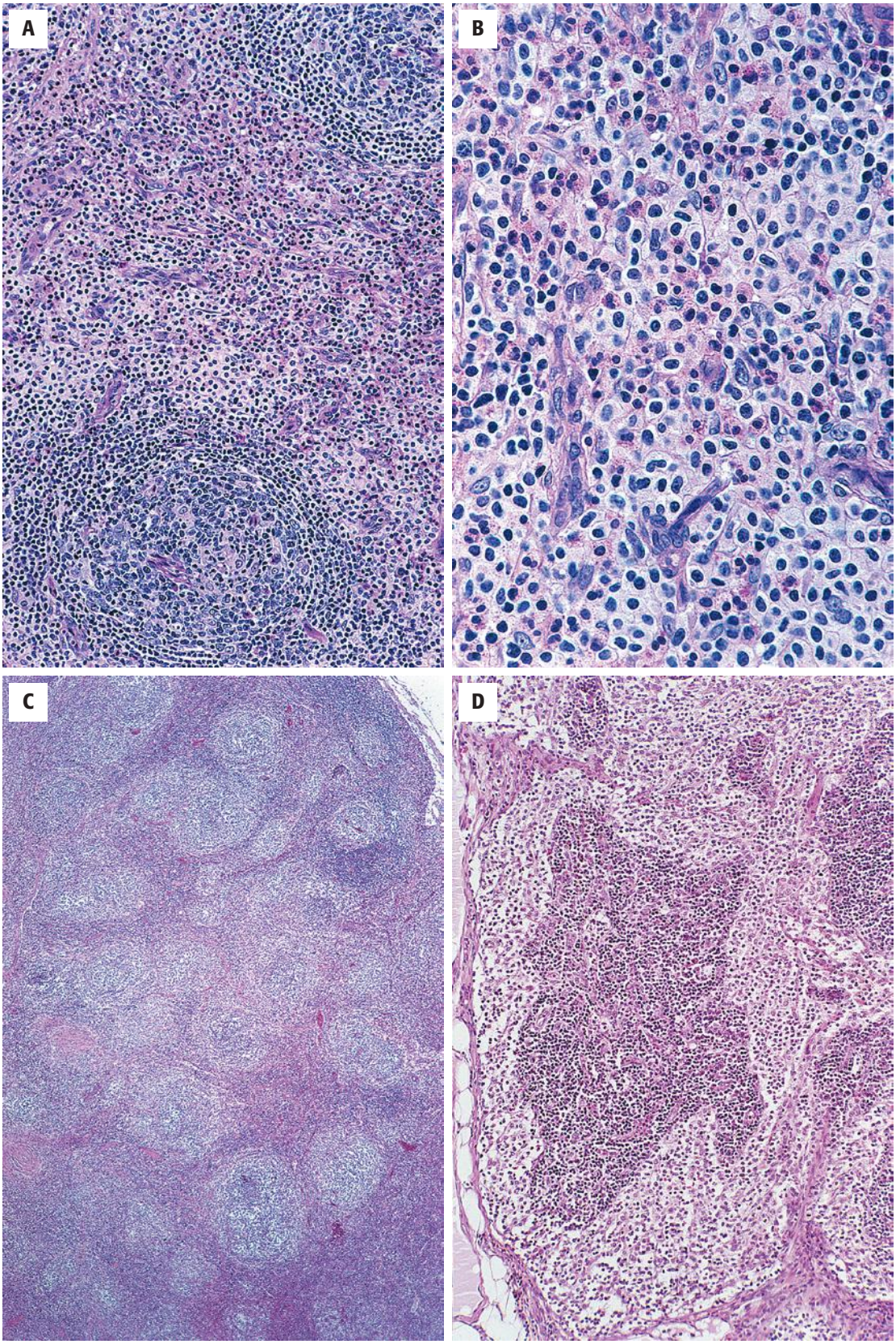


FIG. 20.13

Histologic features of the lymph node in systemic mastocytosis. A, Lymph node section shows mixed cellular infiltration in the perifollicular and paracortical areas, associated with increased vascularity (H&E, original magnification ×160). Scattered aggregates of mast cells are present predominantly in the perifollicular regions. B, Tight mast cell aggregate surrounded by many eosinophils and vessels (H&E, original magnification ×400). The mast cells have abundant clear cytoplasm, a distinct cytoplasmic border, and oval or reniform nuclei with a delicate chromatin pattern. C, Lymph node section shows mast cell replacement of germinal centers, giving an appearance resembling a follicular lymphoma (H&E, original magnification ×250). D, A lymph node section from a case with mast cell leukemia shows marked sinusoidal infiltration by atypical mast cells with abundant clear cytoplasm, giving an appearance resembling a sinus histiocytosis (H&E, original magnification ×96).

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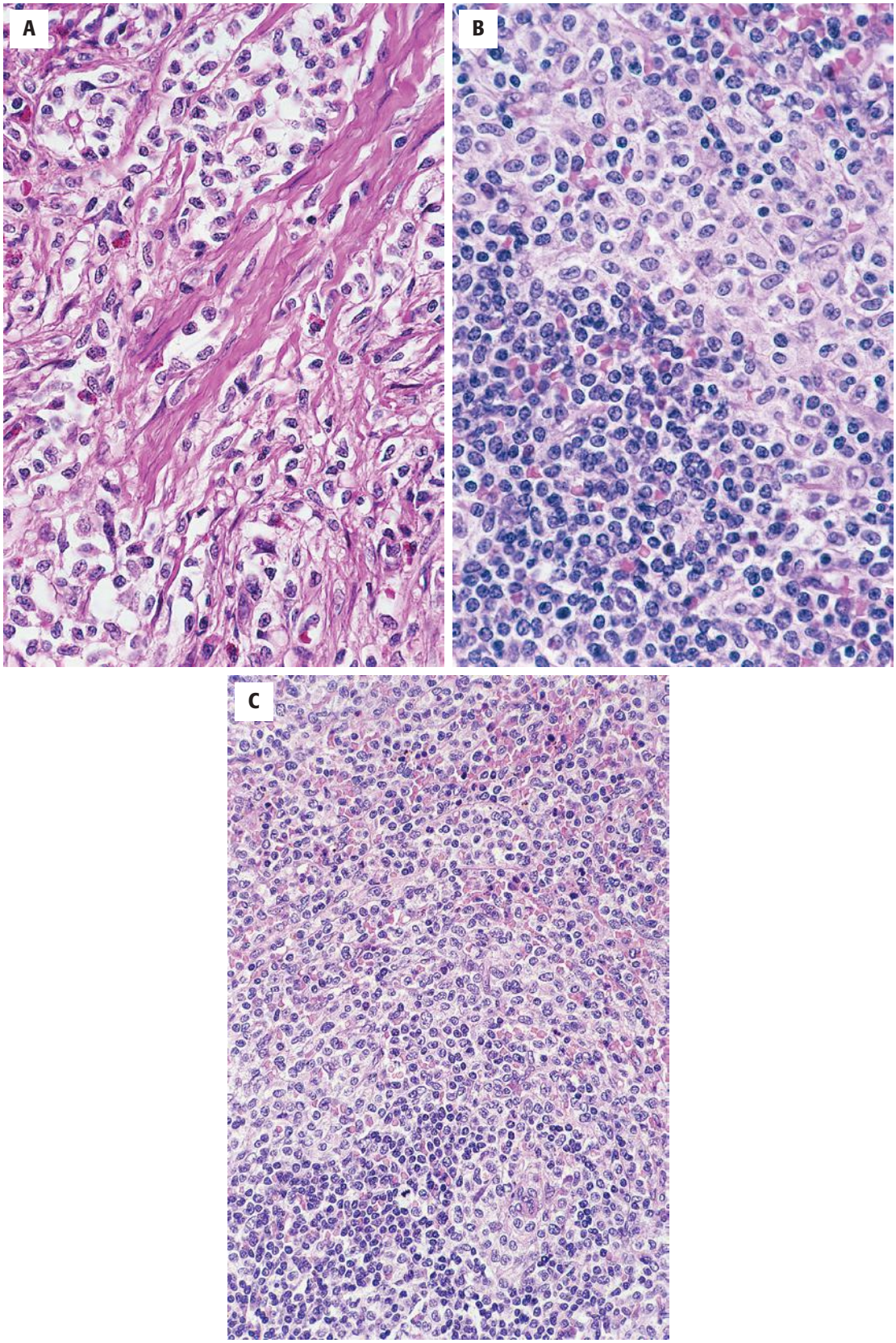


FIG. 20.14

Histologic features of the spleen in systemic mastocytosis. A, High-power view of splenic section shows fibrosis and thickening of splenic trabeculae associated with mast cell infiltrates (H&E, original magnification ×400). B, Splenic white pulp shows clusters of mast cells with abundant clear cytoplasm located at the edge of the marginal zone of this splenic lymphoid follicle (H&E, original magnification ×400). C, Splenic section from a case with mast cell leukemia shows diffuse atypical mast cell infiltration in the red pulp and a small perivascular cluster of immature mast cells in the white pulp (H&E, original magnification ×240).

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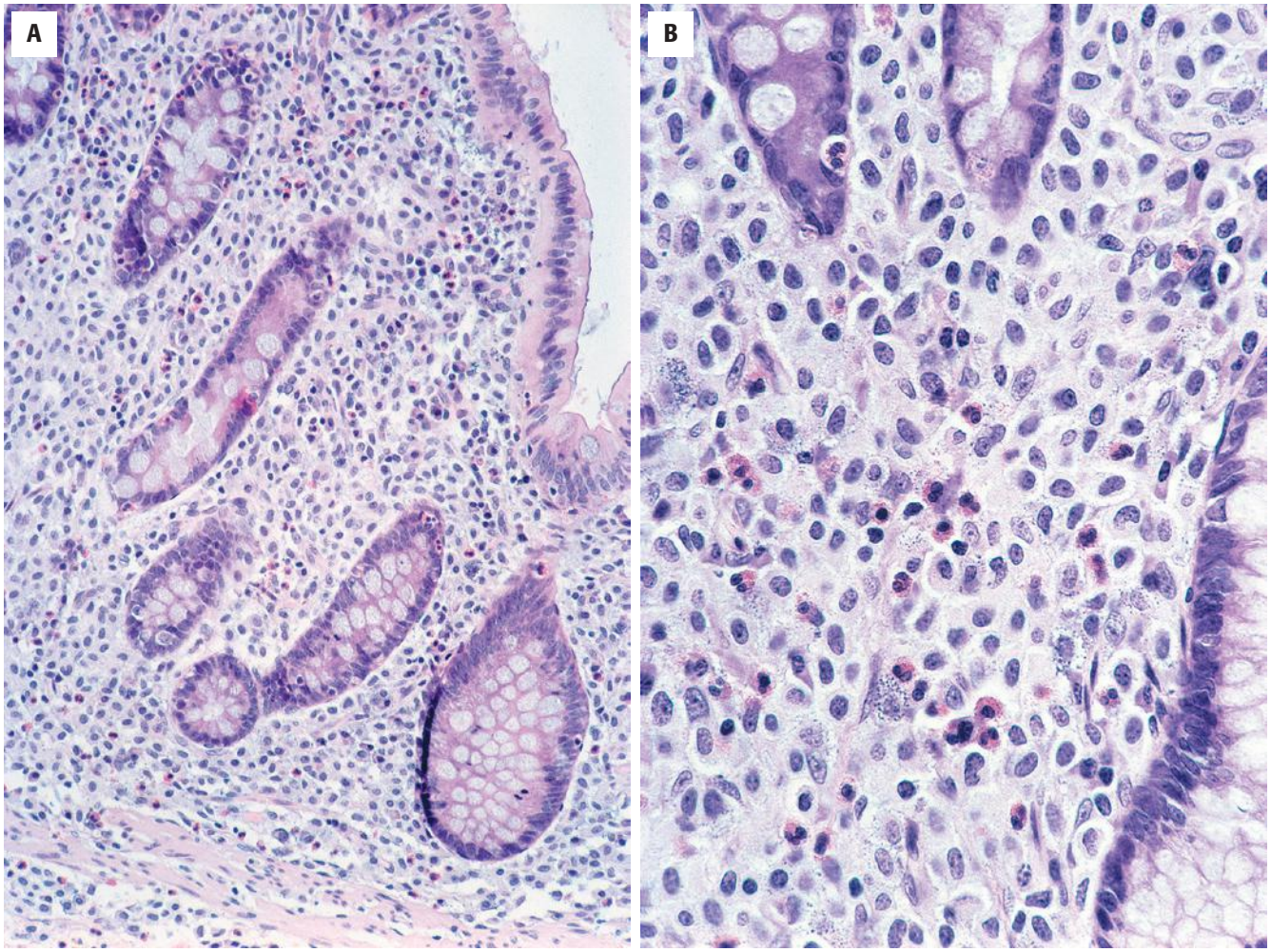


FIG. 20.15

Histologic features of the gastrointestinal tract in systemic mastocytosis. A, Colonic section shows extensive mast cell infiltration in the lamina propria and in the submucosa (H&E, original magnification ×160). B, Colonic section shows extensive mast cell infiltration intermixed with a few scattered eosinophils in the lamina propria (H&E, original magnification ×400).

their metachromatic properties. MCs contain granules rich in aminocaproate esterase (see Fig. 20.4B and D) and chloroacetate (see Fig. 20.3B), which can be demonstrated easily in smears or paraffin sections. Aminocaproate esterase-positive granules are only found in MCs, whereas chloroacetate esterase-positive granules are also present in neutrophils. Of note, decalcification and conventional tissue processes can diminish or totally abolish the metachromatic granules of MCs; they can be better preserved by plastic embedding without decalcification.

Immunohistochemical Stains

Tryptase, KIT (CD117), and CD25 (IHC stains are recommended for the initial workup for SM (see Table 20.1; Fig. 20.16). However, tryptase is not entirely specific for MCs. Abnormal basophils in chronic myeloid leukemia and acute basophilic leukemia or myeloblasts in rare AMLs may show focal positive IHC staining for tryptase; tryptase-positive blasts and abnormal basophils positive for tryptase in bone marrow show variable to dim staining and lack the distinct strong staining found in MCs. An exception is neoplastic mast cells in the GI tract where tryptase expression can be diminished and variable. MCs can be distinguished from basophils by their distinctive strong CD117 expression. Although myeloblasts also may express CD117, their expression is much dimmer than that of MCs. The aberrant CD2 or CD25 expression, or both, by neoplastic MCs may also be used to differentiate them from normal bone marrow MCs (Fig. 20.16). The CD25 expression is found in more than 80% of SM cases. Conversely, CD2 expression is best seen by flow cytometry than IHC. Recently, both CD30 and CD123 have been described in neoplastic MCs (Fig. 20.17); their expression can vary from strong staining to dim and variable staining to no staining in MCs. Nuclear phosphorylated STAT5 has been shown to be present in mast cells in patients with SM, likely reflecting the abnormal KIT tyrosine kinase activity central to the pathogenesis of SM.

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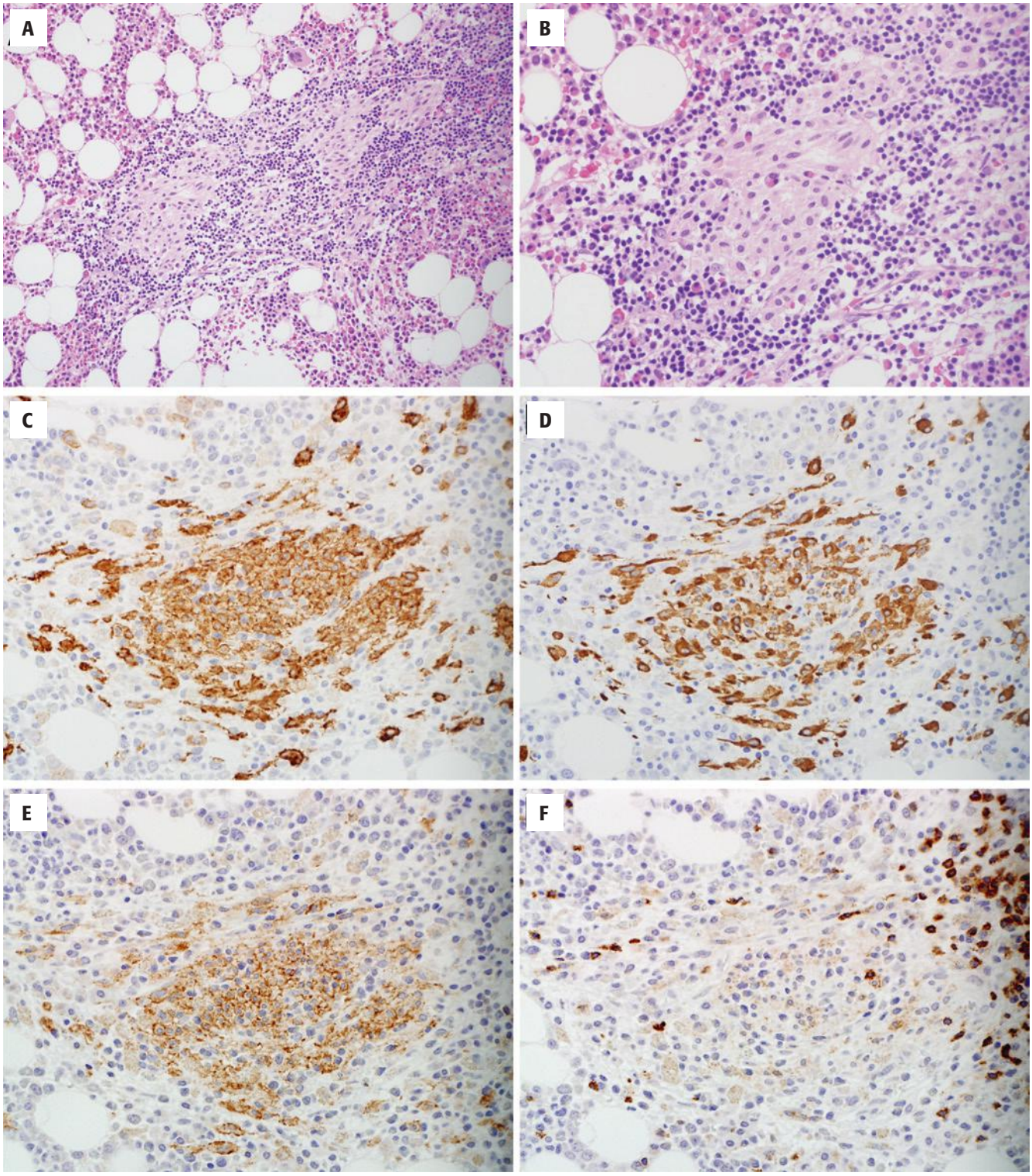


FIG. 20.16

Immunohistochemical stains of neoplastic mast cells. A, B, Bone marrow biopsy shows spindle-shaped mast cells intermixed with lymphocytes (H&E, original magnification ×200 and ×400, respectively). Mast cells express CD117 (C, original magnification ×400), tryptase (D, original magnification ×400), CD25 (E, original magnification ×400). The mast cells are weakly positive to negative for CD2 (F, original magnification ×400).

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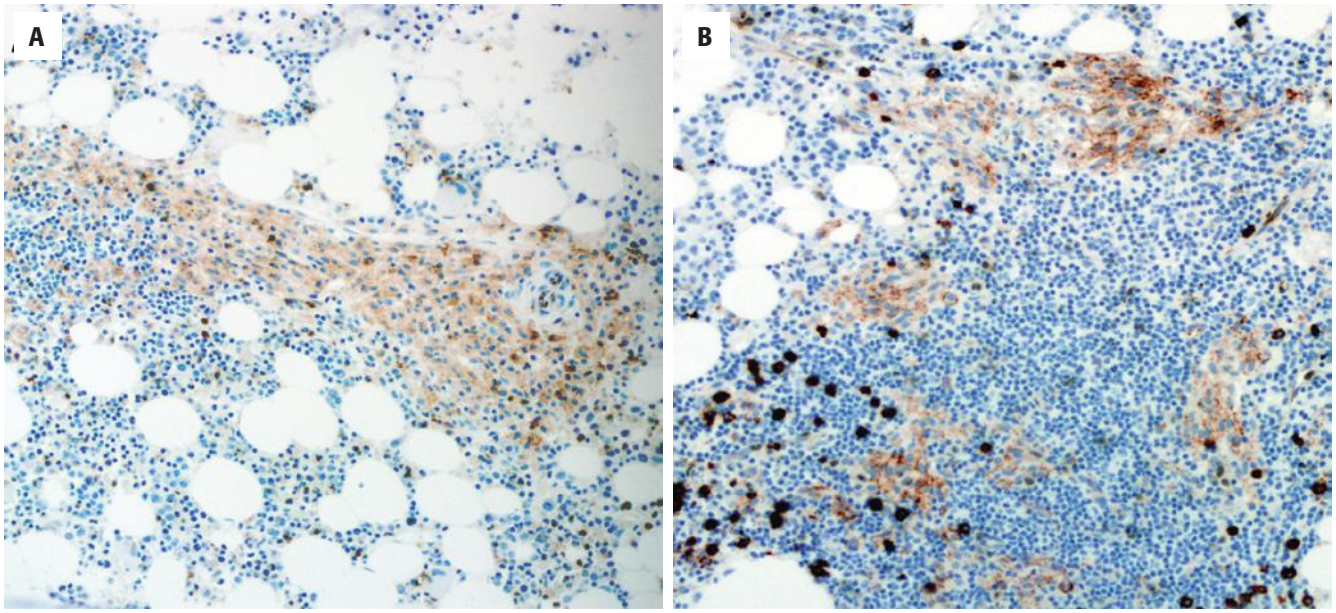


FIG. 20.17
(A) CD30 and (B) CD123 expression by neoplastic mast cells (original magnification ×200).

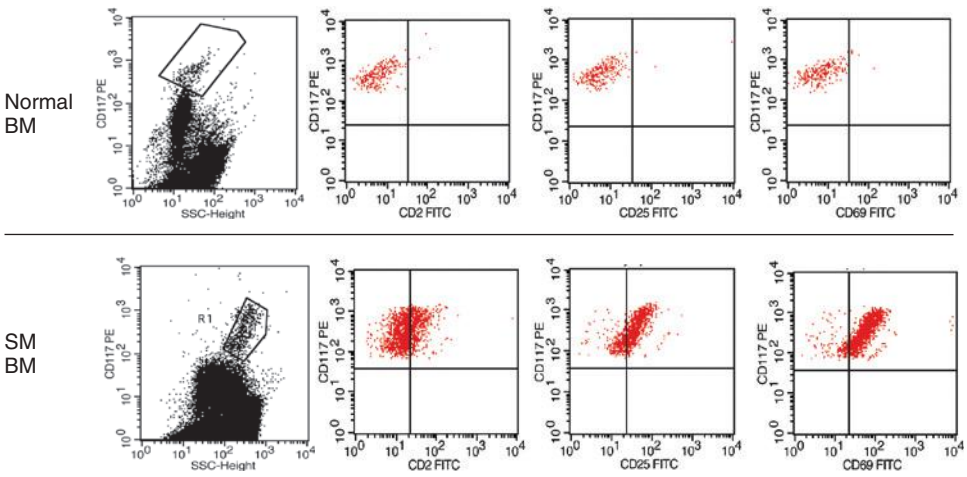


FIG. 20.18
Flow cytometric immunophenotyping of mast cells in normal bone marrow and in bone marrow with systemic mastocytosis. Mast cell population is first gated on the basis of the distinct, bright CD117 expression and side scatter characteristics. Surface expressions of CD2, CD25, and CD69 are then examined. Neoplastic mast cells aberrantly express CD2, CD25, and bright CD69.

Flow Cytometric Immunophenotyping

Flow cytometry has the advantage of simultaneously analyzing multiple MC surface markers using various fluorescent-conjugated monoclonal antibodies. Although bone marrow MCs are rare (normal range, 0.002% to 0.008%), they can be distinguished immunophenotypically from other myeloid precursors by their strong CD117 and negative CD34 surface expressions. Bone marrow MCs also invariably express CD9, CD11c, CD29, CD33, CD43, CD44, CD45, CD49d, CD49e, CD51, CD54, and CD71 and the high-affinity immunoglobulin E receptor. Both CD2 and CD25 have been shown to be constantly absent in normal and reactive MCs. Conversely, neoplastic MCs from SM show unique immunophenotypic characteristics, including aberrant surface expression of CD2 and CD25 antigens, together with abnormally high levels of the activation-related antigens CD30, CD35, CD63, and CD69 (Fig. 20.18). Therefore, a minimal standard flow cytometry panel should include CD2, CD25, and CD117. The MC population can be first gated by its bright CD117 and typical forward/side light scatter characteristics. CD2 and CD25 full or partial expression by MCs is considered evidence of an aberrant MC clone (Fig. 20.18). CD2 expression usually is weaker and less frequent than CD25 expression; consequently, a more sensitive fluorochrome, such as phycoerythrin, is preferred.

Molecular and Cytogenetic Features

KIT and *PDGFRA/B* belong to the class III receptor tyrosine kinases characterized by five immunoglobulin-like domains in the extracellular ligand-binding region, a sole transmembrane domain, a juxtamembrane domain, two intracellular kinase domains divided by a kinase insert (KI) domain, and

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a C-terminal domain (Fig. 20.19). The *KIT* and *PDGFRA* genes are located on chromosome 4q11-q13; the *PDGFRB* gene is located on chromosome 5q31-q33.

Activating Mutation of KIT in Mastocytosis

KIT is expressed in MCs, myeloblasts, granulocytic and erythroid precursors, GI stromal cells, melanocytes, and germ cells. Its ligand, SCF, is expressed by fibroblasts, endothelial cells, bone marrow stromal cells, and cells of the reproductive system. Binding of SCF to the surface domain of KIT induces receptor dimerization, transphosphorylation, and tyrosine kinase activation and subsequently leads to MC differentiation, proliferation, and migration.

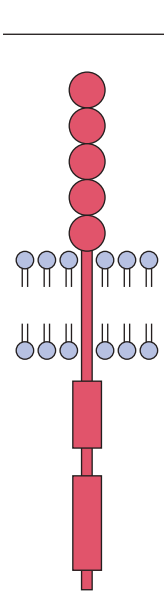


FIG. 20.19
KIT protein domains and corresponding amino acid sequences.

The identification of SCF as the essential growth factor for MCs led to the discovery of the pathogenetic role of KIT in mastocytosis. In HMC-1, a human MCL cell line, 2 *KIT* mutations have been identified—one at codon 560 (juxtamembrane domain), causing substitution of glycine for valine, and another at codon 816 in the enzymatic pocket/activation loop domain, causing substitution of valine for asparagine (Table 20.2). The enzymatic pocket-type mutations at codon 816 induce ligand-independent phosphorylation of KIT and are termed *activating mutations*, recognized by the 2016 WHO classification scheme as a minor criterion. In contrast, juxtamembrane domain mutations cause disruptions of the regulation of phosphorylation and kinase activity of KIT and are termed *regulatory-type mutations*.

Soon after these discoveries, various mutations of *KIT* were identified in mastocytosis. Among the mutations found, the most common is the mutation asparagine to valine at codon 816 (*KIT D816V*) in more than 80% of SM cases. This mutation is found in most patients with ISM, as well as a subset of patients with ASM, SM-AHN, or MCL, but it is less common in typical pediatric cases of CM. In general, the KIT mutants at the activating loop are resistant to imatinib mesylate (see Table 20.2). Interestingly, the *F522C* or *I817V* mutation-related SMs usually have round neoplastic MCs

TABLE 20.2
Summary of *KIT* Mutations in Mastocytosis and Non–Mast Cell Neoplasms

Function Domain	Exon	*KIT* Mutation	Nature of Mutation	Mastocytosis Variant (Frequency %)	Non–Mast Cell Myeloid Neoplasms	Nonhematologic Neoplasms (Frequency %)	Imatinib Mesylate
Extracellular	8	del D419	Unknown	Familial SM (<5)	AML with inv(16)	Familial GIST (<5)	Sensitive
	9	K509I	Unknown	Familial SM (<5)		NA	Sensitive
Transmembrane	10	F522C	Regulatory	WDSM (<5)	NA	NA	NA
	10	A533D	Regulatory	Familial CM (<5)	NA	NA	Resistant
Juxtamembrane	11	V559I	Regulatory	SM (<5)	NA	NA	Resistant
	11	V560G	Regulatory	SM (<5)	NA	NA	NA
Activation loop	17	R815K	Unknown	Pediatric CM (<5)	NA	NA	NA
	17	D816V	Activating	SM (>90)	AML with inv(16) or t(8;21)	Germ cell tumor (3-10)	Resistant
	17	D816Y	Activating	SM (<5)	AML	Germ cell tumor (<5)	Resistant
	17	D816H	Unknown	SM-AML (<5)	AML	Germ cell tumor (<5)	Resistant
	17	D816F	Activating	SM (<5)	NA	NA	Resistant
	17	I817V	Unknown	WDSM (<5)	NA	NA	NA
	17	InsV815-I816	Unknown	SM (<5)	NA	NA	NA
	17	D820G	Unknown	SM (<5)	NA	NA	NA
	17	E839K	Inactivating	CM (<5)	NA	NA	NA

AML, Acute myeloid leukemia; *CM,* cutaneous mastocytosis; *GIST,* gastrointestinal stromal tumor; *NA,* not applicable; *SM,* systemic mastocytosis; *WDSM,* well-differentiated systemic mastocytosis.

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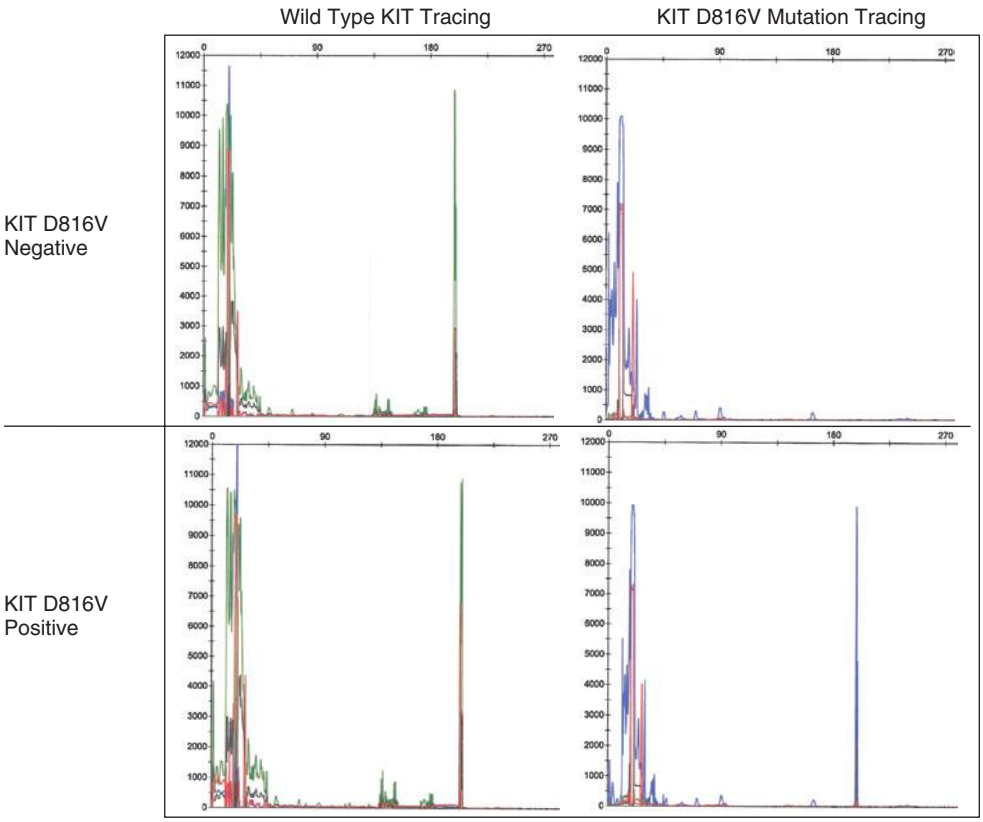


FIG. 20.20

KIT D816V mutation analysis with allele-specific oligonucleotide polymerase chain reaction with fragment analysis (on an ABI 3130xl genetic analyzer). *Left panels* (green) are the tracings of wild-type *KIT*; *right panels* (blue) are tracings of *KIT D816V* mutation. The *upper panels* are tracings of a normal negative control bone marrow aspirate sample. The *lower panels* are tracings of a bone marrow aspirate specimen of a patient with systemic mastocytosis (SM).

and extensive bone marrow involvement. Because they morphologically resemble normal MCs, they were described as *well-differentiated SM* in the literature.

In suspected SM, bone marrow aspirate or cells detached from marrow smears, or paraffin-embedded biopsy material can be used to test for *KIT* mutations. Even though peripheral blood can be used for *KIT* mutation analysis, its sensitivity is less than that of bone marrow aspirate, especially in ISM, unless supersensitive techniques are used; these are available in a few places in Europe but are not in common practice. Commonly used molecular techniques to detect the most frequent *D816V* mutations include real-time polymerase chain reaction (PCR) with restriction fragment length polymorphism, peptide nucleic acid-mediated PCR, and allele-specific PCR (Fig. 20.20). Negative results on *D816V* mutation analysis may be caused by sampling issues due to patchy bone marrow involvement or SM with different *KIT* mutations, especially in the more aggressive forms. For indolent SM, *KIT* sequencing may be indicated to identify rare variants that could potentially be sensitive to imatinib mesylate. With respect to decalcified bone marrow, formalin fixation and EDTA decalcification are preferred for *KIT* mutation testing as harsher decalcification techniques and alternative fixatives are not optimal for nuclei acid preservation. Importantly, *KIT* mutations are not specific for mastocytosis because they can be present in other hematologic and non-hematologic malignancies, such as GI stromal tumors (GISTs), melanomas, and germ cell tumors (see Table 20.2).

FIP1L1-PDGFRA Fusion Tyrosine Kinase in Hypereosinophilic Syndrome and Mastocytosis

Platelet-derived growth factor (PDGF) was originally purified from platelets. Later, it was found to be produced by various cells, including endothelial cells, vascular smooth muscle cells, activated monocytes and macrophages, osteoclasts, erythroblasts, and plasma cells and to exert its effects on mesenchymal cells, such as fibroblasts. Two different PDGF chains A and B form three different PDGF dimeric isoforms—the AA and BB homodimers and the AB heterodimer. Similarly, two different PDGF receptors exist: the α receptor (PDGFRA) binds all the PDGF isoforms, and the β receptor (PDGFRB) binds only PDGF-BB. The binding of the ligand induces dimerization of α- and/or β-subunits leading to three possible receptor dimers. Dimerization is a prerequisite for receptor autophosphorylation and kinase activation, resulting in the signal being transduced to the interior of the target cell by a series of tightly regulated molecular interactions.

The identification of the *FIP1-like-1–PDGFRA* (*FIP1L1-PDGFRA*) fusion in cases of hypereosinophilic syndrome and chronic eosinophilic leukemia adds to the growing list of activated fusion tyrosine kinases linked to the pathogenesis of myeloproliferative neoplasms. This fusion results in a gain-of-function fusion protein deriving from a cryptic interstitial deletion of genes rather than a reciprocal chromosomal translocation. This interstitial chromosomal deletion involves an approximately 800-Kb DNA segment, including the cystein-rich hydrophobic domain 2 locus at 4q12.

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Demonstration of the loss of one of the two cystein-rich hydrophobic domain 2 alleles with interphase fluorescence in situ hybridization can be used as a surrogate for the *FIP1L1-PDGFRA* fusion.

Besides eosinophilia, MC proliferation is a distinct feature of *PDGFRA*-associated neoplasms; it may also be seen in *PDGFRB* and *FGFR1*-associated neoplasms. The MCs may be loosely distributed or may be in loose, noncohesive clusters. Occasional clusters of spindle-shaped and CD25-expression atypical MCs present with features that are indistinguishable from SM. Patients with *PDGFRA*-positive eosinophilic disorders are responsive to low-dose imatinib mesylate treatment.

Differential Diagnosis

The pathologic lesions of SM, which are most distinctive in the bone marrow, typically consist of clusters or aggregates of MCs (mononuclear cells with abundant cytoplasm) on sections stained with H&E. These lesions usually are associated with various degrees of eosinophilic infiltrates. In some cases, the intense eosinophilia may overshadow the MC infiltrates, making it difficult to distinguish the process from myeloid neoplasms associated with mutations in *PDGFRA*, *PDGFRB*, and *FGFR1*. In this situation, the tryptase immunostain is very helpful in highlighting the atypical MC infiltrates that frequently are distributed in loose aggregates rather than tight clusters (see Fig. 20.8B).

Monocytic nodules, also known as *plasmacytoid dendritic cell nodules*, in chronic myelomonocytic leukemia may appear as aggregates of mononuclear cells, resembling MC clusters. However, such lesions usually do not have eosinophilic infiltrates and should not immunostain positively for tryptase.

SM with CD30 expression in the bone marrow has been mistaken for classic Hodgkin lymphoma where the neoplastic mast cells were thought to be Reed–Sternberg variants. A panel of IHC stains will distinguish between these two entities.

In cases of MCL, the heterogeneous pattern seen in cytoplasmic granulation, nuclear morphologic features, and interstitial infiltration of the bone marrow might sometimes resemble that of other hematologic malignancies, such as AML, acute basophilic leukemia, MML and hairy cell leukemia. Cytochemical and IHC studies (see Table 20.1) are helpful in the differential diagnosis of these entities.

Since the pathologic manifestations of SM in the spleen and lymph node include a broad spectrum of histologic findings, many entities must be considered in the morphologic differential diagnosis.

In the spleen, SM can be confused with an MPN or hairy cell leukemia, especially when diffuse red pulp infiltration occurs in the spleen of patients with MCL. Furthermore, MCs also possess tartrate-resistant acid phosphatase, a diagnostic tool for hairy cell leukemia. The differential diagnosis of SM requires a constellation of MC-specific IHC studies (see Table 20.1).

SM infiltrates in lymph nodes may mimic many malignant conditions. They may resemble T-cell lymphoma because of the paracortical distribution, the clear cytoplasm of the MCs in some cases, and the associated vascular proliferation and eosinophilia (Fig. 20.13A and B). However, MCs in SM infiltrates are relatively monomorphous, in contrast to neoplastic T cells, which often have a heterogeneous cellular composition with variable proportions of small, intermediate, and large lymphoid cells. When MC infiltrates replace the lymphoid follicles, the pattern can give a striking resemblance to a follicular hyperplasia or lymphoma (Fig. 20.13C). However, careful examination of the cytologic features of the cells in the follicles shows that the MC cytoplasm is more abundant; it is granular and stains metachromatically. The presence of associated eosinophilia and extramedullary hematopoiesis may be helpful clues that the process is not a follicular hyperplasia or lymphoma. The marked sinusoidal infiltration by MCL in a lymph node needs to be distinguished from sinus histiocytosis (Fig. 20.13D). This distinction may be difficult morphologically because the hypogranular MCs closely resemble histiocytes. However, histochemical staining for chloroacetate esterase or IHC staining for CD117, tryptase, or CD25 can result in the characteristic staining of the MCs in the sinuses. MCs also can resemble Langerhans histiocytes because of their eosinophilic cytoplasm, occasional folded nuclei, and frequent association with eosinophils. However, Langerhans cells have a more characteristic nuclear groove and can be distinguished from MCs by S100 and CD1a immunostains.

Prognosis and Treatment

Cutaneous mastocytosis in children usually regresses spontaneously before or during puberty. In adults, mastocytosis skin lesions are often associated with SM, which in adults is markedly heterogeneous in its clinical display, bone marrow histologic appearance, underlying molecular lesion, and treatment response. Patients with ISM usually have a normal life expectancy. Infrequently, ISM progresses into a more aggressive disease. In ASM, increased bone marrow MCs and eosinophil content, an elevated serum alkaline phosphatase level, anemia, and hepatosplenomegaly portend a poor prognosis. MCL and MCS are clinically very aggressive disorders. Patients with MCL or MCS usually have an overall survival of only weeks to months. By comparison, the clinical course of SM-AHN is usually dictated by the associated hematologic disorder.

In general, treatment of mastocytosis is indicated when patients have MC MRS, UP, or organ dysfunction from ASM. MRS and UP can accompany both indolent and ASM but occur less frequently in SM-AHN. Cytoreductive therapy is usually reserved for advanced MC disease, including ASM, SM-AHN, and MCL (Fig. 20.21).

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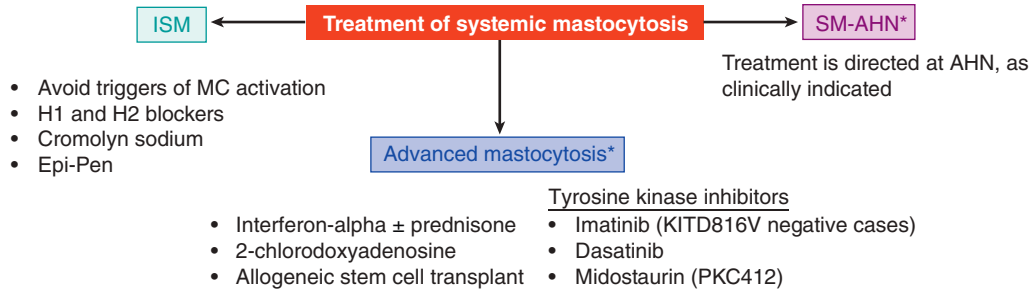


FIG. 20.21

Diagram of treatment options of systemic mastocytosis.

Noncytoreductive drugs for MRS include oral histamine$_1$ and histamine$_2$ antagonists, as well as cromolyn sodium. In addition, patients with a propensity to vasodilatory shock should wear a medical alert bracelet as well as carry an epinephrine auto-injector for self-administration of subcutaneous epinephrine. For UP/MCPM, both intralesional and topical corticosteroids produce substantial response. Oral-based psoralen ultraviolet A (UVA) photochemotherapy is also effective in UP/MCPM.

Rare patients with SM with juxtamembrane *KIT* mutations are sensitive to imatinib, which should be considered first-line therapy for these patients. However, the vast majority of SM patients have the D816V *KIT* mutation, and they are resistant to imatinib. For advanced SM patients, interferon-α is often the initial drug of choice. Cladribine (2-chlorodeoxyadenosine), a purine nucleoside analogue, is an alternative option for interferon-resistant disease (see Fig. 20.21). Recently, molecularly targeted therapies have been evaluated in clinical trials (see Fig. 20.21). They include dasatinib (a dual SRC/ABL inhibitor), midostaurin (a multitargeted kinase inhibitor), masatanib, AP23464 (an adenosine triphosphate–based inhibitor), MLN518 (a quinazoline-based inhibitor), indolinone compounds, OSI-930 (a thiophene-based inhibitor), and nilotinib (an aminopyrimidine-based inhibitor). These newly targeted therapeutic compounds may have roles in the treatment of mastocytosis. Also, allogeneic stem cell transplantation is being increasingly used in appropriate patients with advanced SM.

The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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21

Multiple Myeloma and Other Plasma Cell Neoplasms

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INTRODUCTION

Monoclonal expansion of plasma cells and their precursors gives rise to a spectrum of plasma cell neoplasms ranging from indolent to aggressive. These include monoclonal gammopathy of undetermined significance (MGUS; 67%), multiple myeloma (14%), solitary plasmacytoma of bone or extraosseous tissue (3%), primary amyloidosis (AA; 9%) and immunoglobulin (Ig) chain deposition diseases (>1%), POEMS (polyneuropathy, organomegaly, endocrinopathy, M component, and skin changes) syndrome, and the more recently recognized TEMPI syndrome (telangiectasias, elevated erythropoietin level and erythrocytosis, monoclonal gammopathy, perinephric fluid collections, and intrapulmonary shunting).

The past decade saw remarkable advances in our understanding of the biology of myeloma and improved patient survival. Virtually all myelomas arise from precursor lesions such as MGUS and smoldering stage through stepwise accumulation of genetic lesions and altered bone marrow microenvironment. Genomic and single-cell sequencing have revealed intra-clonal heterogeneity in myeloma and how clonal selection affects therapeutic efficacy. Highly sensitive multicolor flow cytometry immunophenotyping and deep sequencing now afford a more robust measurement of minimal residual diseases than before, allowing more stringent criteria for complete remission. Improvements in existing and altogether new classes of therapeutics have dramatically improved patient survival. These advances have changed the paradigm of disease model, reshaping the standard of practice in terms of diagnosis and management of patients. This chapter focuses first on an update in myeloma, the prototype of plasma cell neoplasm, and its variants and second with a description of other types of related neoplasms.

PLASMA CELL MYELOMA

Clinical Features

Plasma cell myeloma, a disease of older people, is rare in people under the age of 40 (<2% are younger than age 40). The median age is approximately 69 years. Myeloma is most commonly diagnosed among individuals in the age range of 65 to 74 years followed by those aged 75 to 84 years. The disease also has a higher predilection for blacks than for whites (2:1). The incidence is approximately 7.2 men and 4.3 women per 100,000 among white and 14.8 men and 10.5 women per 100,000 among blacks. The incidence is the lowest among Asians, about two per 100,000.

Presenting symptoms are variable and depend on the extent of tumor burden and activity. Most patients present with bone pain owing to tumor infiltration and pathologic fractures. Fatigue and weakness secondary to anemia, which is by far the most common finding, as well as renal failure, weight loss, and recurrent infection (>2 episodes in 12 months) are also common. Less frequent symptoms include hyperviscosity syndrome, hypercalcemia, and spinal cord compression as a result of vertebral body fracture or epidural tumor mass. Hepatosplenomegaly and lymphadenopathy are uncommon.

The diagnosis of myeloma is based on clinical as well as laboratory findings. It requires 10% or more monoclonal plasma cells in the bone marrow or a biopsy-proven plasmacytoma. However, patients who meet the criteria may be asymptomatic and stable for years without treatment; these patients are considered to have smoldering (asymptomatic or indolent) myeloma (SMM). According to the recently proposed revised International Myeloma Working Group (IMWG) criteria, the diagnosis of symptomatic myeloma requires, in addition to the above plasma cell/plasmacytoma requirements, the presence of one or more myeloma-defining events (MDEs), such as CRAB features (hyper**c**alcemia >11 mg/dL, **r**enal dysfunction (creatinine clearance <40 mL/min or creatinine >2 mg/dL), **a**nemia (<10 g/dL), and destructive **b**one lesions). Furthermore, according to the revised IMWG criteria, three biomarkers identify patients at an ultra-high risk with 80% progression to symptomatic disease within 2 years and therefore are also considered MDEs. Presence of one of these three MDEs in a patient with more than 10% plasma cells or a plasmacytoma also allows a diagnosis of active myeloma: (1) clonal plasma cells of ≥60%, (2) involved to uninvolved serum light chain ratio of ≥100, or (3) more than one focal lesion of ≥5 mm.

SMM is distinguished from MGUS by 10% or more monoclonal plasma cells and/or 30 g/L of M-protein in the

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TABLE 21.1

Revised International Myeloma Working Group Diagnostic Criteria for MM, SMM, and MGUS (2014)

MGUS	Serum M protein <3 g/dL and/or abnormal FLC ratio (<0.26 or >1.65) with increased level of appropriate involved light chain Clonal BM plasma cells <10% Absence of MDEs or amyloidosis
SMM	Serum M protein ≥3 g/dL and/or Clonal BM plasma cells 10%–60% and/or Urinary monoclonal protein ≥500 mg per 24 hours Absence of MDEs or amyloidosis
MM	Clonal BM plasma cells ≥10% or biopsy proven plasmacytoma AND any one or more of the following myeloma defining events (MDEs) ■ Evidence of end-organ damage (CRAB criteria) that can be attributed to the underlying plasma cell proliferative disorder, specifically: – Hypercalcemia: serum Ca^{2+} >0.25 mmol/L (>1 mg/dL) above upper limit of normal or >2.75 mmol/L (>11 mg/dL) – Renal insufficiency: serum creatinine >177 μmol/L (>2 mg/dL) – Anemia: hemoglobin value of >2 g/dL below the lower limit of normal or a Hemoglobin <10 g/dL – Bone lesions: one or more lytic lesion(s) on skeletal radiography, CT, PET-CT ■ Or 1 or more of the following 3 biomarkers – Clonal BM plasma cells ≥60 – Involved: uninvolved serum FLC ratio ≥100 (involved FLC level must be ≥100 mg/L) – >1 focal lesion on MRI study (at least 5 mm)

MGUS, Monoclonal gammopathy of undetermined significance; *SMM,* smoldering multiple myeloma; *MM,* multiple myeloma; *MDEs,* myeloma-defining events; *FLC,* serum free light chain; *BM,* bone marrow.

serum. The incidence of SMM is about 8% to 20% of all cases of myeloma. These cases are usually diagnosed incidentally during routine physical examination or laboratory testing. The designation of SMM by the revised IMWG criteria covers the spectrum of cases with up to 60% of monoclonal plasma cells in the bone marrow as long as MDEs or amyloid are absent (Table 21.1). SMMs also have a much higher risk of progression to symptomatic myeloma than MGUS. The rate of progression of MGUS is at 1% per year, but the rate of progression of SMM is 10% per year for the first 5 years, 3% per year for the next 5 years, and 1% per year for the last 10 years. Although most patients do not progress to overt myeloma within the first 5 years, some may become symptomatic within 2 years.

Solitary plasmacytomas of the bone are tumors of neoplastic plasma cells localized to a single bone at presentation, and they represent 3% of plasma cell neoplasms. The tumor primarily affects the axial skeleton, and the thoracic vertebrae are most frequently involved. Most patients present with localized bone pain, and some may present with neurologic symptoms caused by spinal cord or root compression. The patients are usually free of anemia, hypercalcemia, and renal insufficiency. Serum or urine monoclonal protein is detectable in 60% of patients, usually at low levels. Most patients (70%) eventually develop systemic disease at a median of 2 to 4 years. Patients with solitary plasmacytoma and bone marrow clonal plasma cells less than 10% are classified as plasmacytoma with minimal bone marrow involvement.

Solitary extramedullary plasmacytomas tend to be localized to the head and neck regions, where 80% of cases occur, although they may occur in many other parts of the body such as the gastrointestinal tract, central nervous system, and skin. The majority of the tumors do not produce detectable serum paraprotein (<25%), and the tumors rarely spread (<30%).

It is possible that some of these tumors represent extranodal marginal zone lymphoma with marked plasmacytic differentiation. The 10-year survival rate is 70% compared with 40% in solitary plasmacytoma of the bone. About 15% of patients may subsequently develop myeloma.

The diagnosis of solitary plasmacytoma of the bone or soft tissue is established only after extensive radiologic imaging, especially magnetic resonance imaging and positron emission tomography (PET) scan, to exclude occult systemic disease. Risk of local recurrence and dissemination is high in patients with solitary plasmacytoma of the bone with persistent serum monoclonal proteins after local radiation therapy.

Plasma cell leukemia (PCL), either primary or secondary, is defined as an absolute plasma cell count of more than 2×10^9/L or plasma cells constituting more than 20% of the total leukocyte count in the peripheral blood. Primary PCL represents initial presentation of disease and constitutes less than 5% of newly diagnosed cases of myeloma. These patients are younger than those with myeloma, with a median age of 55 years at diagnosis. Secondary PCL is essentially peripheralized myeloma at the terminal stage of disease as a result of excessive tumor growth, representing 1% of myeloma cases in all. Primary PCL is more common than secondary PCL, constituting 60% of leukemic cases, and is an aggressive disease frequently associated with high tumor burden, extramedullary dissemination, and adverse prognosis, with a median survival of 11 months without novel therapy or 25 months with autologous transplantation. Hepatosplenomegaly is more common (50%) and lytic bone lesions are less common (18%) in primary PCL, compared to secondary PCL (17% and 70%, respectively). Hypercalcemia, cytopenia, and renal insufficiency are more common in both primary and secondary PCL compared with myeloma.

Osteosclerotic myeloma is a rare form of myeloma (<1%) usually seen in POEMS syndrome that is diagnosed based on a constellation of clinical and laboratory findings (polyneuropathy, organomegaly, extravascular volume overload, endocrinopathy, M component, skin changes, papilledema, thrombocytosis/polycythemia). The bone changes are characterized by osteosclerosis rather than lytic lesions. The

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monoclonal protein is usually of the IgA λ type and generally less than 3 g/dL. Lymph nodes may show Castleman disease, and serum vascular endothelial growth factor (VEGF) level is elevated.

TEMPI syndrome (telangiectasias, elevated erythropoietin level and erythrocytosis, monoclonal gammopathy, perinephric fluid collections, and intrapulmonary shunting) is a more recently recognized disease considered to be a paraneoplastic syndrome resulting from monoclonal gammopathy that responds to proteasome inhibitor. The disease is rare.

PLASMA CELL MYELOMA—FACT SHEET

Definition
- Monoclonal proliferation of plasma cells or their precursors leading to tumor formation, excess immunoglobulin monoclonal protein, and osteolytic bone disease, symptomatic or asymptomatic

Incidence and Location
- 1% of all malignancy and 10% of hematologic tumors
- Usually preceded by MGUS
- 7.2 men and 4.3 women per 100,000 among white and twice as often among black per year in the United States
- Over 20,000 new cases are diagnosed and over 11,000 die each year
- Predominantly involves bone marrow but may occur at extramedullary sites

Morbidity and Mortality
- Incurable
- Median survival averages 5 years with dose-intensive therapy and stem cell transplantation; 7 years with immunomodulatory drugs and proteasome inhibitors for standard risk patients
- Rare patients may survive 15 to 20 years

Gender, Race, and Age Distribution
- Male predominance (M:F = 2:1)
- Incidence is higher in blacks (2:1)
- Mostly elderly; median age at onset 69 years, but 2% occur in people younger than 40 years of age

Clinical Features
- Bone pain, anemia, recurrent infection, hypercalcemia, hyperviscosity, and renal failure; spinal cord compression (10%); peripheral neuropathies caused by amyloidosis
- Asymptomatic myeloma is diagnosed incidentally but carries higher risk for progression to symptomatic myeloma than MGUS

Radiologic Features
- "Punched-out" osteolytic lesions in vertebral bodies, skull, ribs, humerus, and femur

Prognosis and Therapy
- Poor prognostic indicators include high serum β2M, low serum albumin level, high serum LDH, high PCLI or Ki-67, hypodiploidy, gains of 1q, t(4;14), deletion of 13 and del17p/p53
- Conventional chemotherapy agents or novel agents, or immunotherapy with or without stem cell transplantation

Radiologic Features

X-Ray

Skeletal survey with plain films is essential in patients suspected of having myeloma. The classic radiographic image of myeloma is that of multiple lytic, well-circumscribed lesions within the skull, spine, and pelvis (Fig. 21.1). In solitary plasmacytoma of the bone, the lesions appear to be a localized expansion of a single bone and occasionally are associated with a soft-tissue mass. In POEMS syndrome, sclerotic lesions may be observed on radiographs.

Computed Tomography

Computed tomography (CT) is useful in patients suspected of myeloma and in whom the skeletal survey is negative. CT-guided percutaneous biopsy is useful in patients suspected of osseous or extramedullary plasmacytomas.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is superior to CT in detecting soft-tissue lesions and is more sensitive also in detecting early bone lesions. Thus, it is particularly useful in assessing spinal cord compression and extramedullary lesions to determine the extent of involvement. It is critical that thorough staging with MRI be performed in all patients presenting with solitary plasmacytoma of bone to rule out multifocal disease. MRI of spine and pelvis is useful to detect occult lesions (>5 mm) or to identify patients with SMM for evidence of early progression to active myeloma. The typical appearance of a myeloma infiltrate is a low signal intensity on T1-weighted images, which becomes high on T2-weighted sequences. Myeloma lesions also tend to enhance with gadolinium administration.

Positron Emission Tomography—Computed Tomography

Whole-body fluorodeoxyglucose/positron emission tomography-computed tomography (FDG/PET-CT) imaging is not routinely required, but can be used to detect occult lesions with high sensitivity and specificity.

Pathologic Features

Plasma cell enumeration and cytologic evaluation remain essential for classification and prognostication. Bone marrow aspirate tends to underestimate the extent of disease, especially in cases associated with marked fibrosis. A bone marrow biopsy should be performed, at least in the initial evaluation, to assess the extent and pattern of infiltration as well as evidence of myeloma-associated bone changes, amyloid deposition, and fibrosis. Myeloma usually has a high tumor

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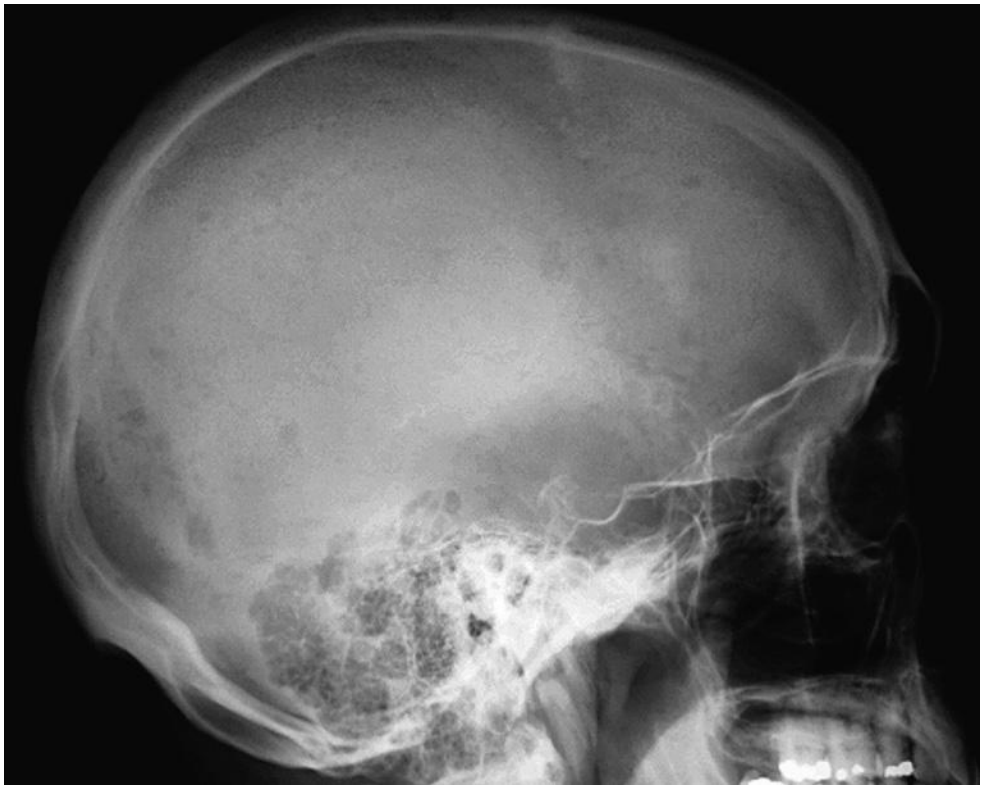


FIG. 21.1
Radiographic image of the skull. "Punched-out" osteolytic lesions characteristic of multiple myeloma are shown.

burden, whereas low-risk SMM and MGUS usually have low numbers of plasma cells that tend to be dispersed in the interstitium or form small clusters. There are also usually a significant number of normal plasma cells admixed in the MGUS.

Most patients usually present with systemic disease, and a biopsy of the iliac crest yields diagnostic material. In a small number of patients, the infiltrate may be patchy, and a negative biopsy result does not exclude the possibility of myeloma. In suspected cases of myeloma, a repeat biopsy of different sites is indicated.

Gross Findings

The involved bone shows a fish-flesh mass with hemorrhage or necrosis.

Microscopic Findings

Bone Marrow

Plasma cell enumeration is performed using a differential count of 200 to 500 cells. Normal plasma cells constitute 1% to 4% of nucleated cells in the marrow and are mature Marschalko type with abundant basophilic cytoplasm, perinuclear hof, and "spoke-wheel" nuclear pattern.

The neoplastic plasma cells often show a variable degree of immaturity or nuclear atypia. Asynchronous maturation of the nucleus and cytoplasm, dispersed chromatin, a high nuclear and cytoplasmic ratio, and prominent nucleoli are features of immaturity. Atypia usually manifests as bizarre nuclear forms and shapes and marked variation in nuclear size. These features are strong evidence of malignancy even if immunoglobulin light chain analysis fails to demonstrate monoclonality as in cases of nonsecretory myeloma. Binucleated or occasionally trinucleated forms may be observed in reactive conditions and are not necessarily indications of malignancy.

A variety of cytoplasmic inclusions may form owing to accumulation of cytoplasmic immunoglobulin, sometimes forming crystalline rods. Russell bodies are the most common cytoplasmic inclusions. Cells containing inclusions are described as grape cells, flaming cells, Mott cells, Gaucher-like cells, and thesaurocytes. Dutcher bodies are nuclear inclusions. In rare cases of monoclonal gammopathies (including multiple myeloma, MGUS, and lymphoplasmacytic lymphoma) crystalline material can be found also in histiocytes—so-called crystal storing histiocytosis. The crystalline material has been shown to occur overwhelmingly in plasma cell neoplasms expressing immunoglobulin kappa light chain.

Two grading systems have been proposed to describe the spectrum of plasma cell morphology in myeloma: the Greipp system and the Bartl system. In the Greipp system (Box 21.1), the plasma cells are divided into four subgroups: mature, intermediate, immature, and plasmablastic. In the Bartl system, six subtypes are described, as shown in Box 21.2. Examples of different Bartl grade tumors are illustrated in Fig. 21.2. The Bartl grading system also describes six patterns of infiltration as interstitial, interstitial with paratrabecular sheets, interstitial/nodular, nodular, packed, and sarcomatous. The Greipp system was developed for use in bone marrow aspirate

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Box 21.1 Greipp Plasma Cell Grading System

Mature Type

Dense chromatin clumping
Nucleus <8 μm
Nucleolus 1 μm
Cytoplasm well developed
Nucleus eccentrically placed with a prominent hof

Intermediate Type

Not meeting criteria of other types

Immature Type

Diffuse chromatin pattern
Nucleus >10 μm or nucleolus >2 μm and abundant cytoplasm
Nucleus eccentrically placed with a hof

Plasmablastic Type

Very high nuclear/cytoplasmic ratio; scanty cytoplasm; and a central, immature, large nucleus with a reticular chromatin pattern, prominent nucleolus, and little or no hof

Box 21.2 Bartl Grading System

Low Grade

Marschalko: eccentric cartwheel nuclei, perinuclear hof, basophilic cytoplasm, few nucleoli or mitotic figures, primarily interstitial
Small cell: smaller than Marschalko; round, lymphocytic nucleus; narrow rim of basophilic cytoplasm; associated with plasma cell leukemia

Intermediate Grade

Cleaved: notched, cleaved nuclei of variable size; high nuclear to cytoplasmic ratio; small hof often present; often associated with packed marrow with fibrosis
Polymorphous: cellular and nuclear polymorphism; 25% may show prominent central nucleoli
Asynchronous: marked nuclear and cytoplasmic maturation asynchrony; >50% cells have large eccentric nuclei, prominent nucleoli abundant basophilic cytoplasm, pronounced nuclear hof

High Grade

Blastic: large nuclei, prominent centrally located nucleoli, and moderate rim of basophilic cytoplasm, faint perinuclear hof

PLASMA CELL MYELOMA—PATHOLOGIC FEATURES

Gross Findings

- Fish flesh, hemorrhagic tumor

Microscopic Findings

- Atypical or immature plasma cells proliferate in large clusters in the bone marrow forming space-occupying lesions associated with increased osteoclastic activity and intramedullary fibrosis; may be associated with amyloid deposition

Ultrastructural Findings

- Prominent Golgi region and dilated rough endoplasmic reticulum; cytoplasmic crystalline inclusions

Fine-Needle Aspiration Biopsy Findings

- Atypical or immature plasma cells

Immunohistochemical Findings

- $CD138^+$, $CD38^+$, κ or λ immunoglobulin light chain; $CD56^+$ (60% to 70%) or CD79a (50% to 70%); $CD117^+$ (20% to 30%) or $CD20^+$ (20%); $MUM1^+$; CD45dim/negative, PAX5/BSAPdim/negative

Differential Diagnosis

- Reactive plasmacytosis
- Acute monoblastic leukemia or idiopathic myelofibrosis
- Non-Hodgkin lymphoma or leukemia with plasmablastic/plasmacytic/plasmacytoid differentiation
- Epithelial tumors

smear samples whereas the Bartl system was developed for use in tissue section. Both have shown poor outcome in patients with plasmablastic or high-grade cytologic features.

In addition to morphologic assessment of the plasma cells, vasculature and bone changes are also essential parts of the overall evaluation. Amyloid deposition and increased osteoclastic activities may accompany the plasma cell infiltrate. Increased bone resorption and bone remodeling are features distinguishing symptomatic myeloma from MGUS.

Peripheral Blood Smear

The smear may have a blue hue and show rouleaux formation of the red blood cells because of a high level of immunoglobulin (Fig. 21.3). Normochromic normocytic anemia is a frequent finding. Leukopenia or thrombocytopenia is usually present at the advanced stage of disease when the tumor burdens are heavy. The circulating plasma cells may resemble plasmacytoid lymphocytes or myeloblasts. Primary PCL is often associated with small-cell morphology resembling low-grade B-cell lymphoma or leukemia with plasmacytoid differentiation.

Ultrastructural Findings

Electron microscopy is usually not required for the diagnosis of myeloma. The typical features of plasma cells are a prominent Golgi region and dilated rough endoplasmic reticulum. Cytoplasmic crystalline inclusions may also be present.

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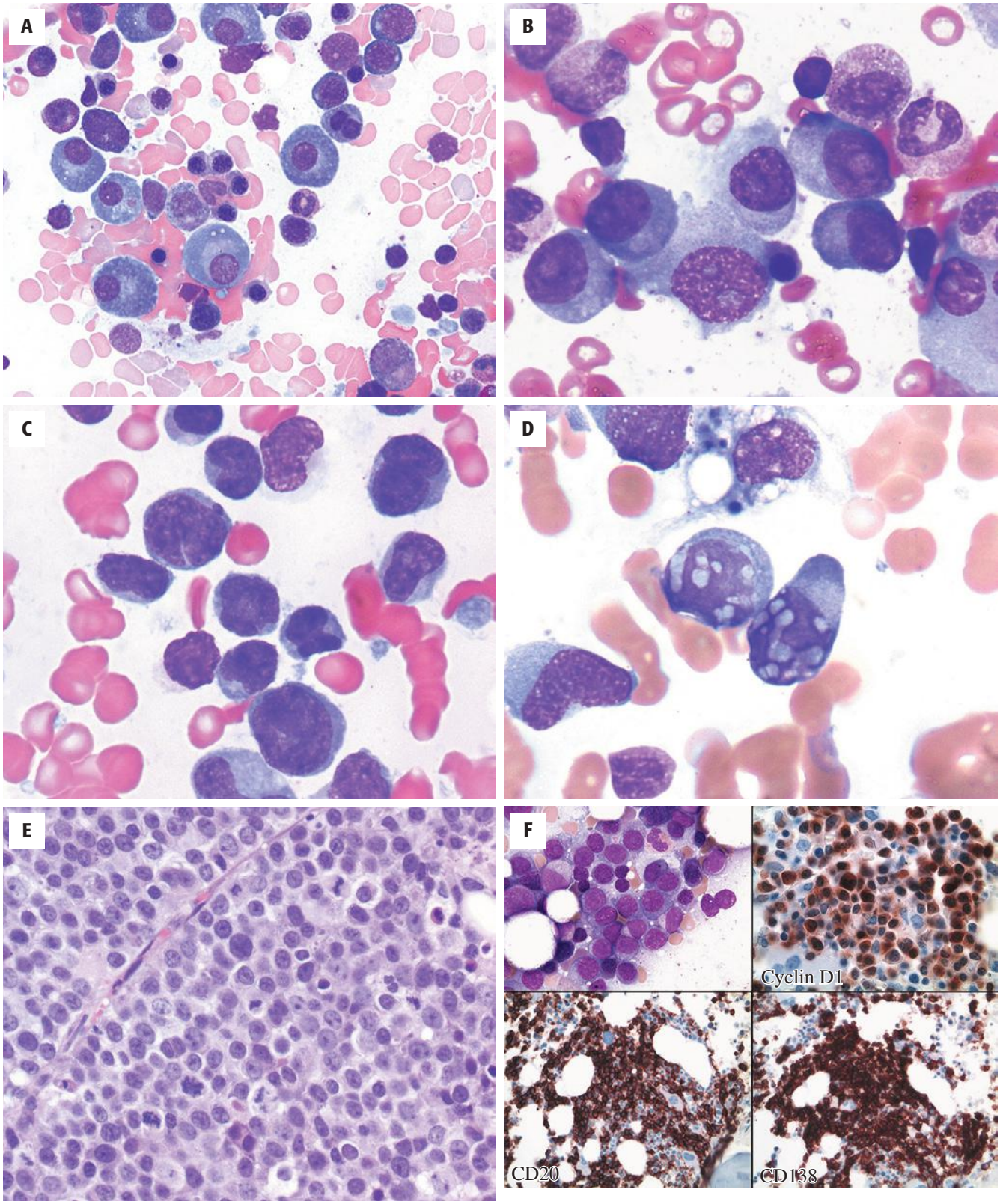


FIG. 21.2

Multiple myeloma. A, Myeloma cells of low-grade cytology. Some plasma cells have cytoplasmic immunoglobulin inclusions (Wright-Giemsa, ×500). B, Myeloma cells of intermediate-grade cytology. The nucleoli are prominent, and cytoplasmic-nuclear maturation is asynchronous. (Wright-Giemsa, ×1000). C, Myeloma cells of intermediate-grade cytology. The cell size and nucleus are polymorphic (Wright-Giemsa, ×1000). D, Plasma cells of intermediate-grade cytology with cytoplasmic immunoglobulin inclusions. E, Bone marrow biopsy of plasmablastic myeloma (hematoxylin and eosin ×400). Note the increased mitotic figures. F, Small-cell variant of plasma cell myeloma resembling lymphoplasmacytic lymphoma (Wright-Giemsa ×1000). The neoplastic cells are positive for CD20, CD138, and cyclin D1 (nuclear and cytoplasmic staining; immunohistochemistry).

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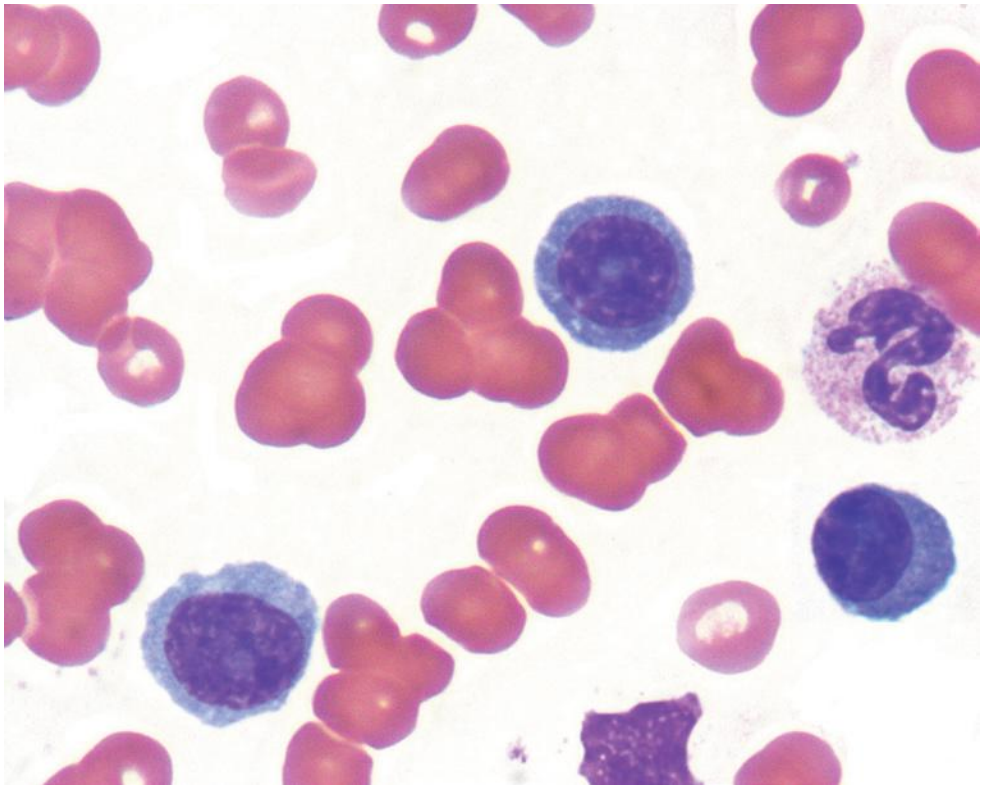


FIG. 21.3
Peripheral blood smear of a patient with plasma cell leukemia.

Ancillary Studies

Laboratory Findings

Laboratory evaluations are essential for diagnosis, follow-up, and prognosis. Complete blood count (CBC) often reveals normocytic normochromic anemia, the most common finding. Serum protein electrophoresis (SPEP), urine protein electrophoresis (UPEP) of an aliquot from 24-hour urine collection, and immunofixation electrophoresis (IFE) are used to detect and quantify monoclonal protein (M protein). Other tests such as β_2-microglobulin (β2M), C-reactive protein, lactate dehydrogenase, interleukin-6, plasma cell labeling index, and bone markers serve as biomarkers for tumor load or tumor activity. Serum chemistry panels include calcium and creatinine to detect hypercalcemia and renal failure. Elevated vascular endothelial growth factor (VGEF) levels support the diagnosis of POEM syndrome.

Most patients have monoclonal serum protein (M component). IgG is most common (55%), followed by IgA (20%), light chain only (20%), IgD (1.5%), IgM (0.5%), and IgE (0.5%). Nonsecretory myelomas account for 2% cases. Urine monoclonal immunoglobulin light chain protein, also termed the *Bence Jones protein*, can be detected in 80% of patients.

M protein (or M component) appears as a discrete band on SPEP or as a narrow spike or peak usually in the β or γ region of the densitometer tracing but may be anywhere between α1 to post-γ regions (Fig. 21.4). In light chain–only myeloma, a distinct band or spike may not appear on SPEP, and hypogammaglobulinemia may be the only finding. In IgD or IgE myeloma, the routine SPEP, which uses antibodies against IgG, IgA, and IgM, will not detect an abnormal band. Using antibody against IgD and IgE will confirm the diagnosis. IFE determines the type of M protein and detects M proteins beyond the sensitivity level of SPEP (<5.0 g/L).

M protein is used to confirm the diagnosis and for follow-up to assess the response to treatment. Serial quantification of M protein by densitometer tracing allows easy assessment of tumor load reduction. Clinically, partial remissions are defined as more than 75% of reduction of serum M protein or more than 95% reduction of urine M protein, and the complete remission (CR) is defined as absence of M protein and bone marrow plasma cells less than 5%.

In patients treated with myeloablative therapy followed by bone marrow transplantation, reconstitution of humoral immunity may create a transient oligoclonal or monoclonal pattern that should be distinguished from the original M protein band. Comparing the IFE pattern before and after therapy may help to determine whether residual disease is present.

Total serum IgG, IgA, and IgM are measured by nephelometry, a tool more useful when the M protein is small. Suppression of uninvolved immunoglobulin is generally an indication of overt disease but can be seen in MGUS and thus is not a reliable discriminator of the two conditions. Discordant readings between nephelometry and densitometer tracing, with a lower reading by the latter, are usually the result of high levels of γ-globulin.

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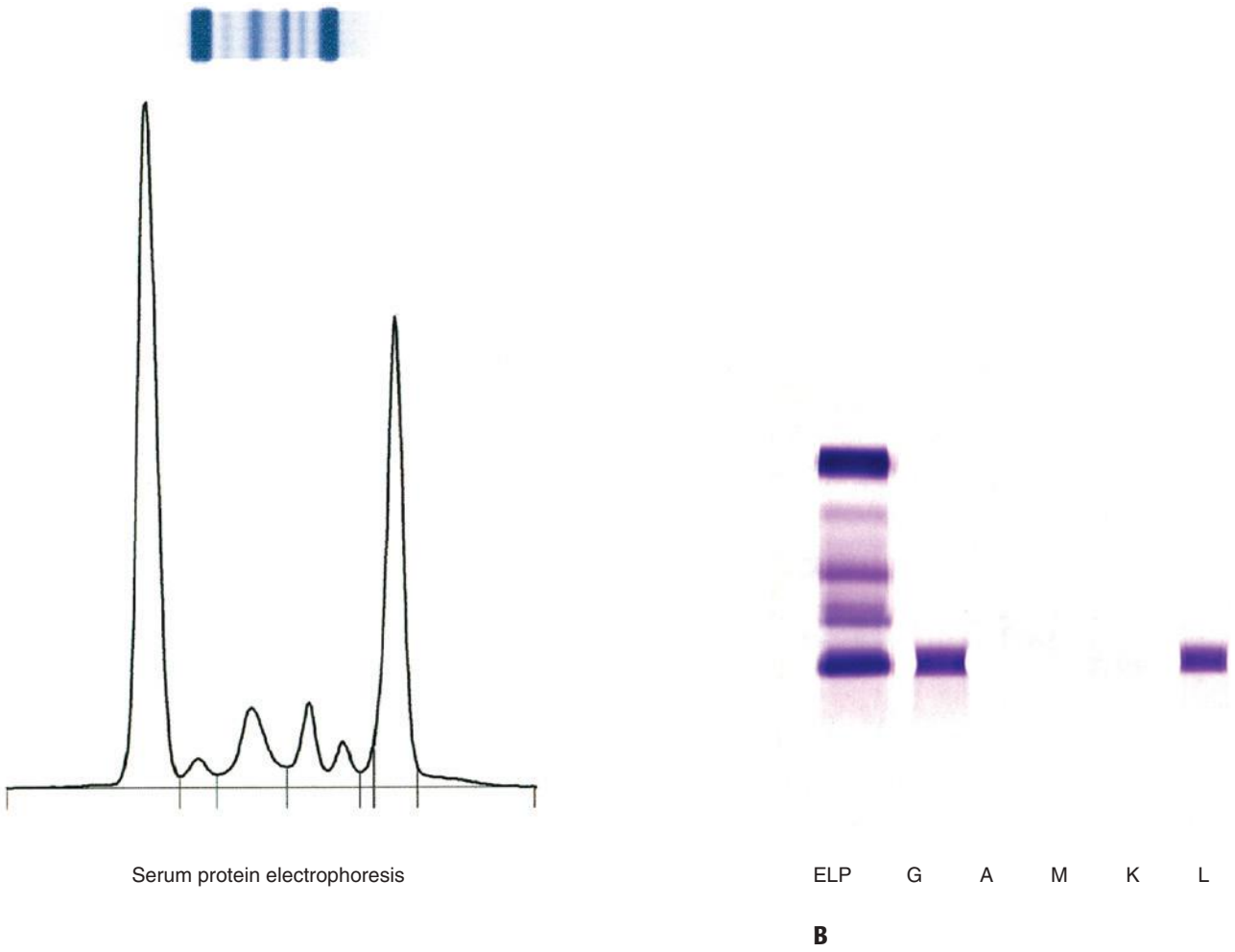


FIG. 21.4

Electrophoresis. A, Serum protein electrophoresis demonstrates an M-protein peak. B, Immunofixation electrophoresis confirms it to be monoclonal IgG λ type.

Serum free light chains (FLC) are measured by nephelometric methods, and the ratio of κ and λ light chains is calculated. FLC ratio is most useful in cases with minimal serum M protein and nonsecretory or light chain–only myeloma and in monitoring patients in clinical remission when the M protein levels are low. It is increasingly replacing UPEP for detection of Bence Jones protein. Abnormal FLC ratio at initial diagnosis also predicts risk for progression from asymptomatic to symptomatic myeloma. It is also one of 3 biomarkers designated in MDEs to diagnose active myeloma when the involved and uninvolved FLC ration is equal or greater than 100. It is also useful in diagnosis of MGUS, amyloidosis (AL) and light chain deposition disease (LCDD) or light chain–only MGUS when the M component is low. FLC is also used to assess response to therapy as serum M protein levels. Stringent complete remission (CR) is defined as normal FLC ratio in addition to the traditional definition of CR.

β2M, reflecting cell turnover and renal function, is used as a prognostic marker. Patients with renal failure have elevated β2M confounding interpretation. C-reactive protein is an acute phase reactant that is produced by the liver under the control of cytokines such as interleukin-6. Its level correlates with disease activity in myeloma. Lactate dehydrogenase is usually elevated in patients with extensive lymphadenopathy and indicates a poor prognosis.

In the International Staging System (ISS) introduced in 2005, β2M and albumin (ALB) levels are used to define three stages of symptomatic (active) myeloma:

1. β2M <3.5, ALB ≥3.5
2. β2M <3.5, ALB <3.5, or β2M 3.5–5.5
3. β2M ≥5.5

β2M levels are measured in mg/dL; ALB levels are measured in g/dL.

The ISS has been recently updated to incorporate cytogenetic findings and serum lactate dehydrogenase (LDH) level. In the revised ISS, stage I disease includes ISS stage I with a normal level and absence of high-risk cytogenetics such as del[17p], t[4;14], or t[14;16]. R-ISS III includes ISS stage III with either high-risk cytogenetics or elevated LDH. All others fall into R-ISS II. The 5-year overall survival (OS) rates are 82% in R-ISS I, 62% in R-ISS II, and 40% in R-ISS III, respectively.

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The plasma cell labeling index measures synthesis of DNA by using a monoclonal antibody (BU-1). The antibody BU-1 reacts with bromodeoxyuridine that is incorporated into the DNA synthesis by the cells in S phase. Patients with standard risk myeloma usually have a plasma cell labeling index of <3% and more than 3% in the high risk subgroup. But this assay is not widely used and Ki-67 (threshold of 10%) is more commonly used as a surrogate for measuring proliferation.

Biochemical markers of bone resorption provide more sensitive information about bone turnover. These include amino- and carboxy-terminal cross-linking telopeptide of type I collagen (NTX and CTX, respectively) or CTX generated by matrix metalloproteinases cross-linked carboxyterminal telopeptide of type I collagen (ICTP). They can be used to monitor disease and treatment response.

Fine-Needle Aspiration

CT-guided percutaneous biopsy or fine-needle aspiration is useful in obtaining tissues from deep-seated plasmacytomas. Other situations in which fine-needle aspiration may be used include abdominal fat pad aspiration and diagnosis of amyloid deposition.

Cytochemistry

The plasma cells are reactive with acid phosphatase and nonspecific esterase. These stains are rarely used clinically for the purpose of identifying plasma cells.

Flow Cytometry Immunophenotyping

Although in many instances flow cytometry immunophenotyping is not required for the diagnosis, it has been increasingly utilized for several reasons. In morphologically challenging tumors or nonsecretory myeloma, identification of monoclonal plasma cells or aberrant marker expression on plasma cells facilitates the diagnosis. The aberrant markers can also be used to detect minimal residual disease (MRD) or as potential targets of therapy. In recent years 8 to 10 color multiparameter flow cytometry (MPF) for MRD detection has been increasingly used to measure depth of CR and as a surrogate for measuring efficacy of newer therapeutic agents.

CD138 is the best marker for identification of benign and malignant plasma cells. CD38 is less specific than CD138 as it is also expressed in T cells and some B cells. The new anti-CD38 antibody daratumumab (Darzalex) therapy will render the routinely used antibody non-reactive. Plasma cell gating is best achieved by using a combination of CD138, CD38, CD45, and light scatter pattern. Additional markers such as CD19, CD27, CD28, CD56, CD81, CD117, and cytoplasmic light chain further discriminate benign from aberrant plasma cells. CD38 is bright in benign plasma cells and often becomes dimmer in malignant plasma cells. Benign plasma cells are typically positive for CD19, CD27, and CD45 and negative for CD117 and CD56. Malignant plasma cells usually show decreased or absent CD19, CD27, or CD45 but increased CD28, CD56, and CD117. However, CD45 and CD20 can be expressed at moderate or higher intensity in 10% and 20% of cases, respectively. CD56 is positive in 60% to 70% of cases. CD117 is aberrantly expressed in 20%–30% of cases and is a useful marker for malignancy. Myeloid markers CD13 and CD33 or other markers such as CD10 can also be identified in a small subset of cases. Indeed, in plasmablastic cases a known diagnostic pitfall is mistaking a plasma cell neoplasm expressing myeloid markers for acute myeloid leukemia. PCL tends to be positive for CD20 (50%) and negative for CD117 and CD56 compared to myeloma. About 90% of secondary PCL cases express CD28 as compared with 30% in primary PCL. The plasma cells express cytoplasmic but usually not surface Ig light chain.

Assays designed to detect MRD by flow cytometry are usually based on the differential expression patterns of surface markers between benign and malignant plasma cells (Fig. 21.5). The international consensus panel proposed for MRD assay includes CD138, CD38, CD45, CD19, CD27, CD56, CD81, and CD117. The detection sensitivity reaches 1 in 10^5 or higher in cases with aberrant marker expression and when at least 2 million events are analyzed. MRD is a continuous variable with progressively longer survival when using a scale of more than 0.01%, 0.01% to 0.001%, or less than 0.001% of MRD. Sensitivity of the flow cytometry–based assay is comparable to allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) and has a wide applicability, but is one log less sensitive than next generation sequencing based MRD assay.

Flow cytometry can also measure tumor ploidy or DNA index (DI). DI of less than 0.95, 0.95–1.05, more than 1.05, and 1.75–2 defines hypodiploid, pseudodiploid, hyperdiploid, and near tetraploid myeloma, respectively.

Immunohistochemistry

As mature plasma cells are difficult to distinguish from lymphoid and erythroid cells on regular hematoxylin and eosin sections, immunostains for plasma cell markers should be performed on the biopsy specimen, at least in the initial evaluation, to assess the extent and pattern of involvement.

The most useful antibody for identifying plasma cells in frozen or paraffin-embedded tissue is syndecan-1 (CD138; see Fig. 21.2F). When combined with κ and λ, syndecan-1 provides a more accurate assessment of tumor load. This is particularly important in cases of SMM, which required a minimal number of 10% monoclonal plasma cells. A caveat is that immunoblastic/plasmablastic large B-cell lymphoma and plasmablastic lymphoma are also positive for CD138. A positive result may represent bone marrow involvement by lymphoma rather than myeloma.

Ki-67 (MIB-1) is used more frequently than a plasma cell labeling index as a surrogate marker for proliferation. Ki-67 of more than 10% is considered an indication of intermediate or high proliferation activity. Dual staining of Ki-67 with κ and λ or CD138 allows distinction between proliferating plasma cells and other marrow elements, especially the rapidly dividing erythroid precursors. Most cases also express CD79a and MUM-1. CD20 is expressed in one fourth of cases and does not reliably distinguish myeloma from other mature

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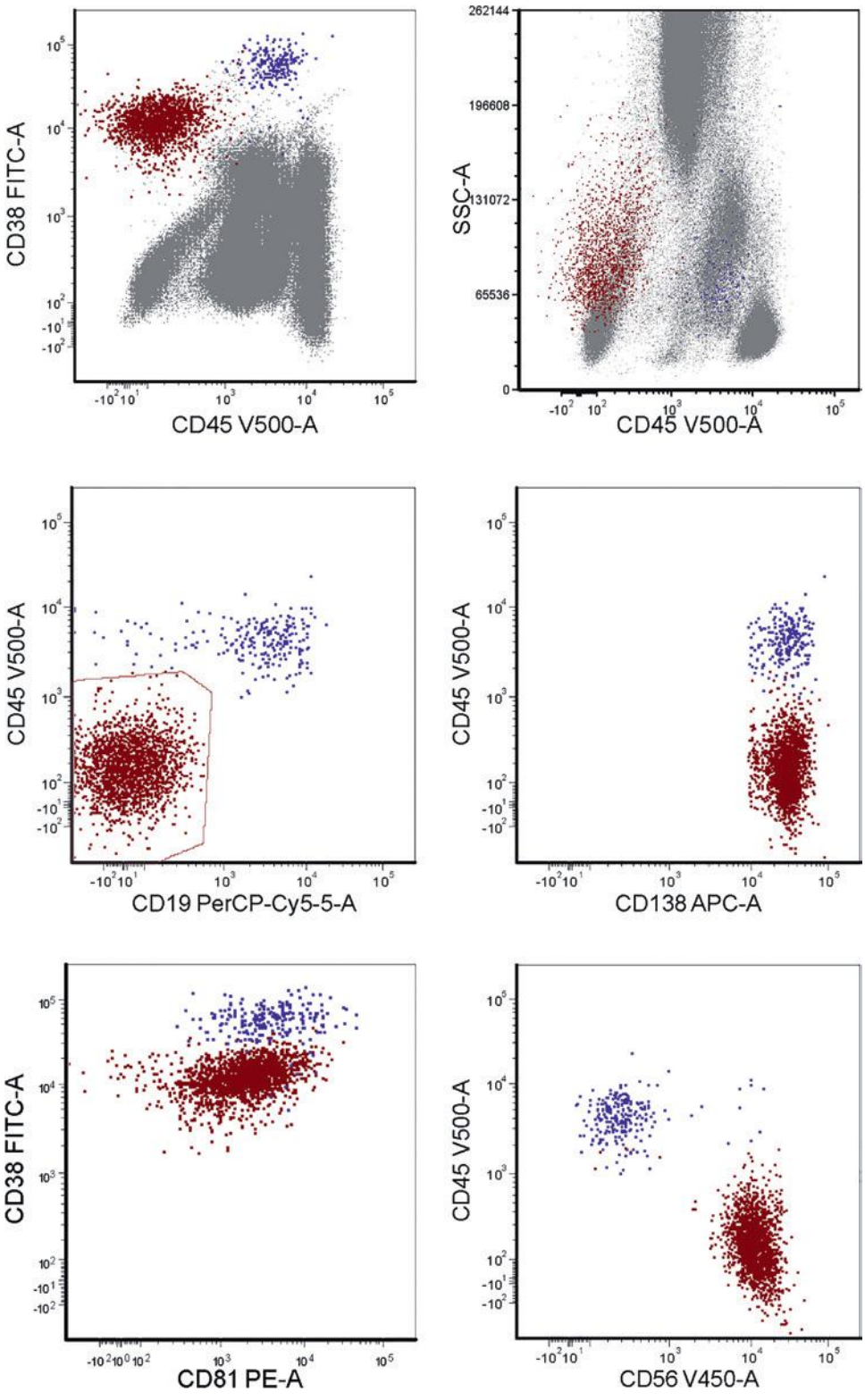


FIG. 21.5

Flow cytoemtry immunophenotyping of normal and aberrant plasma cells. Comparison of neoplastic *(brown)* versus normal plasma cells *(blue)*. The neoplastic/aberrant plasma cells are $CD38^{+}$ (decreased intensity compared to the normal plasma cells), $CD45^{-}$, $CD19^{-}$ $CD56^{+}$ and $CD81^{+}$ (decreased) compared to normal plasma cells which are $CD19^{+}$ $CD45^{+}$.

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TABLE 21.2

The Five Most Common Partner Genes Involved in Translocations of Immunoglobulin Heavy Chain Gene

Chromosome	Gene	Percentage
11q13	CCND1	15
4p16.3	FGFR3 and MMSET	15
16q23	C-MAF	5
6p21	CCND3	3
20q11	MAFB	2

B-cell neoplasms. PAX5 is usually negative or weakly positive with occasional exceptions. In 40% to 45% of cases, cyclin D1 is expressed, typically in a nuclear and cytoplasmic pattern. BLIMP-1 encoded by *PRDM1* gene is negatively correlated with PAX5 and can also be used to identify cells with plasmacytic/blastic differentiation.

Cytogenetics

Conventional cytogenetic analysis detects structural or numeric chromosomal abnormalities, usually complex, at diagnosis in only approximately 30% of cases. In most instances, the low proliferating tumor cells yield no informative result. However, genetic aberrations can be demonstrated in nearly all myelomas by interphase fluorescence in situ hybridization (FISH) analysis. Plasma cell enrichment by CD138 magnetic beads may enhance the yield of interphase FISH.

Translocations involving the switch region of Ig heavy-chain locus on chromosome 14q32 are the most common recurrent cytogenetic abnormalities, occurring in about 50% of cases. Over 50 partner genes have been described; the five most common ones representing 40% of all cases with *IGH* translocation are located at 11q, 4p, 16q, 6p, and 20q. The genes and their functions are mostly related to expression of cyclin D family protein and cell cycle regulation (Table 21.2). These five *IGH* translocations are also detectable in MGUS and therefore are considered primary and possibly initiating events.

The breakpoint in t(11;14) involves part of the *CCND1* gene that is located more centromeric than that in mantle cell lymphoma. The t(11;14) is more common in primary PCL (71%) compared with secondary PCL (23%). The t(4;14) interrupts both fibroblast growth factor (FGF) receptor 3 gene (*FGFR3*) and multiple myeloma SET domain (*MMSET*) genes and is best identified by FISH because the breakpoint involves the telomeric part of the chromosome 4. The t(14;16) and t(14;20) result in overexpression of *MAF/MAFB*, regulating *CCND2*, and therefore overexpression of cyclin D2.

Besides structural aberrations, numeric aberrations are also common. Trisomy most frequently involves odd chromosomes 3, 5, 7, 9, 11, 15, 19, and 21, whereas monosomy and partial deletions affect mainly chromosomes 6, 13, 16, and 22. Gains of 1q are the most common structural aberrations in myeloma, detectable in up to 40% of those with an abnormal karyotype. Monosomy 13 or interstitial deletion of 13 (13q14) is detectable in 15% to 20% of newly diagnosed myeloma by conventional cytogenetics and 50% of cases by interphase FISH. Del13 can also be found in MGUS. The t(4;14), del(13q), and gain(1q) tend to cosegregate.

Based on the number of chromosomes, myeloma cases can be broadly divided into two groups: the hyperdiploid (HRD; 48-75 chromosomes) and non-hyperdiploid (NHRD myeloma; <48 or >75 chromosomes). HRD represents about half of cases and frequently carries trisomies. The five recurrent translocations described above and del13 are by far more common in NHRD than HRD, suggesting that different molecular genetic pathways are involved in pathogenesis of the two subgroups.

Other genetic aberrations described in myeloma include *MYC* rearrangement, usually involving non-*IGH* partners, occurring in 15% to 20% of cases; amplification of 1q21 (resulting in putative cyclin-dependent kinase subunit 1 (*CKS1B*) gene amplification) in 30% to 40%. Deletion of *TP53* is identified in 10% of newly diagnosed myelomas but is much higher in PCL, in approximately 50% of pPCL and 70% of sPCL. Unlike the five primary translocations and 13q deletions, *MYC* rearrangement or *RAS* mutations are rare in MGUS. Many of these genetic abnormalities have prognostic implications to be mentioned below (see Prognosis and Therapy).

Gene Expression Profiling, Next-Generation Sequencing, Single-Nucleotide Polymorphisms, and Micro-RNA Microarrays

The advent of new genomic technology allows global investigation of genes altered in myeloma and has resulted in new ways of classifying myeloma. The translocation/cyclin D (TC) classification scheme proposes eight molecular subgroups—6q21, 11q13, D1, D1+D2, D2, non, 4p16, and maf—based on the predominant activation pattern of cyclin D protein and the pattern of *IGH* translocations identified by DNA microarray. The University of Arkansas for Medical Sciences (UAMS) molecular classification of myeloma identifies seven distinct gene expression clusters, including the translocation clusters MS, MF and CD-1/2, a hyperdiploid cluster, a cluster with proliferation-associated genes (PR) and a cluster marked by a low incidence of bone disease (LB). More recently, the European group introduced EMC-92-gene signature that identified additional molecular clusters including NFκB, CTA and PRL3. Other technologies, such as complementary genomic hybridization (CGH)-based mapping array, also allow investigation of key candidate genes in the context of complex chromosomal gain and loss. Single nucleotide polymorphism (SNP) array has found additional prognostic markers including del(12p13.31) as an independent adverse marker and amplification of (5q31.1) as a favorable marker. Similarly, micro-RNA–based array and proteomics have also shed light on mechanisms controlling myeloma growth and disease progression. Micro-RNA may also serve as a biomarker for diagnosis, prognosis, and monitoring of residual disease.

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Although some of these techniques are largely still in the realm of clinical research, GEP and aCGH have been increasingly incorporated into clinical practice.

Next-generation sequencing has not only confirmed known recurrent mutations but also discovered new mutations in myeloma. Mutations of *NRAS* (20% to 30%), *KRAS* (15% to 20%), and NF-κB (6% to 7%) pathway components such as *TRAF3, cIAP1/2, CYLD*, and *NIK* are most common, followed by *CCND1* (12%), *TP53* (10%), *BRAF* (5%), *ATM* (3%), *ATR* (1.5%), or *MYC* (1%). Mutations in *CCND1* and DNA repair pathway alterations (*TP53, ATM, ATR,* and *ZNFHX4* mutations) are negatively associated with survival. In contrast, mutations in *IRF4* and *EGR1 on 5q*, a target of IMiD drugs, are positively associated with survival. The prognostic impact of *RAS* pathway mutations remains controversial, but they can be exploited further for targeted therapy. These mutations also correlate with cytogenetics. Patients with *TP53* mutations are mostly seen in those with del17p, whereas *CCND1* mutation are frequent in t(11;14). *EGR1* is seen mostly in the hyperdiploid cases, and t(11;14) is associated with *KRAS* and *IRF4* mutations. Besides mutations, studies have found p16 methylation in 20% to 30% and p18 methylation in 20% to 30% of myeloma. Hypermethylation of tumor suppressor genes (*GPX3, RBP1, SPARC,* and *TGFBI)* is associated with an adverse outcome. Adverse mutations, such as abnormal copy number changes or translocations, also have accumulative effects, with more aberrations correlating with a worse outcome.

Single-cell sequencing has identified marked intra-clonal heterogeneity and clonal evolution upon therapeutic intervention in myeloma. Focal lesions from different loci may harbor divergent genetic hits, and different subclones may compete in Darwinian evolution during the course of disease. The so-called clonal tide has profound therapeutic implications as drug-resistant subclones, such as those with deleted or mutated *TP53,* detectable only as a minor subset at diagnosis, may be "selected" to expand after therapy. To eradicate the myeloma, tumor stem cells, or driver mutations will inevitably need to be addressed in order to overcome the challenge of clonal heterogeneity and evolution.

Differential Diagnosis

A variety of reactive conditions are associated with plasmacytosis and sometimes up to 30% of marrow cells. Autoimmune disorders, hepatitis C, human immunodeficiency virus infections, angioimmunoblastic T cell lymphoma, and Hodgkin lymphoma are a few examples. Nuclear immaturity and marked atypia are not features of reactive plasmacytosis and are strong indications of malignancy. Reactive plasmacytosis is also frequently associated with increased eosinophils, mast cells, and megakaryocytes. Benign plasma cells usually occur in small clusters of five or six cells in perivascular areas, whereas the myeloma cells proliferate in sheets; infiltrate and replace normal hematopoietic elements with increased bone resorption. Demonstration of monoclonality by immunoglobulin light chain analysis confirms a neoplastic process. Identification of aberrant marker expression such as CD56 or CD117 provides additional supporting evidence of malignancy.

Large immature myeloma cells may resemble monoblasts, react with nonspecific esterase, and may express myeloid-associated markers such as CD33, causing confusion with acute monoblastic leukemia. CD138 is an extremely helpful marker for the distinction of the two entities. The monoblasts are negative for CD138.

The polymorphous subtype of myeloma is frequently associated with fibrosis. The tumor cells show marked nuclear irregularity and can be confused with primary myelofibrosis. Again, demonstration of CD138 expression by the infiltrating cells confirms a plasmacytic origin.

Monoclonal protein can be detected in a variety of non-Hodgkin lymphomas or chronic lymphocytic leukemia. Since a small amount of monotypic B cells of identical idiotype can be detected in patients with myeloma, the differential diagnosis of lymphoma with marked plasmacytic differentiation versus myeloma arises. In the bone marrow, chronic lymphocytic leukemia with plasmacytoid differentiation or lymphoplasmacytic lymphoma (LPL)/Waldenström macroglobulinemia (WM) is the most commonly entertained differential diagnosis. The neoplastic plasma cells in myeloma are usually $CD19^-$, $CD45^-$, $CD56^+$, whereas plasma cells in LPL tend to coexpress CD19, CD45, and lack CD56. Of note, myeloma cases with *IGH-CCND1* translocation are often $CD20^+$ with lymphoplasmacytic morphology. Thus, cases of suspected LPL in bone marrow should be examined for this translocation or cyclin D1 overexpression by immunohistochemistry to exclude plasma cell myeloma. Furthermore, in LPL samples obtained after chemotherapy, the plasma cells persist and become the predominant component whereas the lymphoid cells are eliminated. In this setting, detection of IgM paraprotein strongly favors a diagnosis of LPL/WM. *MYD88* L265P or *CXCR4* mutations are identified in more than 90% of WM and a small number of other low-grade B-cell lymphoma, such as splenic marginal zone lymphoma and chronic lymphocytic leukemia, and support lymphoma rather than myeloma.

When evaluating small extramedullary tissue biopsy specimens, such as gastrointestinal or ocular tissue, immunohistochemistry is often the only available tool for immunophenotyping, and the differential diagnosis often includes marginal zone B-cell lymphoma with marked plasmacytic differentiation. Expression of CD79a by the neoplastic cells is not a reliable marker for the distinction of the two entities. Pax5/BSAP and CD19 are usually absent in myeloma and can be very useful in these settings. In the rare cases of myeloma expressing CD20 and Pax5/BSAP, evidence of CD138 and immunoglobulin heavy chain IgG or IgA type expression may be helpful. The presence of small numbers of monocytoid B cells and remnant reactive germinal centers favors extranodal marginal zone lymphoma.

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The distinction of a high-grade extramedullary plasmacytic neoplasm from immunoblastic/plasmablastic lymphoma can be extremely difficult. Immunophenotype may be indistinguishable. Epstein-Barr virus–encoded RNA in situ hybridization is usually positive in plasmablastic lymphoma and negative in myeloma. Correlation with other clinical features such as serum M protein and radiographic imaging may be helpful in difficult cases.

Plasmacytosis in MGUS usually is less than 10% of total cellularity and lacks cytologic atypia. Immunoglobulin κ-to-λ ratio may fall within the normal range when analyzed by immunohistochemistry, as the polytypic plasma cells are frequently intermixed. Flow cytometry allows better discrimination of monotypic plasma cells from polytypic plasma cells in this setting.

Localized plasmacytoma of bone needs to be distinguished from metastatic tumor. In this situation, CD138 is not useful, as epithelial tumors also express this marker. Demonstration of immunoglobulin light chain expression and restriction is critical.

Patients with TEMPI syndrome may show nonspecific erythroid hyperplasia and mild nonspecific megakaryocytic hyperplasia. There is usually no clustering of megakaryocytes or fibrosis. The syndrome should be suspected when erythrocytosis coexists with plasma cell dyscrasia. Elevated erythropoietin and a normal VGEF level allow the diagnosis and distinction from POEMS syndrome. The disease can be monitored by following erythropoietin level.

Prognosis and Therapy

With the novel agents such as the immunomodulatory (IMiD) drugs thalidomide, lenalidomide, and proteasome inhibitor bortezomib, the median survival of patients with standard risk myeloma is now 7 years, a remarkable improvement compared to approximately 3 years with standard therapy (melphalan and prednisone) and 5 years with dose-intensive therapy and autologous stem cell transplantation. In transplant-eligible patients, the induction usually consists of a two- or three-drug combination of either an IMiD or a cytotoxic drug (cyclophosphamide) plus a proteasome inhibitor and steroid followed by autologous transplant and maintenance by either IMiD and/or a proteasome inhibitor. Rarely, allogeneic transplant is given in the clinical trial setting for chemoresistant patients. Patients ineligible for transplant are treated with novel agents with or without melphalan.

Newer generations of IMiDs (pomalidomide) and proteasome inhibitors (carfilzomib, ixazomib) further enhance the therapeutic efficacy, especially in refractory cases. The target of IMiD is cereblon. New classes of drugs such as immunotherapy (anti-CD38 antibody, anti-SLAMF7 antibody elotuzumab), histone deacetylase (HDAC) inhibitor (Panobinostat), and antimitotic kinesin spindle protein (KSP) inhibitor (ARRY-520) are also approved. The immune checkpoint PD1 and PD-L1 axis is a new frontier being explored for treatment of refractory myeloma. Adaptive therapy with T cells or ex vivo expansion of natural killer (NK) cells or vaccines is being tested in clinical trials. Patients with *RAS/MAPK* pathway mutation may benefit from MEK inhibitors or *BRAF* mutations to BRAF inhibition. Mutations in the NF-κB pathway could also be targeted therapeutically. Despite these advances, myeloma remains an incurable disease, and survival is highly variable among different patients. Risk-stratified therapy is the key to improve survival.

There has been great interest in SMM in recent years partly because more effective and less toxic therapeutic agents are now available allowing optional early intervention. It remains controversial, though, as to how patients should be treated. Currently the treatment for these patients is usually provided in the setting of clinical trials.

Besides age, performance status, disease stage, molecular genetics, and cytogenetics underscore the tumor biology and therefore form the cornerstone of risk stratification. An abnormal karyotype identified by conventional cytogenetics is indicative of highly proliferative tumors and inferior survival. Furthermore, deletion of chromosome 13 or 13q14, hypodiploidy, and monosomy 17/p53 are associated with a poor prognosis. Detection of t(4;14), t(14;16), or t(14;20), deletion of *TP53* by FISH predicts an inferior survival but deletion of chromosome 13 detected by FISH in the absence of an abnormal karyotype is not predictive of an inferior survival. Gain of chromosome 1q (1q+) (35% to 40%) and loss of chromosome 1p (10%), frequently occur together and are now also considered adverse risk factors. Combinations of ≥3 CA confer ultra-HR with <2 years survival. Bortezomib partly overcomes the adverse effect of t(4;14), and this genetic is now listed as an intermediate-risk factor in the Mayo Clinic risk model. Deletion of 1p involves possibly *CDKN2C,* which encodes for the cyclin-dependent kinase inhibitor 2C (p18), causing increased proliferation, or *FAM46C* at 1p12. The definition of high-risk factors has evolved with the novel anti-myeloma therapy discussed below. The risk factors also have an accumulative effect on survival, with more predicting worse outcome. The R-ISS which incorporates stage of diseases based on ISS with genetics and LDH levels (Table 21.3) is a robust risk model for symptomatic myeloma patients. More recent studies found that the combined GEP signature and R-ISS could further refine risk stratification.

Laboratory parameters that correlate with poor prognosis include the proliferation index, which can be measured by plasma cell labeling index (≥3%), S-phase (≥1%) by flow cytometry, or Ki-67 labeling index by immunohistochemistry. Other laboratory values include elevated serum β2M (>3.5 mg/dL), C-reactive protein (CRP), high LDH, and hypoalbuminemia. CRP is a surrogate marker for interleukin-6, a growth factor for myeloma cells.

Other unfavorable clinical features include IgA, IgD, or IgE myeloma, extramedullary disease, and leukemic presentation or increased circulating plasma cells (5%).

Depth of response to therapy also predicts outcome as mentioned before. Persistent MRD after induction or transplant reflecting drug resistance is correlated with an

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TABLE 21.3
Revised International Staging System (R-ISS)

Stage I	Stage II	Stage III
ISS I and standard-risk[a] cytogenetics by FISH and normal LDH	Not fitting criteria for stage I or III	ISS III and either high-risk cytogenetics[b] by FISH or high LDH

FISH, Fluorescence in situ hybridization; *LDH*, lactate dehydrogenase.
[a]Standard-risk cytogenetics by FISH = no high-risk abnormality.
[b]High-risk cytogenetics by FISH = del(17p) and/or t(4;14) and/or t(14;16).

inferior survival. MRD can be measured by next-generation sequencing (NGS) of immunoglobulin genes, multi-parameter flow cytometry (MFC), and sensitive imaging techniques (e.g., PET-CT). NGS is highly sensitive, and MFC is widely applicable.

In patients with SMM, features associated with a high risk of progression include 50% to 60% of plasma cells, an adverse cytogenetic finding, more than 95% of marrow plasma cells being aberrant plasma cells by flow cytometry, IgA type, and decreased levels of uninvolved immunoglobulin. Patients having these features have a progression rate of 25% in 2 years compared to 5% for those lacking these features.

Development of new anti-myeloma drugs aims at targeting interaction of myeloma cells and their bone marrow microenvironment. Adhesion molecules on extracellular matrix proteins and bone marrow stromal cells regulate the homing of myeloma cells. The adherence of myeloma cells to stromal cells leads to the overproduction of several cytokines and growth factors such as IL-6, insulin-like growth factor 1 (IGF1), tumor necrosis factor-α (TNF-α), stromal-cell–derived factor-1α (SDF-1α), and VEGF through autocrine and paracrine loops. These then activate multiple signaling pathways including JAK/STAT, PI3k/Akt/NF-κB, and Wnt, which in turn promote angiogenesis, survival, and drug resistance of myeloma cells. Conversely, myeloma bone lesions develop as a result of increased cytokines, such as macrophage inflammatory protein 1α (MIP1α), released by myeloma cells, causing overexpression of the receptor activator of NF-κB ligand (RANKL) by osteoblasts and reduction of its decoy receptor osteoprotegerin. The imbalance of the two molecules leads to increased bone resorption and decreased bone formation, which then releases a range of cytokines further promoting myeloma growth. Drugs such as bisphosphonates (pamidronate and zoledronic acid) that regulate bone metabolism are effective in anti-myeloma and improving survival. New agents such as anti-interleukin-6 antibody, histone deacetylase inhibitors, immune checkpoint inhibitors, and chimeric antigen receptor T cells (CART-BCMA), are in clinical trials. In keeping with advances in patient outcomes, these new therapeutic strategies may eventually render myeloma a controllable chronic, if not curable, disease.

MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE

MGUS is an asymptomatic disorder in which a monoclonal immunoglobulin is secreted by low proliferating plasma cells in the bone marrow. The M protein is often detected incidentally during routine laboratory evaluation. There is no evidence of myeloma, primary amyloidosis, or non-Hodgkin lymphomas at the time of diagnosis. MGUS, however, can evolve to any of these conditions eventually. The current criteria for diagnosis of MGUS are as follows: serum M protein less than 30 g/L, bone marrow plasma cells less than 10%, and absence of myeloma defining events such as CRAB.

Clinical Features

The incidence of MGUS increases with age, occurring in 3% of the population older than age 50 years, 5% older than age 70 years, and 10% older than age 80 years. MGUS is more prevalent in men (4.0%) than in women (2.7%). Like myeloma, blacks are more affected than whites (14.8% vs. 7.8%), and Asians have the lowest rate. Relatives of patients with MGUS have increased risk for MGUS and myeloma, suggesting a genetic susceptibility.

It is important to distinguish whether MGUS is a result of lymphoid neoplasm or plasma cell neoplasm because they have different clinical implications. Currently, three major types of MGUS are recognized: non-immunoglobulin M (IgM) MGUS, IgM-MGUS, and light chain–MGUS. The frequency of each type also appears to be slightly different among white and black populations, but overall IgG type is most common (65% to 81%) followed by IgA (5% to 19%) or IgM (2% to 7%), biclonal (2% to 17%), and light chain–only type (3%). The IgM-MGUS is related to lymphoproliferative disorders rather than to myeloma and may evolve to LPL/WM, other non-Hodgkin lymphoma, amyloidosis, or chronic lymphocytic leukemia. The light chain type, also known as Bence Jones proteinuria of undetermined significance, is defined by an abnormal FLC ratio of less than 0.26 or more than 1.65 without evidence of Ig heavy chain expression by IFE. It may evolve to light chain–only myeloma.

Bence Jones protein is usually absent or present in small amounts in about two thirds of patients. It does not indicate progression to myeloma. Although asymptomatic, patients with MGUS are at a higher risk for bone fractures, deep vein thrombosis, and MGUS-related peripheral neuropathy than their healthy counterparts.

Pathologic Features

Bone marrow biopsy and aspirate show a mild increase in plasma cells, usually less than 10% (median, 3%) that are

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MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE—PATHOLOGIC FEATURES

Microscopic Findings
- Interstitial mild plasmacytosis (<10%) of mature forms devoid of marked atypia; no associated bone changes or fibrosis

Immunohistochemical Findings
- May not show distinct immunoglobulin light chain restriction due to coexistence of polyclonal plasma cells

Differential Diagnosis
- Smoldering myeloma (≥10% monoclonal plasma cells and/or ≥30 g/L M protein)

scattered throughout the interstitium. Large aggregates of plasma cells are not seen, and bone trabeculae are unremarkable. Lack of marked cytologic atypia and an infiltrative pattern help to distinguish MGUS from myeloma.

Ancillary Studies

The size of the M protein is usually less than 15 g/L in 80% and more than 20 g/L in only 4% to 5%. Flow cytometry identifies both immunophenotypically aberrant and normal plasma cells.

By FISH analysis, aneuploidy is a consistent finding in MGUS. Monosomy 13 and 14q32 translocations are detectable in 21% to 45% and 50%, respectively, with the former being usually detected in a subset of clonal population. It is not useful for prediction of progression.

Mutation profiles are usually less complex than those of myeloma but mutations of N-RAS and *K-RAS* seen in myeloma have been reported in MGUS also.

Differential Diagnosis

No known molecular or cytogenetic markers reliably distinguish MGUS from myeloma. Microarray analysis reveals significant overlap between gene expression patterns of MGUS and myeloma. The progress of MGUS to myeloma is likely a result of stepwise accumulation of genetic changes. *MYC* rearrangement is observed in 3% to 4% of MGUS/SMM and 15% of myeloma. They are likely involved in disease progression.

On the morphologic ground, plasma cells in MGUS generally lack nuclear atypia and immaturity seen in myeloma. The plasma cells are usually singly dispersed in the interstitium.

The IgM type of MGUS is distinguished from the so-called IgM-related disorders, such as primary cold agglutinin disease, cryoglobulinemia, or IgM MGUS-associated neuropathy, by the absence of symptoms attributable to the M protein. In IgM-related disorders, the patients may present with minimal

MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE—FACT SHEET

Definition
- Asymptomatic plasma cell neoplasms associated with low levels of serum IgM protein and low proliferating cells
- Three types: IgG/A, IgM, and light chain

Incidence and Location
- 1% and 3% of individuals >50 and >70 years, respectively; involving bone marrow

Morbidity and Mortality
- 1% annual risk and 25% cumulative probability of evolving into overt myeloma, plasmacytoma, lymphoplasmacytic lymphoma/Waldenström macroglobulinemia, or amyloidosis

Gender, Race, and Age Distribution
- Elderly, male predominance
- Incidence is higher in blacks (2:1)

Clinical Features
- Asymptomatic

Radiologic Features
- No evidence of lytic bone lesion

Prognosis and Therapy
- Stable unless progresses to overt myeloma or other related diseases; progression may be gradual or sudden. Increasing M protein levels, plasmacytosis, and abnormal FLC ratio are at higher risk
- Managed by observation only during the stable phase

serum M protein but with no evidence of lymphoma. However, the patients suffer from more debilitating complications such as autoimmune hemolytic anemia, cryoglobulinemia, and peripheral neuropathy owing to IgM binding to the myelin-associated glycoprotein and cryoglobulinemia. Distinguishing IgM MGUS from Waldenstrom macroglobulinemia requires clinicopathologic correlation. Presence of an IgM paraprotein >30 g/, obvious bone marrow involvement by a lymphoplasmacytic proliferation (10% or more lymphoma/plasma cells), or signs/symptoms attributable to WM other than described for IgM-related disorders would favor WM over IgM MGUS.

Prognosis and Therapy

The incidence of MGUS progression to myeloma or related disorders is about 1% each year. Cumulative incidence of progression is about 10% at 5 years, 15% at 10 years, and 20% at 15 years. The interval from recognition of MGUS to diagnosis of myeloma ranges from 1 to 32 years (median 10.4 years).

Nearly all myelomas are preceded by a period of MGUS, which may progress suddenly or gradually through a

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smoldering phase to symptomatic myeloma. It is therefore critical to identify patients at risk for progression for better management. Because MGUS and myeloma share common molecular genetic features, the current models of risk stratification are based on clinical or laboratory features. Progressive increase in the size of M protein predicts a higher risk. In the model proposed by the Mayo Clinic group, patients with low-risk MGUS have less than 15 g/L M protein, IgG type, and a normal FLC ratio. Patients with intermediate or high risk have a more than 15 g/L M protein IgA-type and an abnormal FLC ratio: less than 0.26 or more than 1.65. As with SMM, the model proposed by the Spanish group that predicts a higher risk for progression of MGUS is that the ratio of aberrant plasma cells to normal plasma cell 95% or greater and DNA aneuploidy.

Beside myeloma, other diseases that may evolve in patients with MGUS include primary amyloidosis or plasmacytoma. Patients with MGUS are usually observed without chemotherapy and monitored regularly for progression, with frequency dictated by risk as determined by the models just described. Overtreating these patients is harmful because of the risk of myelodysplastic syndrome.

PRIMARY AMYLOIDOSIS

Amyloidosis is a syndrome caused by accumulation of insoluble proteins with a β-pleated sheet configuration. Amyloidosis can be primary, secondary, or familial according to the cause. The four major types of amyloids are AL, AA, AF, and Aβ2m. Serum amyloid P component is present in all four types of amyloidosis regardless of underlying cause. Rare cases of amyloidosis are hereditary, due to mutations of transthyretin or fibrinogen α-chain. ATTRwt and ATTR mutated are associated with normal or mutated transthyretin (V122I TTR variant).

Primary amyloidosis (AL) is associated with plasma cell neoplasm whereas secondary amyloidosis (AA) is associated with chronic inflammation such as in rheumatoid arthritis. AF is found in familial amyloidosis, and Aβ2m is related to hemodialysis. The AL contains a part of the variable region of the light chain, most often of the λ type (75%) but sometimes of the κ type (25%). AA contains serum amyloid A (pre-albumin), a protein derived from an acute phase reactant. This section focuses on primary amyloidosis.

Clinical Features

The incidence of AL is eight per million person-years with a median age of 63 years at onset. There is a male predominance. The symptoms are vague and nonspecific in the early stage. The most common complaints are fatigue and malaise. Amyloid infiltrates cause organ damage and organ dysfunction. Thus, the clinical presentations are related to the dominant organ involved and the extent of organ systems involved. Renal involvement results in nephrotic syndrome or renal failure. Cardiac involvement with amyloid deposition in the extracellular space and the conduction system results in restrictive cardiomyopathy and arrhythmia. Gastrointestinal tract infiltration can cause macroglossia, hemorrhage, malabsorption, diarrhea, and obstruction. Vascular damage results in facial and periorbital purpura (the raccoon sign). Soft-tissue accumulation may lead to carpal tunnel syndrome and to the "shoulder pads" sign. Deposits in nerves result in sensorimotor peripheral neuropathy. Amyloid deposition may result in mass formation, so-called amyloidoma. Coagulopathy owing to binding of the amyloid fibers with factor X may cause severe bleeding complications. The major causes of death are congestive heart failure and fatal arrhythmia. Misdiagnosis as temporal arteritis or Sjögren's syndrome may delay the correct diagnosis and therapy.

PRIMARY AMYLOIDOSIS—FACT SHEET

Definition
- Plasma cell neoplasms associated with abnormal light deposition in the tissue as amyloid fibers causing organ damage and dysfunction

Incidence and Location
- Eight per million person-years; ~2000 new cases each year
- Predominantly involves heart, kidney, liver, and nervous system

Morbidity and Mortality
- Median survival is 3 years; major vital organ failure

Gender and Age Distribution
- Male predominance; median age is 63 years at onset
- 60% of patients aged 50 to 70 years at diagnosis; 10% <50 years

Clinical Features
- Symptoms are related to the dominant organ involved and the extent of organ systems involved
- Restrictive cardiomyopathy, nephrotic syndrome, hepatic failure, gastrointestinal symptoms, and neurologic dysfunctions

Radiologic Features
- Echocardiography demonstrates concentric thickening of the ventricular wall and the septum

Prognosis and Therapy
- Cardiac involvement predicts poor prognosis
- Chemotherapy or novel therapy with or without stem cell transplantation to eliminate neoplastic clone

Pathologic Features

Grossly, the involved organs appear to be waxy and stiff. On hematoxylin and eosin sections, the amyloid fibers have an

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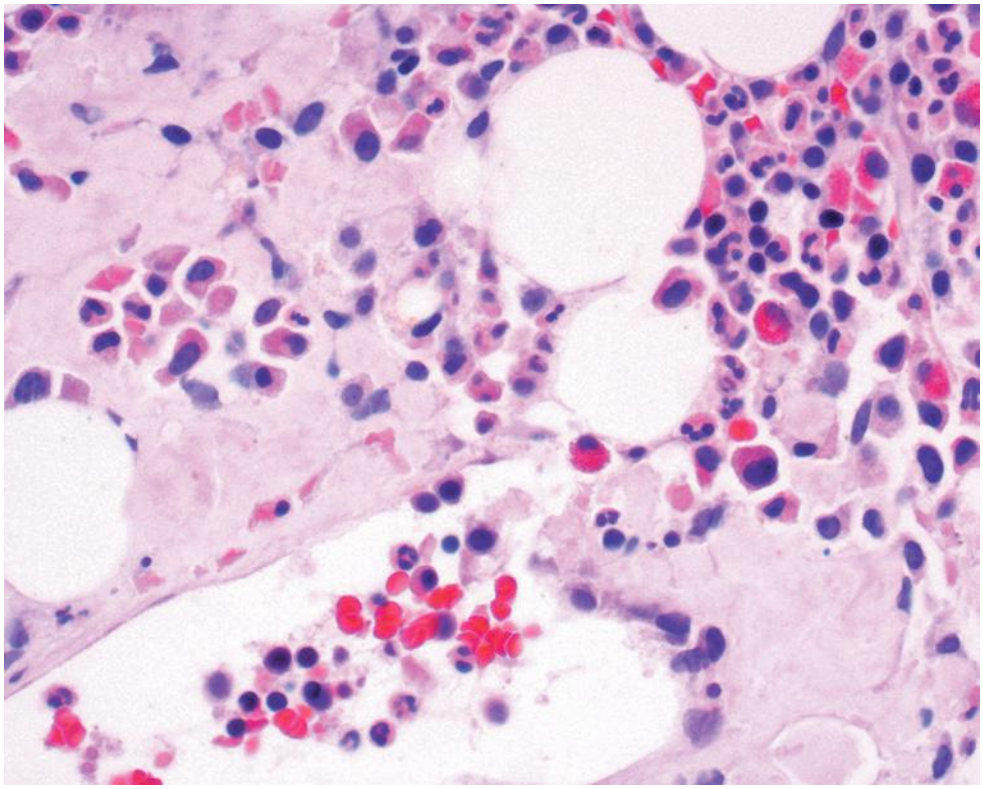


FIG. 21.6
Amyloid deposits in the bone marrow medullary space (×400).

amorphous, eosinophilic appearance often associated with cracking artifact and multinucleated giant cell reactions (Fig. 21.6). The amyloid fibers appear apple-green birefringent when stained with Congo red and viewed under polarized light.

Primary amyloidosis is essentially a small number of plasma cells in the range of MGUS associated with amyloid deposition in tissue that causes damage. All three types of MGUS, non–immunoglobulin M (IgM) MGUS, IgM MGUS, and light chain MGUS can give rise to AA. As such, the organ dysfunction caused by amyloid deposition should not be taken as evidence of end organ damage meeting the criteria for active multiple myeloma. The limited numbers of plasma cells are best appreciated by immunohistochemistry or flow cytometry immunophenotyping. A low level of serum M protein can be detected in 80% of patients. Evaluation of bone marrow suspected of plasma cell neoplasms should always include vasculature and periosteum, where the amyloid deposits are likely to occur. Other locations are bone marrow medullary cavities.

Abdominal subcutaneous fat pad aspiration is the most often used diagnostic test. In patients who have a serum M component and are suspected of primary amyloidosis, combined fat pad and bone marrow biopsy with Congo red stains yields a diagnosis in 90% of cases, whereas each method alone may detect up to 80% and 50% of cases, respectively. Direct biopsy of the involved organ is rarely required and carries the risk of bleeding. Primary amyloidosis is most commonly associated with two particular immunoglobulin λ light chain variable regions, the 3r (λIII) and the 6a (λVI).

PRIMARY AMYLOIDOSIS—PATHOLOGIC FEATURES

Gross Findings
- Waxy, stiff

Microscopic Findings
- Eosinophilic, amorphous deposit in the medullary space, vessel walls, or the soft tissue that are stained red by Congo red stain and apple-green birefringent in polarized light

Ultrastructural Findings
- Nonbranching linear fibrils 7 to 10 nm in diameter and of variable length

Fine-Needle Aspiration Biopsy Findings
- Amorphous waxy, basophilic material on Diff-Quick stain or cynophilic clumps of material with fissures on Papanicoloau (Pap) stain

Immunohistochemical Findings
- Positive for κ or λ light chain and amyloid P component

Laboratory Findings
- Low level of M protein by IFE or/and abnormal FLC ratio
- Measurement of troponin, brain natriuretic peptide

Differential Diagnosis
- LCDDs or HCDDs

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Ancillary Studies

Immunohistochemistry or flow cytometry immunophenotyping using a panel of antibodies including CD138, in conjunction with immunoglobulin light chains, will highlight plasma cells and determine monoclonality. The AL amyloid can be detected using antibody specific for AL or amyloid P component. By electronic microscopy, amyloid fibers appear as a mass of nonbranching linear fibrils 7 to 10 nm in diameter and of variable length. In one third of patients with the AL, no distinct spike is visible by SPEP. IFE is more sensitive in patients with a low level of M protein and should be performed to confirm its presence. Serum FLC assays can determine the type of the M protein and confirm the diagnosis. They are also useful for monitoring response to therapy. Mass spectrometry of amyloid deposits isolated by laser capture microdissection is becoming the standard to confirm the protein identity of amyloid deposists prior to definitive therapy.

Differential Diagnosis

As AL is treated with chemotherapy and/or novel agents with or without autologous transplantation, it is critical that other forms of amyloidosis be excluded. This can be challenging when non-AL coexists with MGUS with minimal plasmacytosis in the bone marrow, mimicking AL. A useful hint to the presence of AL is amyloid deposition in the bone marrow. Congo red stain should be routinely performed in the bone marrow biopsy of all patients suspected of AL. Another feature of AL is its heterogeneous clinical presentation with multiorgan involvement. Molecular studies with PCR amplification and DNA sequence analysis will detect patients with mutant transthyretin or fibrinogen A α-chain variants. Mass spectrometry allows specific typing of amyloid fibers and is considered the gold standard.

The diagnosis of primary amyloidosis requires direct evidence of amyloid-induced organ damage as a result of monoclonal plasma cell proliferation. All of the following criteria need to be fulfilled: presence of amyloid-related systemic syndrome (e.g., renal, liver, heart, gastrointestinal tract, or peripheral nerve involvement), positive amyloid staining by Congo red or electron microscopy (EM) in any tissue, confirming that the amyloid is composed of Ig chain by immunostaining, mass spectroscopy, or other techniques, and detection of monoclonal plasma cell proliferation such as serum or urine M protein, abnormal FLC ratio, or monotypic plasma cells in bone marrow.

The distinction between AL and light or heavy chain deposition diseases is discussed in the following section.

Prognosis and Therapy

The median survival of patients with AL is 3 years. The patients usually succumb to progressive organ failure. The prognosis largely depends on the dominant organ involvement. Cardiac involvement predicts a poor prognosis. Using N-terminal pro–brain natriuretic peptide (NTproBNP), serum troponin T, and serum FLC ratios, patients are stratified into 4 subgroups with median survivals of 94.1, 40.3, 14.0, and 5.8 months, respectively. Elevated levels of growth differentiation factor-15 (GDF-15), a newer marker, >7575 pg/ml also predict a poor outcome. Stem cell transplant is a preferred therapy, but only 20% of patients are eligible. Those ineligible for transplant are treated with various agents to eliminate the neoplastic cells.

MONOCLONAL LIGHT AND HEAVY CHAIN DEPOSITION DISEASES

Monoclonal light chain deposition diseases (LCDD) and heavy chain deposition diseases (HCDD) are caused by deposition of abnormal heavy or light chains secreted by neoplastic plasma cells. In LCDD, HCDD, or combined LCDD and HCDD, the abnormal proteins do not have a sheet structure or contain the P component but are deposited in tissue, as in amyloidosis, causing organ damage and dysfunctions. LCDD and HCDD are frequently underdiagnosed. In adult patients with unexplained nephrotic syndrome, the index of suspicion should be high for amyloidosis or LCDD.

The κ light chain, especially products of $V_{\kappa I}$ and $V_{\kappa IV}$, is more often involved in LCDD than is the λ light chain. Multiple mutations of the variable region alter amino acid sequence and enhance their tissue affinity. Free light chains filtered through the glomeruli tend to deposit in the kidney when the amount exceeds the degradation capacity of the renal tubular epithelia. Free heavy chains usually do not circulate in blood. Deposition of heavy chains usually results from structural deletion of the CH1 constant domain of the heavy chain, which then prevents its association with heavy chain binding protein leading to deposition in the kidney.

Clinical Features

Combined LCDD and HCDD and isolated HCDD are rare. The patients with LCDD are usually younger (average age 55–60 years) and predominantly female compared with patients with amyloidosis. The patients usually present with acute renal failure with profound proteinuria or hematuria. Less commonly, the patients present with cardiomyopathy or hepatic or pulmonary insufficiency.

Pathologic Features

Monoclonal immunoglobulin deposits in tissue as amorphous eosinophilic material resembling amyloid but does not stain for Congo red. The diagnosis of LCDD is usually established by renal biopsy with appropriate ancillary studies. In the

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LIGHT OR HEAVY CHAIN DEPOSITION DISEASES—FACT SHEET

Definition
- Abnormal immunoglobulin secreted by neoplastic plasma cells that are not amyloid material deposit in tissue

Incidence and Location
- Rare; tend to be underdiagnosed, involving major organs as in amyloidosis, with kidney being the most common

Morbidity and Mortality
- Median survival 1 to 2 years; most often the patients die of organ failure

Gender and Age Distribution
- 55 to 60 years; predominantly female

Clinical Features
- Nondiabetic nephrotic syndrome most common presentation

Radiologic Features
- Nonspecific

Prognosis and Therapy
- Poor; treated with chemotherapy or novel therapy to eliminate monoclonal plasma cells

LIGHT CHAIN DEPOSITION DISEASES—PATHOLOGIC FEATURES

Gross Findings
- Waxy, stiff

Microscopic Findings
- Eosinophilic amorphous material that does not stain for Congo red; linear ribbon-like deposition along the basement membrane of the glomeruli and the vasculature

Ultrastructural Findings
- Discrete, electron-dense, punctate deposits

Fine-Needle Aspiration Biopsy Findings
- Amorphous material similar to amyloid, but Congo red–stain negative

Immunohistochemical Findings
- The deposits are positive for κ or λ light chain by immunofluorescence stain

Differential Diagnosis
- Amyloidosis, diabetic nephropathy

kidney, LCDD is characterized by nodular glomerulosclerosis, resembling diabetic nephropathy. Deposition in the renal vasculature results in proliferative vasculopathy. The renal tubules show interstitial disease.

As in AL, most cases (50% to 60%) of LCDD have minimal plasmacytosis in the bone marrow. Immunohistochemistry is usually required to demonstrate monoclonality of the plasma cells. The bone marrow is involved with overt myeloma only in 10% of patients.

Ancillary Studies

By immunofluorescence, abnormal immunoglobulin appears along the renal glomerular and tubular basement membrane as linear ribbon-like deposition. By electron microscopy, the abnormal immunoglobulin typically appears as discrete, electron-dense, punctate deposits. These deposits are usually demonstrated in the kidney biopsy specimen. Similar changes can also be observed in the dermal–epidermal junction of the skin biopsy specimens.

Serum Protein Electrophoresis and Immunofixation

Serum M protein is usually minimal in most patients, but it helps to establish the diagnosis of plasma cell neoplasms. Serum FLC ratio is very useful in diagnosis of LCDD.

Differential Diagnosis

The differential diagnosis is amyloidosis. The abnormal immunoglobulin in LCDD or HCDD is Congo red negative.

Prognosis and Therapy

The overall prognosis is poor, with an overall survival of 1 to 2 years, regardless of plasma cell tumor load.

HEAVY CHAIN DISEASES

Heavy chain diseases (HCD) comprise a group of rare B-cell neoplasms that exhibit marked lymphoplasmacytic differentiation and an excessive amount of a monoclonal heavy chain devoid of light chain in the serum or/and urine. The major part of constant-1 (CH1) domain of the heavy chain responsible for binding light chain is lost during the process of somatic hypermutation. The clinical presentations of HCD are variable and depend on the type of monoclonal immunoglobulin heavy chain produced. The three major types of HCD, in decreasing order of frequency, are α-, γ-, and μ-chain diseases. The α-chain disease is associated with extranodal marginal zone B-cell lymphoma. The γ-chain disease has features of marginal zone lymphoma or lymphoplasmacytic lymphoma. The μ-chain

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disease is considered a variant of chronic lymphocytic leukemia.

α-CHAIN DISEASE

Clinical Features

Also known as Mediterranean lymphoma, α-heavy chain disease (α-HCD) typically affects young adults in the second and third decades of life from the Mediterranean area, northern African, or the Middle East. Environmental factors such as poor hygiene and malnutrition with repeated acute infectious diarrhea and chronic parasitic infestations have been implicated. Presenting symptoms are most often gastrointestinal, such as abdominal pain, diarrhea, and malabsorption. Severe disease may cause malnutrition and local complications such as bowel obstruction or perforation. Recent studies suggest that *Campylobacter jejuni* may be an important pathogenic micro-organism, and the early phase of disease may respond to antibiotics. Rarely, the patient presents with the respiratory form with dyspnea, diffuse pulmonary infiltrates, or hilar lymphoadenopathy. Endoscopic examination shows infiltrative and nodular patterns of mucosal abnormality.

Pathologic Features

The neoplastic infiltrate primarily involves the proximal small bowel at the level of the duodenum or jejunum and may spread to the mesenteric lymph nodes and show features of extranodal marginal zone B-cell lymphoma. These tumors are also termed *immunoproliferative small intestinal disease* (IPSID). In the early stage of the disease, the lamina propria is infiltrated by small mature lymphocytes, plasmacytoid lymphocytes, and plasma cells producing only α-chain protein. Lymphoepithelial lesions may be present with associated villous atrophy. As the disease progresses, the infiltrate extends to the submucosa, and large immunoblasts are increased. In the advanced stage, the infiltrate becomes transmural, and the tumor may transform to immunoblastic large B-cell lymphoma.

Ancillary Studies

SPEP is usually normal or shows only hypogammaglobulinemia without a distinct spike. Sometimes the abnormal protein appears as a broad band extending from the α2-β region. IFE with antibody specific for α is needed to confirm the diagnosis.

HEAVY CHAIN DISEASES—FACT SHEET

Definition
- B-cell neoplasms associated with secretion of monoclonal immunoglobulin heavy chain devoid of light chain; α-HCD, γ-HCD, and μ-HCD are the three most common types

Incidence and Location
- Rare; sites of involvement variable depending on the type of disease; α-HCD involves small intestine or mesenteric lymph nodes, γ-HCD and μ-HCD involve bone marrow or other extramedullary sites

Morbidity and Mortality
- Markedly variable ranging from months to years

Gender and Age Distribution
- Young adults in α-HCD, elderly males in γ-HCD and μ-HCD

Clinical Features
- Gastrointestinal symptoms in α-HCD, chronic lymphocytic leukemia, or Waldenström macroglobulinemia–like presentations in γ-HCD and μ-HCD with hepatosplenomegaly and peripheral lymphocytosis. Autoimmune disorders common in γ-HCD

Radiologic Features
- Nonspecific

Prognosis and Therapy
- Variable; early stages of α-HCD are managed with oral antibiotics; early stages of γ-HCD and μ-HCD are observed only; advanced stages of all three types of HCD diseases require chemotherapy or novel therapy

HEAVY CHAIN DISEASES—PATHOLOGIC FEATURES

Gross Findings
- Mass formation; ulcer in α-HCD

Microscopic Findings
- Marginal zone B-cell lymphoma in α-HCD with infiltrate of mature lymphoplasmacytic cells associated with villous atrophy; lymphoplasmacytic lymphoma in γ-HCD and μ-HCD associated with lymphocytosis

Ultrastructural Findings
- Nonspecific

Fine-Needle Aspiration Biopsy Findings
- Lymphoplasmacytic infiltrate

Immunohistochemical Findings
- The lymphoid cells are positive for pan B-cell markers

Differential Diagnosis
- Chronic lymphocytic leukemia

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Prognosis and Therapy

Survival varies from a few months to several years, depending on the stage of the disease. Antibiotics are given regardless of whether organisms are identified clinically to eradicate any possible infectious agents. Refractory disease is treated with surgical debulking of the tumor mass, focal radiation, and/or rituximab plus fludarabine or other combination chemotherapy with or without autologous transplantation.

γ-CHAIN DISEASE

Clinical Features

γ-HCD, also known as Franklin's disease, is a heterogeneous disease with variable clinical presentation. It affects mostly elderly people in their 60s and has a higher predilection among women. B type of symptoms, anemia, recurrent infection, lymphadenopathy, and hepatosplenomegaly are common presenting symptoms. There are usually no lytic bone lesions. Autoimmune diseases may occur in as many as one third of patients manifesting as autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, Sjögren's syndrome, rheumatoid arthritis, systemic lupus erythematosus, or thyroiditis. The abnormal protein consists primarily of the Fc region of the heavy chain with a normal carboxy terminal end followed by an internal deletion of the V and the entire CH1 domain.

Pathologic Features

The lymph nodes, bone marrow, and spleen are frequently involved. Some patients present with only bone marrow disease (localized medullary disease) and others localized extranodal sites such as skin, thyroid or parotid gland, upper airway or gastrointestinal tract, or conjunctiva (localized extramedullary disease). The morphologic features are heterogeneous and variable compared to α- or μ-HCD. The neoplastic infiltrate is composed predominantly of lymphoplasmacytoid cells similar to those seen in extranodal marginal zone (mucosa-associated lymphoid tissue [MALT]) lymphoma or lymphoplasmacytic lymphoma. Those involving spleen are indistinguishable from splenic marginal zone or splenic diffuse red pulp small B-cell lymphoma. Occasionally the neoplastic cells present with a leukemic form resembling chronic lymphocytic leukemia.

Ancillary Studies

Cytopenia, especially anemia and a positive Coomb's test, is common as a result of an autoimmune disorder. SPEP usually detects no abnormal protein as it is often obscured in the β region. IFE confirms monoclonal γ-heavy chain without light chain production. The low molecular weight of the γ-heavy chain allows the abnormal protein to be detected in the urine, usually as a dimer. Despite morphologic resemblance to LPL, *MYD88L265P* mutation has not been identified in an 11-case study of this entity, suggesting a different mechanism of pathogenesis.

Prognosis and Therapy

Survival varies greatly from months to years depending on the clinical features. Those with an indolent disease are often managed with observation only, whereas those with aggressive disease generally require rituximab with or without combination chemotherapy. Localized extranodal disease can be managed with surgical resection or radiation therapy.

μ-CHAIN DISEASE

Clinical Features

μ-HCD is an extremely rare and indolent disease that clinically resembles chronic lymphocytic leukemia. The disease usually affects white men with a median age of 58 years at diagnosis. The patients usually present with hepatosplenomegaly, and lymphadenopathy is identified in 40% of patients. Lytic bone lesions are found in about 20% of patients, and some show carpal tunnel syndrome and amyloid deposition.

Pathologic Features

The peripheral blood shows lymphocytosis. The bone marrow is infiltrated by small lymphocytes, plasmacytoid lymphocytes, and mature plasma cells with cytoplasmic vacuoles, as in lymphoplasmacytic lymphoma/Waldenström macroglobulinemia.

Ancillary Studies

Immunofixation electrophoresis detects excess monoclonal μ-chains in the serum. SPEP may appear unremarkable in about half of patients. Immunofixation reveals excess monoclonal μ-chain as polymers of variable sizes with no associated κ or λ light chain. However, immunoglobulin light chain, usually of κ type, are produced, although not bound to the heavy chain; thus Bence Jones protein can be detected in the

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urine of two thirds of patients with μ-HCD and may be associated with cast nephropathy.

Differential Diagnosis

μ-HCD with marked plasmacytic differentiation needs to be distinguished from myeloma. Those with more peripheralized disease are distinguished from chronic lymphocytic leukemia by immunophenotyping.

Prognosis and Therapy

Owing to rarity of the disease, prognosis and therapy are not well defined and case dependent.

The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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22

Disorders of the Spleen

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INTRODUCTION

The spleen is a secondary lymphoid organ that functions in the development of the immune response and in blood filtration. In the spleen, blood-borne antigens are presented to lymphocytes and macrophages to facilitate antibody development and cytotoxic immune responses. In addition, senescent red blood cells and blood-borne foreign substances are introduced to and removed by macrophages. Finally, the spleen also serves as a reservoir for platelet and granulocytic storage, dynamically adjusting cellular contents in the peripheral blood during times of stress.

NORMAL SPLEEN ANATOMY AND HISTOLOGY

The average adult spleen measures 12 × 7 × 3.5 cm, weighs 150 g, and contains a blood volume of 300 mL. The medial portion is concave and divided into the gastric (anterior) surface, which abuts the hilum and tail of the pancreas, and renal (posterior) surface, which abuts the upper pole of the left kidney and adrenal gland. The main blood supply to the spleen is the splenic artery. At the hilum, the splenic artery branches into the superior polar, superior middle, inferior middle, and inferior polar splenic arteries. Venous drainage is primarily through the splenic vein, which arises from the hilum and courses along the superior aspect of the pancreas to join the superior mesenteric vein forming the portal vein. The left gastroepiploic veins also provide ancillary drainage for the spleen.

The spleen is divided into two visually distinct components: red pulp and white pulp. The red pulp is a three-dimensional network of cords and sinuses that forms approximately three fourths of the splenic volume. The cords are reticular fibers, a matrix of collagenous connective tissue, with admixed myofibroblasts and fibroblasts; scattered lymphocytes, plasma cells, and macrophages are also seen. Between these cords lie the splenic sinuses, which are filled with peripheral blood cells and macrophages; these sinuses are lined by plasma cells and plasmablastic cells that migrate from follicles. In contrast, the white pulp constitutes the remaining one fourth of the spleen and consists of B and T lymphocytes that are found either around vessels or as independent follicles. Lymphocytes around vessels form the periarteriolar lymphoid sheath and are predominantly T cells, whereas lymphoid follicles are composed predominantly of B cells with some admixed T cells. Generally, follicles are found adjacent to the periarteriolar lymphoid sheath but can also be seen in isolation within the red pulp. Germinal centers are not a usual component of the white pulp in adulthood (Fig. 22.1). Finally, the marginal zone constitutes the area between the white and red pulps and contains both lymphocytes and macrophages; it is here that the presentation of peripheral blood elements to lymphocytes and macrophages occurs.

TRAUMA AND CONGENITAL ABNORMALITIES

Both trauma and congenital abnormalities of the spleen result in distinct clinical and pathologic findings, which warrant discussion here.

SPLENIC RUPTURE

Splenic rupture can be classified as spontaneous or delayed. Spontaneous splenic rupture is more common in males and typically occurs in relation to infarction, thrombocytopenia, tumors, lymphoma, or leukemia. Delayed splenic rupture is defined as development of splenic hemorrhage more than 7 days after an injury and is thought to be the result of an evolving initial splenic contusion. Symptoms of acute or delayed splenic rupture include abdominal pain with referred shoulder pain, abdominal distention, and signs of bleeding or shock. Ruptured spleens, even those removed acutely, characteristically show reactive white pulp changes in addition to subcapsular hemorrhage. Emergent splenectomy is the only definitive therapy.

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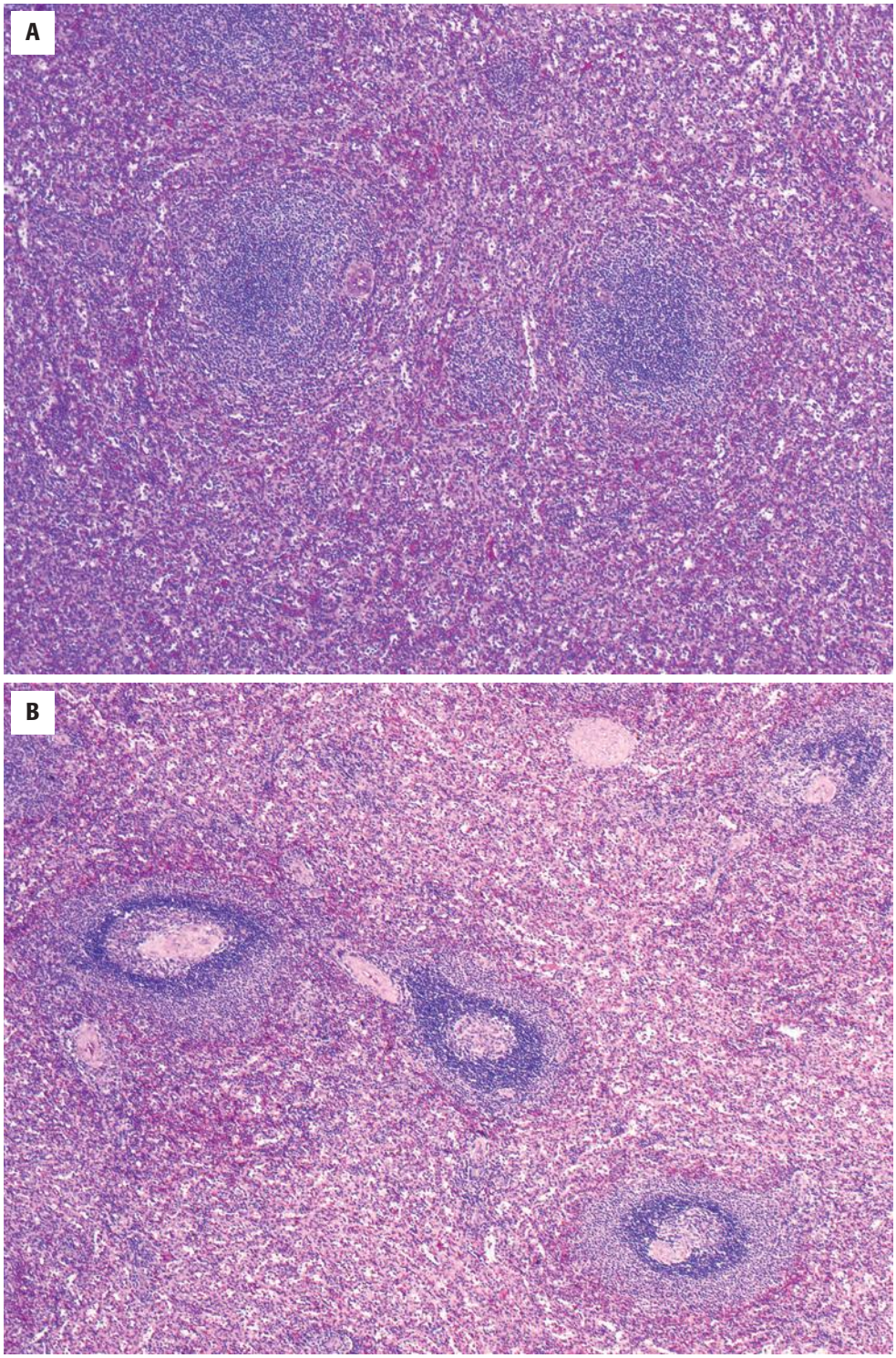


FIG. 22.1

Normal and reactive spleen. A, The normal adult spleen shows aggregates of white pulp small lymphocytes without distinct germinal center formation. The red pulp, containing the splenic cords and sinuses, resides between the lymphoid component. B, In an adult with autoimmune or infectious conditions, including immune thrombocytopenic purpura, the splenic white pulp is evenly distributed but expanded with distinct germinal centers, mantle zones of dark staining cells, and an outer marginal zone of medium-sized cells with more abundant, pale staining cytoplasm. These distinct zones are also seen in normal spleens of children.

SPLENOSIS

One consequence of splenic rupture is splenosis, which is dissemination of splenic tissue to a separate anatomic site. This phenomenon is also a common sequela of trauma or surgery. It occurs in up to 75% of trauma patients undergoing splenectomy and is thought to result from seeding or hematogenous spread of tissue, or both. Splenosis usually occurs in the abdominal and pelvic cavities, but intrathoracic, subcutaneous, intrahepatic, and intracranial cases have been described. The heterotopic splenic tissue generally demonstrates abnormal histology; it lacks trabecular structures and

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has poorly formed white pulp, although the red pulp is normal. Functionally these splenosis nodules are capable of clearing senescent erythrocytes but do not offer protection against encapsulated bacteria. Splenosis nodules are often detected on computed tomography (CT), raising concern for malignancy; however, they are benign and removal is required only in symptomatic cases.

ACCESSORY SPLEEN

A relatively common congenital variation is that of an accessory spleen, which is seen in up to 20% of otherwise healthy individuals and is found in greater frequency (up to 30%) in patients with hematologic disorders. It is thought to result from inadequate fusion of primary lobules during the second or third trimester of fetal development. Most patients with an accessory spleen have only one, although multiples are not uncommon. In the majority of cases, an accessory spleen is found near the hilum or in the supporting ligaments or greater omentum. Other notable locations are (in order of frequency) the gastrocolic ligament, pancreatic tail, greater omentum, greater curvature of the stomach, the splenocolic ligament, the small and large bowel mesentery, the left broad ligament in women, and the left spermatic cord in men. Accessory spleens are histologically and functionally identical to the native spleen. As such, if a patient fails therapeutic splenectomy, the possibility of an accessory spleen should be considered.

ASPLENIA

Asplenia is congenital or acquired. Congenital asplenia is rare and inherited in a predominantly autosomal-dominant pattern. It may first be detected due to pneumococcal sepsis in an infant or newborn. Although it can occur in isolation, it is also associated with heterotaxy syndromes. The most common of these syndromes is the Ivemark syndrome, in which right-sided organs are duplicated and organs that are normally present on the left side are absent. Other associated conditions include Pearson syndrome, Stormorken syndrome, Smith-Fineman-Myers syndrome, ATR-X syndrome, Fanconi anemia, and autoimmune polyendocrine syndrome type 1. However, the most common cause of asplenia is secondary to trauma, infarction, or surgery. Regardless of etiology, the most important consequence of asplenia is increased susceptibility to infection by encapsulated organisms, most commonly *Streptococcus pneumoniae. Neisseria meningitidis, Escherichia coli, Haemophilus influenzae, Staphylococcus* species, and *Streptococcus,* which occur at rates ranging from 7% to 12% in asplenic patients. Interestingly, this increased susceptibility is caused by defective immunoglobulin (Ig) M and opsonin production.

Asplenia is evident in the peripheral blood smear, which demonstrates anisopoikilocytosis of red blood cells including acanthocytes, echinocytes, and target cells as well as red blood cell inclusions such as Pappenheimer and Howell-Jolly bodies.

SPLENIC-GONADAL FUSION

Another congenital anomaly is that of splenic-gonadal fusion, which results from fusion of the splenic anlage with the gonadal mesoderm of the left urogenital fold (Fig. 22.2). Typically a disorder of young males (male-to-female ratio, 20 : 1), it often manifests as a scrotal mass identified during physical examination for cryptorchidism or during inguinal hernia repair. The condition exists in two forms. The first more common form is characterized by a continuous cord of splenic or fibrous tissue between the normal spleen and ectopic mass. The second, discontinuous type, shows no connection between the spleen and a mass. Associated cryptorchidism, micrognathia, and limb defects are commonly associated with continuous splenic-gonadal fusion but are rarely seen with the discontinuous form. Less commonly, cardiac defects, spina bifida, cleft palate, and imperforate anus can be seen. Typically, the ectopic splenic tissue is confined to the tunica vaginalis. It is well demarcated from the gonad and has a fibrous capsule and a burgundy red-to-purple cut surface. Histologically it is identical to the native spleen, although fibrosis, thrombosis, calcification, fat degeneration, or hemosiderin deposits can be observed.

SPLENIC CYST

One of the most common benign tumors of the spleen is the splenic cyst. These tumors have a male predominance and typically appear in the third decade of life. They are designated as primary (true) or secondary (false). Primary cysts represent approximately 20% of all splenic cysts. They are unilocular and have a firm, fibrous, trabecular wall that is lined by mesothelial cells or squamous epithelium (Fig. 22.3). Notably, the epithelial lining of primary cysts may be patchy, with denuded areas present that may simulate a secondary cyst. Primary cysts can be further subdivided into parasitic and nonparasitic types. Parasitic cysts, though uncommon, are typically attributable to *Echinococcus* species and are readily identified by the presence of parasite scolices in the cyst contents. Nonparasitic primary cysts appear to arise from congenital inclusions of capsular mesothelium. Interestingly, patients with primary cysts may have elevations of CA19-9 and carcinoembryonic antigen. Treatment in symptomatic cases requires a complete splenectomy because incomplete resection often leads to recurrence.

Secondary cysts represent approximately 80% of splenic cysts and are often associated with a history of abdominal trauma. These cysts are unilocular and thin walled and differ from primary cysts by the complete absence of an epithelial lining; therefore they are unlikely to recur even if only partially resected.

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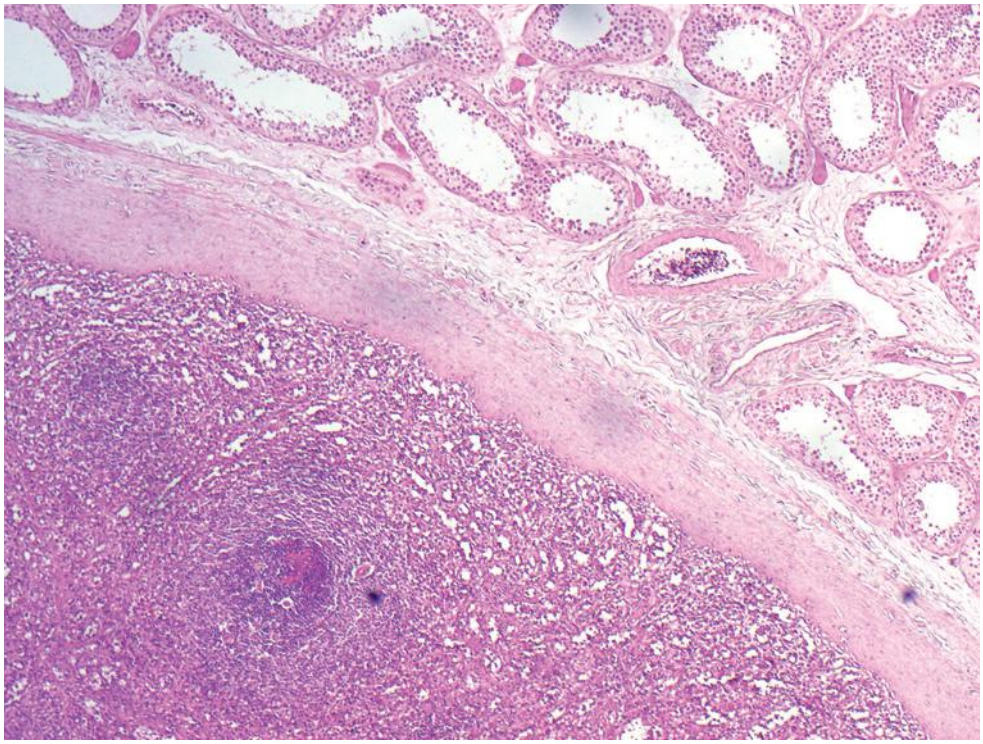


FIG. 22.2
Splenic-gonadal fusion. Seminiferous tubules (top right), usually without spermatogenesis, are present fused with normal splenic tissue.

SPLENIC CYST—FACT SHEET

Clinical Features
- Primary cyst: male predominance; third decade of life
- Secondary cyst: often associated with history of trauma

Gross Features
- Primary cyst: unilocular with a firm fibrous trabeculated wall
- Secondary cyst: unilocular with a nontrabeculated thin wall

Morphology
- Primary cyst: wall lined by mesothelial cells or squamous epithelium
- Secondary cyst: wall with absence of epithelial lining

Prognosis and Therapy
- Treatment is a simple splenectomy
- Incomplete resection in primary cysts leads to recurrence

INFLAMMATION OF THE SPLEEN

Inflammation of the spleen commonly results in splenomegaly. Generally it is a response to local or systemic infectious or noninfectious diseases.

FOLLICULAR HYPERPLASIA

Germinal center formation in the white pulp is a normal finding in the childhood spleen, but its presence is abnormal in adults (see Fig. 22.1B). Reactive follicular hyperplasia can be seen as a result of a systemic infection as in bacterial sepsis, measles, typhoid fever, and acquired immunodeficiency syndrome (AIDS). In addition, autoimmune conditions such as rheumatoid arthritis and idiopathic thrombocytopenic purpura (ITP) can lead to follicular hyperplasia. The histologic findings are those of a balanced and proportional expansion of all lymphoid compartments: follicles, mantle zone, marginal zone, and peripheral arteriolar sheaths. Disproportionate expansion of B cells in the mantle or marginal zones should be cautiously evaluated, because these can be subtle indications of lymphoma involvement.

SPLENIC ABSCESS

Splenic abscesses are rare, with a predicted frequency of less than 0.7% based on autopsy studies, and may be single or multiple. The most common etiology is hematogenous seeding of bacteria from a secondary site (e.g., heart, lung, urinary tract). Other well-known predisposing conditions are splenic trauma and hemoglobinopathies. Most abscesses are well circumscribed with a thick nonepithelialized fibrous wall and central accumulation of necrotic tissue associated with acute inflammatory cells. Preserved splenic tissue surrounding the abscess may show white pulp hyperplasia. All abscesses should be cultured for bacterial and fungal organisms; however, most cases are associated with gram-negative bacilli, *Staphylococcus aureus,* or *Streptococcus* species, although *Salmonella* species is commonly seen in patients with sickle cell disease. Fungal infection, though rare, can be seen. *Candida, Aspergillus,* and *Cryptococcus* species are the most common culprits.

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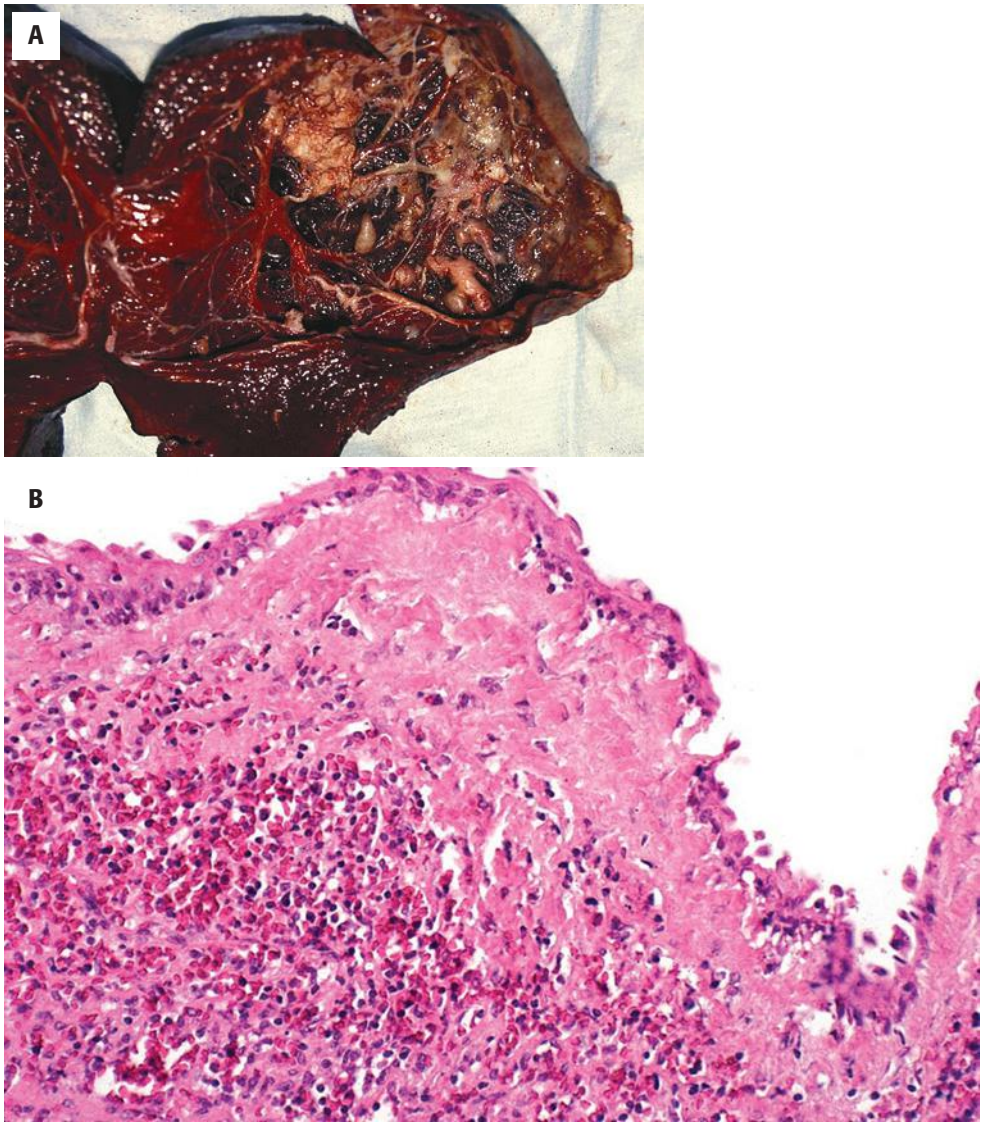


FIG. 22.3

Primary splenic cyst. A, Grossly, the cyst is trabeculated without serious or slightly bloody fluid contents. B, The cyst wall shows an epithelial lining, which should at least be focally present in all primary cysts. The lining may be mesothelial, squamous, or a mixture of cell types.

INFECTIOUS MONONUCLEOSIS IN THE SPLEEN

Nearly half of all patients with infectious mononucleosis demonstrate splenomegaly. Although most patients show no significant complications, a well-known adverse outcome from Epstein-Barr virus (EBV) infection of the spleen is splenic rupture secondary to trauma; however, in most cases of rupture the severity is minor. The morphologic findings of infectious mononucleosis are varied, but the spleen can enlarge because of expansion of the red pulp and white pulp with a spectrum of immunoblasts, reactive lymphocytes, and plasma cells. In dramatic cases, Reed-Sternberg–like cells may be seen. In such cases, evaluation of these cells with immunohistochemical stains for CD30 and CD15 must be made with caution, because CD30 will be positive in the reactive immunoblasts. These cells should generally not express CD15. In addition, the histologic picture can mimic that of other non-Hodgkin lymphomas. In these cases, careful evaluation of the splenic architecture should provide necessary information, because lymphomas will unequally expand the spleen, unlike infectious mononucleosis, in which the underlying balance of white and red pulp elements will be retained.

GRANULOMAS

Infection with mycobacteria, fungus, *Brucella* species, or EBV can result in extensive necrotizing granulomas that typically involve the white pulp and are generally well circumscribed. However, granulomas can also be seen as malignancies such as hairy cell leukemia and both Hodgkin and non-Hodgkin lymphomas. In addition, sarcoidosis, uremia, and selective IgA deficiency can result in splenic granulomas. Finally, lipogranulomas—collections of vacuolated histocytes

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surrounded by plasma cells and lymphocytes—are a relatively common benign finding in North America but not so in other parts of the world. Such geographic stratification together with other correlative data have led to the hypothesis that lipogranulomas are caused by differences in dietary intake or packaging of foods, ultimately resulting in absorption of mineral oils through the intestine and distribution throughout the body and to the spleen.

METABOLIC DISEASES WITH SPLENIC MANIFESTATION

The most common congenital metabolic diseases with significant pathologic manifestations within the spleen are the lysosomal storage diseases, which demonstrate morphologic findings similar to those seen in the marrow (see Chapter 6). In general, these congenital enzyme deficiencies develop early in infancy or childhood and result in splenomegaly.

GAUCHER DISEASE

The most common of the lysosomal storage diseases is Gaucher disease, which is an autosomal recessively inherited deficiency of glucocerebrosidase (β-glucosidase). The spleen is typically diffusely enlarged, and the cut surface is pale and filled with large collections of Gaucher cells, which are histiocytes with eccentric nuclei, and abundant tissue paper–like blue-gray foamy cytoplasm (Fig. 22.4A). These cells are best appreciated on smear or touch preparations. The major differential diagnosis is that of other lysosomal storage diseases and, in particular, Niemann-Pick disease; however, one should also consider hematologic malignancies, many of which demonstrate Gaucher-like cells. Prognosis is variable and depends on the subtype of Gaucher disease. Patients can undergo recombinant enzyme replacement therapy to prevent complications.

NIEMANN-PICK DISEASE

Niemann-Pick disease is rare, occurring in an estimated 1/120,000 live births; it is inherited in an autosomal-recessive pattern. It results from a deficiency of either acid sphingomyelinase (types A and B) or Niemann-Pick C proteins (types C and D). Although Niemann-Pick disease can be typed according to enzyme deficiency, the disease is also separated into five categories based on clinical features (type IA, IS, IC, IIS, and IIC). In the case of types IA and IS, the spleen is characteristically massively enlarged, often 10 times normal size. Cut cross sections demonstrate homogeneously pale tissue. Microscopic examination reveals diffuse expansion of the red pulp by sphingomyelin-laden foamy macrophages with characteristic small, uniform, mulberry-like cytoplasmic globules.

OTHER METABOLIC DISEASES INVOLVING THE SPLEEN

The mucopolysaccharidoses (MPS) are progressive diseases resulting from the inability to effectively process glycosaminoglycan. These are inherited autosomal recessively with the exception of MPS 2, or Hunter syndrome, which is X-linked recessive. Hepatosplenomegaly is a common presentation. Microscopic examination of the spleen will demonstrate plump vacuolated histiocytes. A diagnosis can generally be made by urinanalysis, which reveals increased concentration of glycosaminoglycan fragments. Most patients can be screened initially with an enzyme assay and by sequencing known mutations. Tangier disease, which is caused by defective cholesterol transport, presents in childhood with hepatosplenomegaly and premature coronary atherosclerosis. Tissues demonstrate accumulation of foamy cholesterol-laden macrophages, particularly in the spleen. In Tay-Sachs disease, hexosaminidase A deficiency, there is accumulation of GM2 ganglioside in the heart, liver, and spleen leading to plump vacuolated histiocytes. Involvement of the central nervous system with vacuolated neurons is also predominant. In addition, nondescript foamy histiocytes are seen in many other metabolic diseases, including Fabry disease, Wolman disease, and von Gierke disease. Finally, sea blue histiocytes may be found nonspecifically in many lysosomal storage disorders and in Hermansky-Pudlak syndrome, chronic myeloid leukemia, red blood cell disorders, and autoimmune disorders. These histiocytes are associated with massively increased cell destruction, and in these cases the macrophage cytoplasm is filled with insoluble lipid pigment, called *ceroid* (see Fig. 22.4B). Compared with Gaucher cells, these cells are stained more intensely blue with Wright-Giemsa, and the inclusions are globular rather than fibrillary.

HYPERSPLENISM-RELATED DISORDERS

IDIOPATHIC THROMBOCYTOPENIC PURPURA

Therapeutic splenectomy is occasionally performed on patients refractory to medical therapy in order to raise platelet counts. The spleen in ITP is generally unremarkable in gross appearance. Characteristically the white pulp demonstrates attenuated mantle zones with hyperplastic marginal zones containing increased immunoblasts and plasma cells. In cases of ITP, the presence of splenic congestion or white pulp expansion should raise suspicion for infarction or lymphoma, respectively.

THROMBOTIC THROMBOCYTOPENIC PURPURA

Thrombotic thrombocytopenic purpura (TTP) is a rare adult disorder with an incidence of approximately 4 per 100,000

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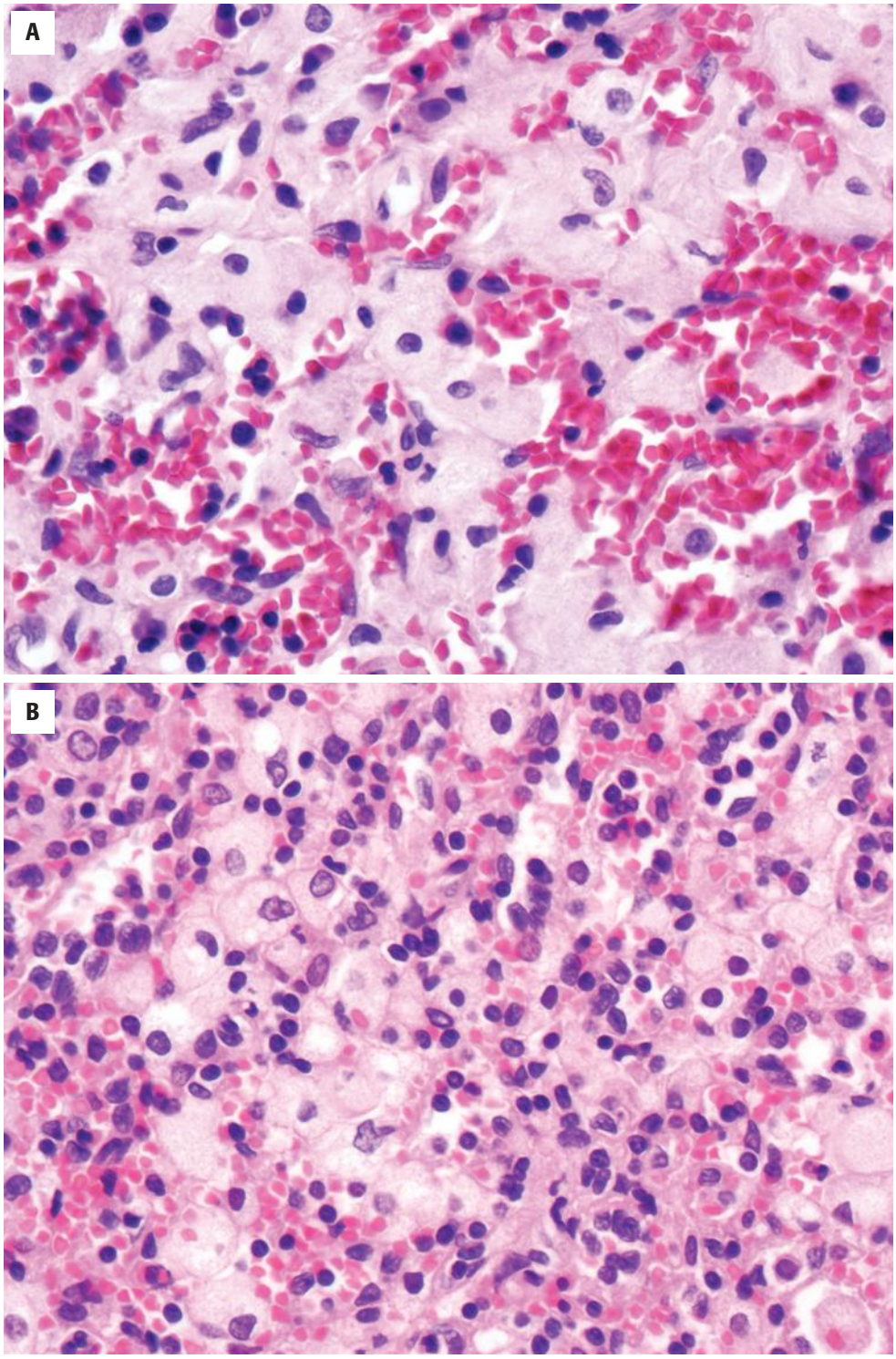


FIG. 22.4

Storage diseases in the spleen. A, Gaucher disease frequently involves the spleen with extensive red pulp replacement by large histiocytes with so-called tissue paper cytoplasm. B, The less specific ceroid histiocytes also involve the red pulp with less distinctive features.

(see Chapter 2). Treatment involves fresh frozen plasma, and splenectomy is uncommonly performed for refractory or relapsing patients. Grossly, the spleens of patients with TTP are not typically splenomegalic (mean weight, 214 g). The histologic features are somewhat varied, but common characteristics are subendothelial deposits, hemophagocytosis, arteriolar thrombi, B-cell hyperplasia, and periarteriolar concentric fibrosis. Hemosiderosis also occurs and can be highlighted with a Prussian blue stain. Less typical findings include extramedullary hematopoiesis, endothelial cell proliferation, infarcts, and blood lakes.

CONGESTIVE SPLENOMEGALY

Portal hypertension ultimately results in congestive splenomegaly. Most cases are the result of splenic, hepatic, or portal vein thrombosis, congestive heart failure, or cirrhosis. Rarely,

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cases of portal hypertension-associated splenomegaly have no discernible cause and are termed *idiopathic portal hypertension*. Grossly, the spleen appears large, firm, and dark. On histologic analysis, the white pulp is decreased and there is marked sinusoidal and venous dilatation with numerous hemosiderin-laden macrophages and red pulp fibrosis. Focal hemorrhage is often seen, sometimes with development of sclerotic nodules. Resolution of splenomegaly is achieved by treating the underlying condition.

OTHER HYPERSPLENISM-RELATED DISORDERS

In hereditary spherocytosis, splenomegaly is common and is the result of massive congestion of splenic cords by spherocytes. Hyperplastic follicles also may be seen. Splenectomy, which is typically reserved for patients with severe disease, corrects the anemia.

Similar to hereditary spherocytosis, acquired immune-mediated hemolytic anemia results in congestion of the splenic cords or sinuses, or both, by numerous erythrocytes, which gives the spleen an overall dark, red, and firm gross appearance. Acquired hemolytic anemias have varied etiologies and may be the result of toxins (bacterial hemolysins), plasma lipid abnormalities, parasites, and immune reaction. The immune-mediated hemolytic anemias are usually Coombs-positive (either autoimmune or alloimmune in the setting of prior transfusion). The lining cells of sinuses are often prominent, and foci of extramedullary hematopoiesis may be observed; splenic infarcts may be found in about one fourth of cases. Splenectomy, typically reserved for cases of autoimmune hemolytic anemia refractory to medical management, achieves remission in approximately 50% of cases.

In sickle cell disease, the two major sequelae in the spleen are autoinfarction and splenic sequestration. Typically, patients with sickle cell disease with sequestration are young, and grossly their spleens are enlarged and show hemorrhagic infarcts. On histologic examination, the spleen shows hemorrhagic necrosis and sickle cells within the splenic cords. Spleens from older patients with sickle cell disease are small and fibrotic, and on microscopy they show white pulp atrophy with infarcts encrusted with iron and calcium (Gamna-Gandy bodies).

SPLENIC LYMPHOMAS–LEUKEMIAS

Splenic lymphomas–leukemias that preferentially localize to the spleen are splenic marginal zone lymphoma, hairy cell leukemia, splenic diffuse red pulp small B-cell lymphoma, hairy cell leukemia-variant (HCLv) of the spleen, hepatosplenic T-cell lymphoma, and micronodular T-cell–histiocyte-rich, large B-cell lymphoma (MTLBL) of the spleen. Interestingly these lymphomas and leukemias are generally not confined to the spleen and involve the marrow and peripheral blood. With the exception of the provisional World Health Organization (WHO) category of splenic diffuse red pulp small B-cell lymphoma and MTLBL, these are discussed in the chapters on small B-cell lymphomas, B-cell leukemias of mature lymphocytes, and peripheral T-cell lymphomas.

SPLENIC DIFFUSE RED PULP SMALL B-CELL LYMPHOMA

Splenic diffuse red pulp small B-cell lymphoma (SRPL) is rare and represents less than 1% of non-Hodgkin lymphomas. Currently SRPLs fall under the ill-defined 2016 WHO designation of splenic B-cell lymphoma–leukemia, unclassifiable. The two best-known provisional entities in this group of lymphoma–leukemias are SRPL and HCLv. The latter has been discussed in Chapter 12. Nearly all patients with SRPL will have disseminated disease, including bone marrow and, rarely, cutaneous involvement. Patients are more frequently male (male-to-female ratio, approximately 2 : 1) and generally are elderly (median age >65 years). Although splenomegaly is common, B-type symptoms are rare. Interestingly, a lymphocytosis is often present, occasionally in association with thrombocytopenia, and laboratory values may demonstrate a high lactate dehydrogenase and β2-microglobulin levels.

Grossly, cases of SRPL demonstrate a homogeneous increase in the size of the spleen, which on cut section appears

SPLENIC DIFFUSE RED PULP SMALL B-CELL LYMPHOMA—FACT SHEET

Clinical Features
- Elderly (>65 years) with male predominance
- Lymphocytosis occasionally with thrombocytopenia
- Splenomegaly is common

Morphology
- Monotonous population of small to medium-sized lymphocytes with round to oval nuclei, single small nucleoli, and pale cytoplasm
- Red pulp expansion by lymphoma cells

Immunophenotype
- $CD20^+$, $CD19^+$, $CD79a^+$, $CD22^+$, $DBA.44^+$, IgG^+, $FMC7^+$, $CD11c^{\pm}$, IgD^-, annexin $A1^-$, $CD25^-$, $CD103^-$, $CD123^-$, $CD10^-$, $BCL6^-$, $CD23^-$, $CD38^-$, $BCL1^-$

Prognosis and Therapy
- Indolent incurable disease that may require no treatment
- Splenectomy with or without chemotherapy

Differential Diagnosis
- Hairy cell leukemia and hairy cell leukemia variant
- Prolymphocytic leukemia
- Chronic lymphocytic leukemia or small lymphocytic lymphoma
- Splenic marginal zone lymphoma
- Lymphoplasmacytic lymphoma

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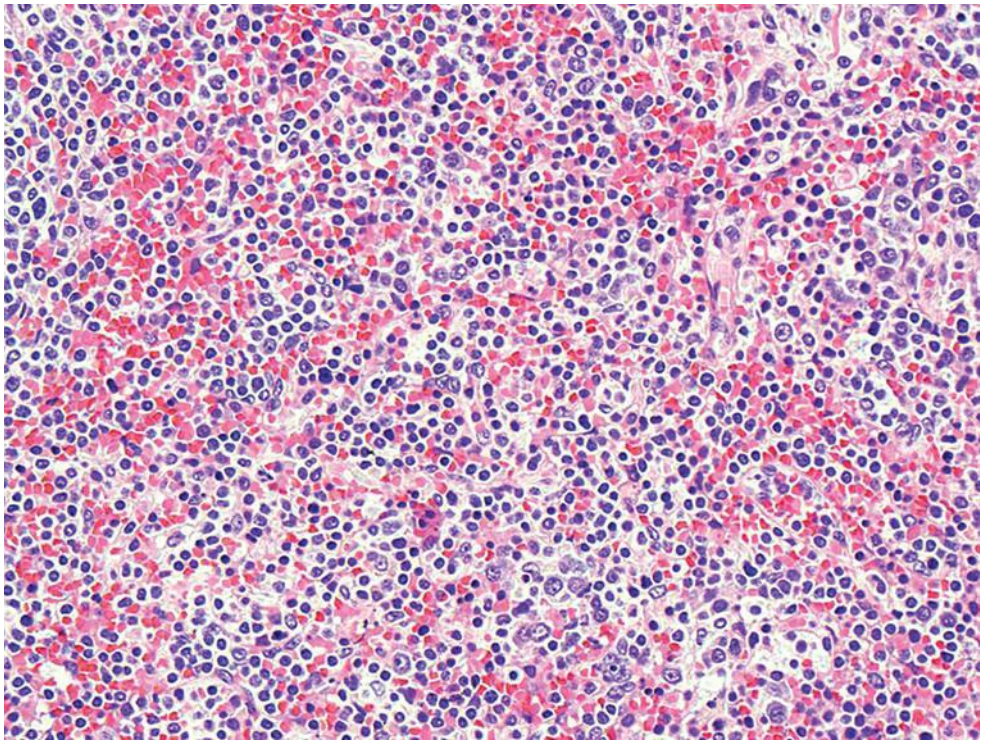


FIG. 22.5

Splenic diffuse red pulp small B-cell lymphoma. The red pulp is infiltrated by small, mostly round B lymphocytes. The morphologic and immunophenotypic features (see text) are similar to the cells of splenic marginal zone lymphoma, but the pattern of infiltration is more similar to hairy cell leukemia.

red-brown and lacks any distinct nodularity. The microscopic features are those of expansion of the red pulp by monotonous populations of small to medium-sized lymphocytes with round to oval nuclei, single small nucleoli, and pale cytoplasm (Fig. 22.5). In contrast, the white pulp may appear atrophic. In the peripheral blood, these tumor cells typically are greater than 25% of lymphocytes and appear small to medium in size with clumped nuclear chromatin, round nuclei, and broad or fine villous cytoplasmic projections. Occasionally cells with prominent central nucleoli may be seen in the blood and raise the differential diagnosis of prolymphocytic leukemia and hairy cell leukemia-variant; however, villous projections are not a feature of prolymphocytic leukemia. As stated previously, bone marrow involvement is characteristic, with lymphoma cells interspersed throughout sinusoids. Neoplastic lymphoid aggregates are not typically seen.

Immunohistochemical studies show that the neoplastic cells express CD20, CD79a, CD19, CD22, and BCL2. In addition, they are generally also positive for FMC7, DBA.44 (CD76), and IgG; a subset of cases also are positive for CD11c. In contrast, MUM1, annexin A1, CD25, IgD, CD5, CD10, CD103, CD123, CD23, and BCL6 are absent. Interestingly, weak expression of CD5, CD43, CD103, CD123, or CD23 may be seen by flow cytometry.

Detailed molecular analyses have shown that these lymphomas have clonal *IGH* rearrangements with few somatic hypermutations and *TP53* alterations are frequently seen, resulting in increased p53 expression.

Treatment is varied; however, as the disease is typically indolent, no treatment at all may be required, although a subset of patients may benefit from splenectomy with or without chemotherapy.

The differential diagnosis includes hairy cell leukemia (HCL), splenic marginal zone lymphoma (SMZL), and HCL variant (HCLv). The blood smear morphology and lack of CD103 and CD25 expression by SRPL allows distinction from HCL, whereas the red pulp pattern of SRPL distinguishes it from SMZL. Expression of CD103 by HCLv and its usual absence in SRPL can help to separate these two entities from each other, but overlap may exist between rare cases of HCLv and SRPL. Blood smear, bone marrow, and spleen samples for morphologic evaluation and full phenotypic characterization are necessary for the best subclassification. Without splenic histopathology, indications of SRPL include appropriate villous lymphocyte blood smear morphology and typical immunophenotype with purely sinusoidal bone marrow involvement. Little is known about the molecular genetics of SRPL but study of 19 cases found mutations in 2 cases (PEST domain mutations that likely activate *NOTCH1*), *MAP2K1* mutations (K57E, K57N) in 2 cases, *SF3B1* P642L in 1 case, *BRAF* K601Q in 1 case, and *TP53* I255S in 1 case with concurrent *TP53* deletion. *MYD88* L265P mutations were not found. Thus some pathway abnormalities seen in other lymphoid malignancies may be shared with SRPL.

MICRONODULAR T-CELL/HISTIOCYTE-RICH LARGE B-CELL LYMPHOMA OF THE SPLEEN

Large B-cell lymphoma can occasionally develop as a primary single mass or multiple masses in the spleen. One rare variant of diffuse large B-cell lymphoma, MTLBL, has been described and constitutes a specific subset with extremely poor prognosis. Patients with MTLBL are more frequently male (male-to-female ratio, 2:1) and exhibit splenomegaly, anemia, and B type

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symptoms. Interestingly, on gross appearance, the spleen, though enlarged, does not show a discrete mass, and only on histologic analysis is clearly diffusely expanded by small discrete micronodules throughout white pulp. Classically, the red pulp is spared. Numerous small T cells and histiocytes are seen with scattered rare (<10%) large atypical B cells within nodules and few, if any, small mature B cells. The large neoplastic B cells can have a somewhat pleomorphic appearance as atypical centroblasts, lymphocytic, and/or histiocytic (L&H) cells, or Reed-Sternberg cells, thus complicating the diagnosis. Immunohistochemical studies demonstrate these B cells to be positive for CD20, CD79a, BCL6, and OCT2 with variable staining for epithelial membrane antigen (EMA) and CD30; these cells are negative for CD15. Importantly, as in T-cell/histiocyte-rich large B-cell lymphoma in nodal sites, follicular dendritic cell networks as demonstrated by CD21, CD23, or CD35 should be absent. Most cases show involvement of the perisplenic lymph nodes and bone marrow by rare atypical large B cells though diffuse sheets of large B cells can also be seen. MTLBL may be misdiagnosed as a reactive inflammatory lesion or other non-Hodgkin or Hodgkin lymphoma if immunohistochemical studies are not performed.

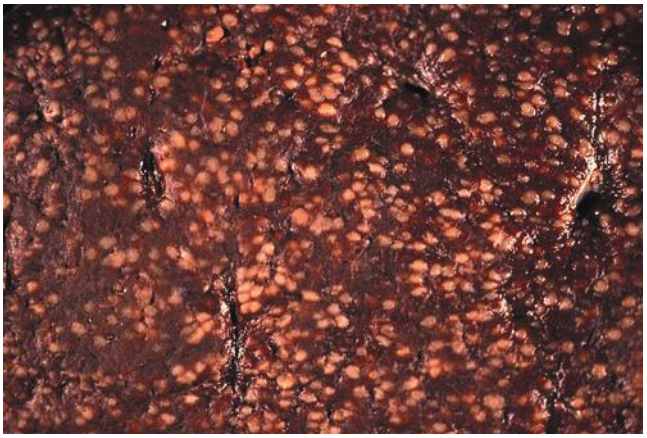


FIG. 22.6

Miliary gross pattern of splenic involvement. This pattern usually represents an expansion of the white pulp and is common with splenic involvement by lymphomas of small B lymphocytes, including splenic marginal zone lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, and follicular lymphoma. In this case, the spleen is involved by chronic lymphocytic leukemia.

LEUKEMIAS SECONDARILY INVOLVING THE SPLEEN

Both myeloid and lymphoid leukemias commonly involve the spleen and result in generalized enlargement and expansion of the red pulp in both the splenic cords and sinuses.

CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA

Involvement of the spleen by chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) with resultant splenomegaly is rarely a presenting complaint; however, it is often a deciding factor in initiating treatment because it heralds development of complications such as ITP, autoimmune hemolytic anemia, and hypersplenism. The spleen demonstrates a miliary gross pattern that is similar to many other small B-cell lymphomas (Fig. 22.6). The cells of CLL/SLL predominantly involve the white pulp; they also infiltrate the red pulp. In cases with significant numbers of prolymphocytes, which can be easily distinguished by their large nucleoli, splenomegaly is often massive. The differential diagnosis includes other small-cell lymphomas, including lymphoplasmacytic lymphoma, mantle cell lymphoma, and follicular lymphoma. With the exception of a subset of cases of lymphoplasmacytic lymphoma, these disorders all infiltrate and expand the splenic white pulp. As such, the diagnosis rests largely on immunophenotypic and molecular studies, with CLL showing dim CD20 and dim surface light chain expression with aberrant CD5 and lymphoid-enhancer-binding factor 1 (LEF1) expression on $CD23^+$ B cells. Patients with CLL and splenic involvement are typically in advanced stage and have shorter survival times. Interestingly, studies have shown improved survival in patients with hemoglobin less than 10 g/dL or platelet count less than $50 \times 10^9/\mu L$ who received splenectomy in addition to standard chemotherapy.

ACUTE MYELOID LEUKEMIA AND ACUTE LYMPHOBLASTIC LEUKEMIA

Splenic involvement by acute myeloid leukemia (AML) and acute lymphoblastic leukemias (ALL) is not uncommon (see Chapters 14 and 15). The spleen is typically normal in size, although splenomegaly can be seen. Involvement and expansion of the red pulp by leukemic blasts are characteristic, and a rare but significant complication of both ALL and AML is rupture of the spleen, which has been reported in cases with extensive leukemic involvement. Involvement of the spleen is almost always seen in conjunction with peripheral blood or bone marrow findings that yield morphologic and immunophenotypic data for classification. In exceedingly rare cases, myeloid sarcoma involving the spleen can be a presenting feature of AML.

T-CELL LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

T-cell large granular lymphocytic (T-LGL) leukemia can also secondarily involve the spleen, and up to 50% or more of patients will exhibit clinically detectable splenomegaly (see Chapter 13). Histologically, there is expansion of the red pulp with an intrasinusoidal infiltration by large granular lymphocytes; these cells are best appreciated on smear or touch preparation. Lymphoid germinal center hyperplasia is commonly seen, and, in most cases, preservation of the white pulp boundaries is maintained; expansion of the mantle zone by LGLs is also a feature. These neoplastic cells generally

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express CD3, CD8, perforin, and granzyme B. Although they characteristically express CD57 in the blood and bone marrow, they frequently lack expression of CD57 in the spleen. Interestingly, CD5 is lost in many cases of splenic involvement. Recently activating *STAT3* and *STAT5b* mutations have been identified in patients with T-LGL leukemia. Even though it is a clonal neoplastic disease associated with cytopenias, LGL leukemia is a relatively indolent disorder.

LYMPHOMAS SECONDARILY INVOLVING THE SPLEEN

As leukemias can secondarily involve the spleen, so too can lymphomas from nodal or extranodal sites. The clinical features of lymphoma involvement in the spleen are diverse, but with extensive involvement the end result is the same: splenomegaly. Although these disorders are described in other chapters, the characteristic splenic features are provided here.

DIFFUSE LARGE B-CELL LYMPHOMA

Diffuse large B-cell lymphoma (DLBCL) is a common non-Hodgkin lymphoma that can secondarily involve the spleen (see Chapter 8). Classically, large nodules of firm white fish flesh–like tissue expand the spleen and may extend beyond the capsule. Regional lymph nodes are typically involved and often enlarged. Histologic sections of the spleen show an expansion of the white pulp by nodules and diffuse sheets of large atypical centroblastic or immunoblastic lymphoid cells with clearing of nuclear chromatin and distinct nucleoli; mitoses are generally abundant. Immunophenotypically, the cells are positive for CD45 and for B-cell antigens such as CD20, CD79a, PAX5, and CD19. As in cases of DLBCL in other anatomic sites, germinal center markers such as BCL6 and CD10 are variably expressed, and a subset of cases may express CD5 and lack germinal center markers. The lymphoma cells are monotypic, and immunoglobulin light chain restriction can often be demonstrated.

In addition, DLBCL may arise de novo or can result from transformation of a low-grade splenic lymphoma such as splenic marginal zone lymphoma (13% of patients in one series). However, in these cases peripheral nodes, rather than the splenic and hilar lymph nodes, are the usual sites of transformation.

Less commonly, DLBCL develops as a splenic red pulp proliferation. Such cases appear to be the splenic manifestation of intravascular large B-cell lymphoma. Reported cases of splenic red pulp DLBCL often express CD5, involve bone marrow with an interstitial/intrasinusoidal pattern, often involve liver with an intrasinusoidal pattern, and have an aggressive course. Importantly, these cases may be confused with red pulp involvement by acute or chronic leukemia, but immunophenotyping studies are useful in proving the mature B-cell lineage of the proliferation.

LYMPHOPLASMACYTIC LYMPHOMA

Lymphoplasmacytic lymphoma (LPL) typically involves both the splenic white and red pulp and often in a diffuse pattern, although the infiltrate may form distinct small nodules in the red pulp (see Chapter 7). Periarteriolar aggregates of plasmacytoid cells, small lymphocytes, plasma cells, and a variable number of immunoblasts are consistent with LPL, and Dutcher bodies, increased mast cells, and hemosiderin may also be features. Rarely, LPL has been associated with Hodgkin lymphoma or progression to DLBCL. Recently *MYD88* mutations have been identified in more than 90% of cases of LPL and are rare to absent in other small B-cell lymphomas with plasmacytic differentiation allowing molecular distinction. However as *MYD88* mutations can be seen in other B-cell lymphomas, LPL remains somewhat a diagnosis of exclusion; other lymphomas in the differential diagnosis to rule out are splenic marginal zone lymphoma with plasmacytoid differentiation, CLL/SLL, and mantle cell lymphoma. The most frequently overlapping entity is splenic marginal zone lymphoma; however, SMZL shows a biphasic pattern, with paler cells suggesting marginal zone differentiation.

MANTLE CELL LYMPHOMA

Splenomegaly is a common clinical finding in mantle cell lymphoma (MCL) owing to a dense infiltration of tumor cells. A number of patterns, including mantle zone hyperplasia, pseudo-nodular growth, and diffuse infiltration, can be seen (see Chapter 7). The red pulp cords and sinuses are typically infiltrated. Tumor cells are characteristically monotonous and small with an irregular cleaved nucleus; however, morphologic variants (blastoid, pleomorphic, small cell, and marginal zone–like) may also be seen. The immunophenotype remains characteristic, and cells express CD5, CD19, FMC7, CD43, and cyclin-D1 and lack CD10 and BCL6. The various growth patterns of MCL introduce SMZL, follicular lymphoma, CLL/SLL, and reactive follicular hyperplasia into the differential diagnosis; however, cyclin-D1 and SOX11 positivity by immunohistochemistry establishes the diagnosis of MCL. In addition, detection of bright CD20 and immunoglobulin light chain expression are useful in excluding CLL/SLL.

Of note, a variant of MCL that lacks nodal involvement but rather shows peripheral blood and frequent splenic involvement has been described. Such cases have hypermutated immunoglobulin heavy chain variable region genes, appear to lack SOX11, and have an indolent clinical course.

FOLLICULAR LYMPHOMA

Follicular lymphoma commonly involves the spleen; however, splenomegaly may not be apparent (see Chapter 7). Typically

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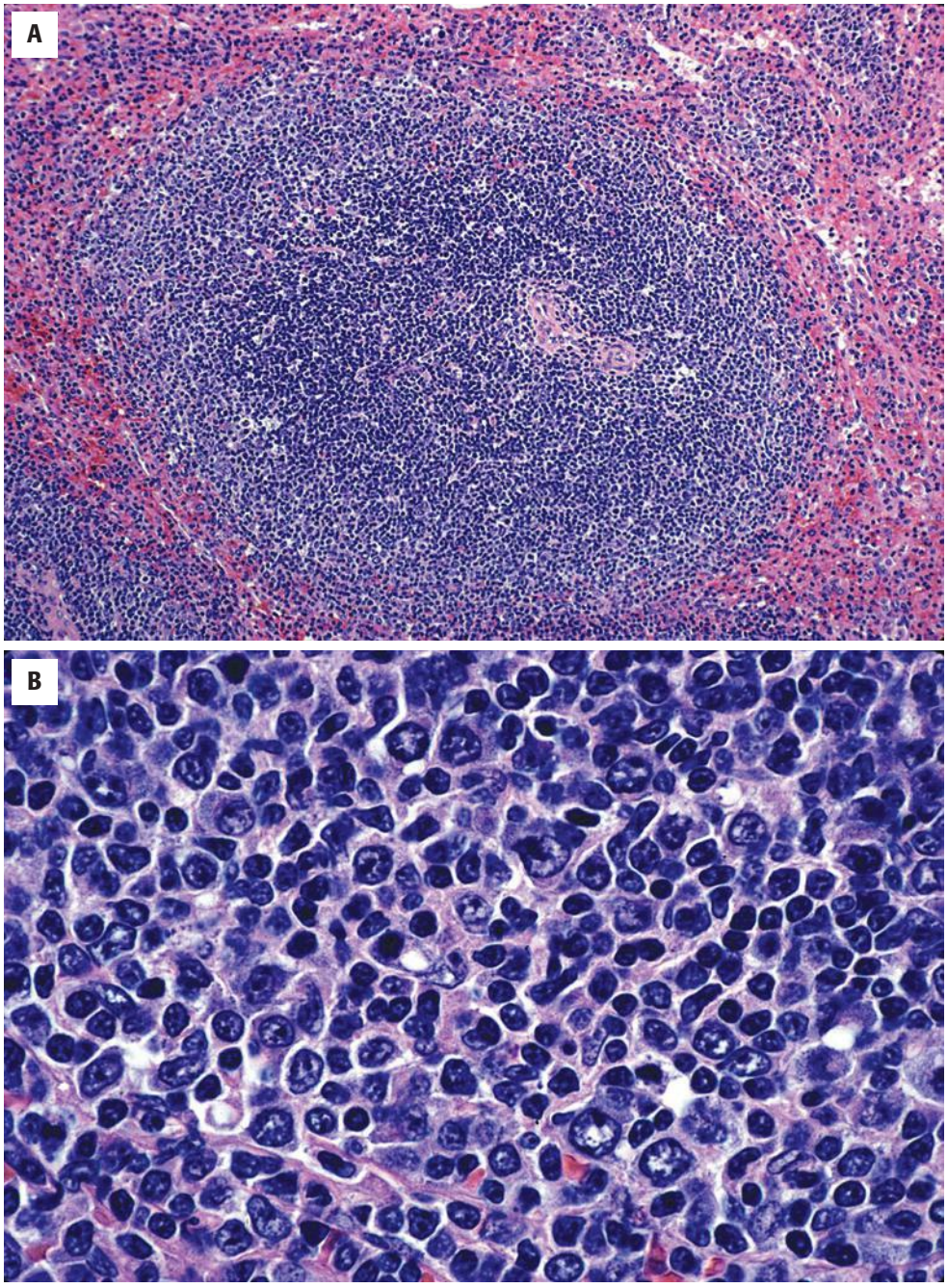


FIG. 22.7

Follicular lymphoma involving the spleen. A, The white pulp is expanded, and there is an expanded outer marginal zone that suggests splenic marginal zone lymphoma. B, The central white pulp cells show a mix of small cleaved and large cells typical of follicular lymphoma. These B cells also expressed CD10 and BCL2, confirming the diagnosis of follicular lymphoma.

the white pulp is expanded throughout the spleen, and mass lesions are absent unless large cell transformation has occurred. The splenic cut surface demonstrates numerous small discrete white nodules, which histologically are often irregular in shape and may be surrounded by a residual mantle and marginal zones (Fig. 22.7). As in other anatomic locations, the lymphoma cells are a variable composition of cleaved centrocytes and centroblasts and may also resemble marginal zone–type cells in the periphery of nodules. Germinal center marker expression is retained (CD10 and BCL6), and, in most cases, aberrant expression of BCL2 in follicles is seen; however, in at least one study of splenic follicular lymphoma in which the presenting disease was primarily in the spleen, BCL2 expression was lost or diminished in a significant number of cases. Interestingly, many cases may also show patterns of involvement by in situ follicular lymphoma. Molecular analysis for t(14;18) *(IGH/BCL2)* or *BCL6* translocations have made the diagnosis more definitive. Misinterpreting an expanded marginal zone component in splenic involvement by follicular lymphoma as splenic marginal zone lymphoma is a differential diagnostic pitfall; however, follicular lymphoma is a more systemic node-based disease when compared to splenic marginal zone lymphoma. In addition, identification of central white pulp cells with the

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characteristic mix of small cleaved and large cells of follicular lymphoma is the best diagnostic clue for follicular lymphoma over splenic marginal zone lymphoma and should prompt immunophenotyping studies and clinical evaluation for further evidence of follicular lymphoma.

HODGKIN LYMPHOMA

The spleen is the most common extranodal site of involvement by Hodgkin lymphoma, and early studies found that more than one third of patients have splenic involvement (see Chapter 11). Typically, the spleen is enlarged, and the cut surface shows several white fleshy tumor nodules. Lesions characteristically first involve the periarteriolar lymphoid sheath and marginal zones of the white pulp. Although the nodular sclerosis subtype is the most common one to involve the spleen, other subtypes can be seen as well, with the exception of nodular lymphocyte-predominant Hodgkin lymphoma, which typically does not involve the spleen. Subtyping of Hodgkin lymphoma, other than the classic type versus the lymphocyte-predominant type, should not be performed on splenic tissue as bands of fibrosis are not specific to the nodular sclerosis type in this organ. In addition, some cases can be associated with granulomas, but the presence of granulomas alone without identifiable tumor cells should not be used as an indicator of involvement by Hodgkin lymphoma. Finally, splenic involvement, an indicator of advanced disease, is usually associated with involvement of the marrow or liver.

OTHER HEMATOPOIETIC PROLIFERATIONS OF THE SPLEEN

MASTOCYTOSIS

Although mastocytosis (see Chapter 20) describes a group of biologically distinct diseases, all are recognized causes of splenomegaly. Mast cells can invade the red pulp, white pulp, or both and form aggregates typically in the red pulp around trabeculae and the capsule (Fig. 22.8). When invasion of the white pulp occurs, mast cells are found in the follicular and parafollicular regions. A characteristic feature of splenic mastocytosis is fibrosis, which is often grossly evident on cut section and tends to surround the mast cell aggregates, imparting a lobular appearance. Mast cells often display cytoplasmic projections in tissue sections, raising the differential diagnosis of hairy cell leukemia; however, the presence of patchy cellular aggregates is characteristic of mastocytosis and is rarely seen in hairy cell leukemia involving the spleen. A definitive diagnosis is made by immunohistochemistry with cells positive for mast cell tryptase and CD117. Aberrant expression of CD25 and CD2 is often seen, and a subset of cases are CD30 positive. Most cases have mutations of the *KIT* gene.

MYELOPROLIFERATIVE NEOPLASMS

Myeloproliferative neoplasms, although not originating in the spleen, frequently involve it. For example, in the case of primary myelofibrosis, massive splenic enlargement is common because of the production of defective red blood cells and platelets that are secondarily removed by the spleen, resulting in splenomegaly and extramedullary hematopoiesis (Fig. 22.9). Splenic infarction can occur at later stages of myelofibrosis (50% of cases) and is often associated with abdominal pain.

VASCULAR AND OTHER NONHEMATOPOIETIC PRIMARY SPLENIC TUMORS

Vascular tumors are the most common tumors of the spleen (Table 22.1) that typically involve the red pulp. They may be diffuse or form a tumor mass and are usually found incidentally on imaging.

SPLENIC HEMANGIOMA

Splenic hemangiomas are benign tumors that are also usually asymptomatic but may cause splenomegaly, abdominal pain, and hypersplenism. Most hemangiomas are localized and form single or multiple tumor nodules that contain cystic blood-filled spaces (Fig. 22.10); these spaces are lined by endothelial cells, and papillary projections may occur in areas along with thrombi. The tumor nodules are usually surrounded by fibrosis and may show calcification. Plain abdominal radiographs, CT scans, and sonograms are nonspecific but all show discrete solid and cystic masses, often with evidence of calcification. Diffuse hemangiomatosis of the spleen, which is less common, often is associated with systemic hemangiomatosis. Massive splenomegaly and coagulopathies are characteristic in these cases. Diffuse hemangiomatosis differs from peliosis by the presence of intervening fibrosis in hemangiomatosis, which is not a feature of peliosis. The differential diagnosis also includes lymphangioma and primary splenic cyst; however, localized lymphangiomas and primary splenic cysts of the spleen usually contain proteinaceous fluid rather than the blood of a hemangioma. In addition, diffuse lymphangiomatosis may be localized to the spleen but is usually a systemic process and most commonly occurs in children and young adults with massive splenomegaly.

LITTORAL CELL ANGIOMA

Littoral cell angiomas are unique to the spleen. They may occur at any age and usually cause mild to moderate splenomegaly. Littoral cell angioma is a tumor presumably derived

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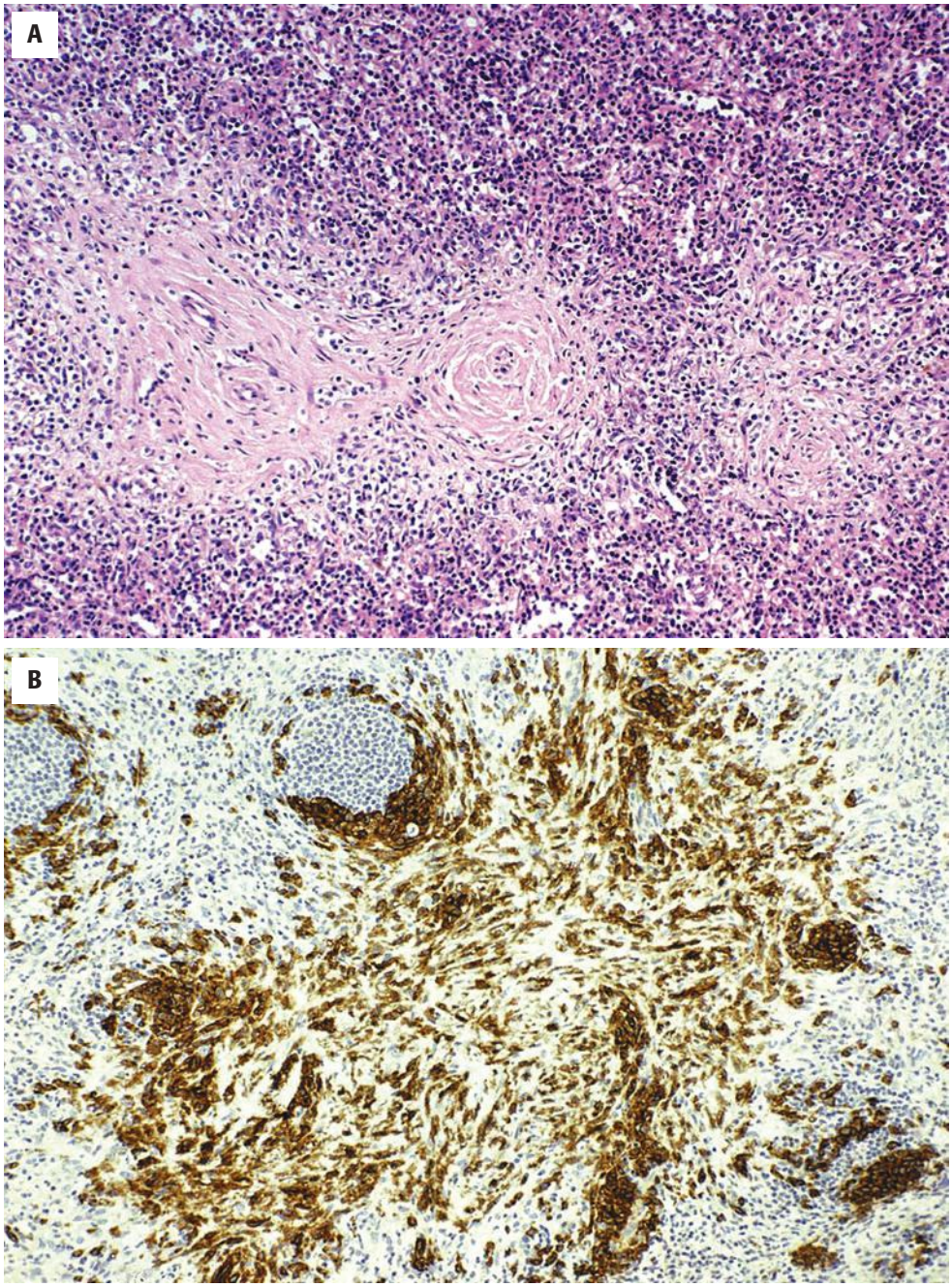


FIG. 22.8

Mastocytosis. A, Patchy areas of fibrosis are present. Within the fibrosis are eosinophils and mast cells with abundant pale or clear staining cytoplasm. B, These cells stain for CD117 (shown here) and tryptase, and they usually show aberrant coexpression of CD25 on tissue sections.

from the littoral cells lining the sinus channels, but the tumor immunophenotype differs slightly from this presumed normal counterpart. Grossly, the spleen shows diffuse multinodularity with spongy dark red nodules that can measure up to 9 cm in diameter; rarely, it develops as a single large mass. Histologically, the vascular spaces are lined by plump cells with nuclear enlargement and often show papillary areas with lining cells sloughing into the vascular spaces (Fig. 22.11). The lining cells of littoral cell angioma have a unique immunophenotype, expressing vascular, histiocytic, and dendritic-associated markers CD31, CD68, CD163, and at least focal CD21. The actual lining cells are $CD34^{-}$, and they do not express CD8, unlike normal splenic sinus lining cells. The differential diagnosis includes hemangioma and angiosarcoma; however, hemangiomas of the spleen express CD34 and usually lack the nuclear enlargement of littoral cell angioma. In addition, angiosarcomas show more cytologic atypia than littoral cell angiomas as well as mitotic figures and necrosis—features that are not present in littoral cell angioma. Most cases of littoral cell angioma are treated with splenectomy without recurrence. Rare cases with foci containing a solid clear cell proliferation have metastasized many years later, and such cases probably represent a rare entity termed *littoral cell hemangioendothelioma*.

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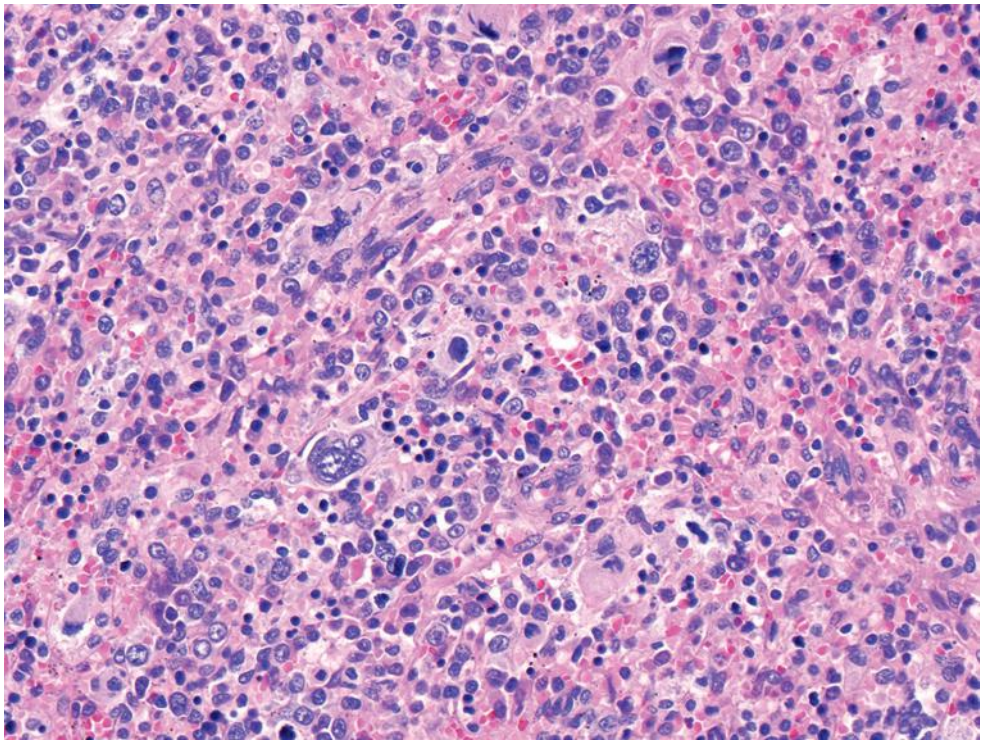


FIG. 22.9

Primary myelofibrosis in the spleen. The spleen is usually massively enlarged with expansion of the red pulp by marrow elements, including many atypical megakaryocytes.

TABLE 22.1

Vascular and Other Nonhematopoietic Primary Splenic Tumors

Entity	Clinical Features	Pathologic Findings	Immunophenotype	Prognosis
Hamartoma	Rare, predominantly elderly, cytopenias common	Nodular lesion; median size, 5 cm; numerous slit-like vascular channels lined by plump-flattened endothelial cells without white pulp	$CD8^+$, vWF^+, $CD31^+$, $CD34^\pm$, $CD21^-$, $CD68^-$	Benign, but possible risk of rupture in larger lesions
Hemangioma	Common benign tumor of the spleen, generally asymptomatic	Nonencapsulated, <2 cm, vascular channels separated by red pulp and fibrous septae	$CD31^+$, $CD34^+$, vWF^+, $CD21^-$, $CD68^-$, $CD8^-$	Benign, but there can be a risk of rupture
Littoral cell angioma	Rare, often incidental finding	Numerous channel-like vascular spaces lined by plump cells that surround fibrovascular cores and luminal macrophages	$CD31^+$, vWF^+, $CD163^+$, $CD68^+$, $CD21^\pm$, $CD34^-$, $CD8^-$	Benign, reported association with secondary malignancies
Lymphangioma	Rare, generally an isolated finding	Often subcapsular, variably sized cystic spaces with flat, bland endothelium filled with proteinaceous fluid	$CD31^+$, $CD34^\pm$, $CD21^-$, $CD8^-$, $D2\text{-}40^+$	May recur if not completely excised
Sclerosing angiomatoid nodular transformation	Rare, occurs in elderly (>50 years), generally asymptomatic; female-to-male ratio, 2:1	Red-tan, unencapsulated mass composed of nodules with slit-like, round, vascular spaces lined by plump endothelial cells and pericytes surrounded by densely collagenous fibrotic or fibrinoid granulomatous tissue	Three vascular patterns: $CD34^-$, $CD31^+$, $CD8^+$ sinusoids; $CD34^+$, $CD31^+$, $CD8^-$ capillaries; $CD34^-$, $CD31^+$, $CD8^-$ veins; CD68 expression is variable	Indolent and benign with no tendency for recurrence after splenectomy
Angiosarcoma	Most common nonlymphoid malignancy of the spleen	Typically multifocal, with irregular anastomosing vascular channels with marked atypia, frequent mitoses, and invasion of surrounding stroma	$CD31^+$, $CD34^+$	Malignant lesion with high rate of dissemination
Inflammatory pseudotumor-like follicular/fibroblastic dendritic cell sarcoma	Rare, with fever and abdominal pain	Scar-like lesion composed of myofibroblastic spindle cells with mixed inflammatory cells (lymphocytes, plasma cells, eosinophils)	Spindle cells: EBV^+, $SMA^\pm$, $Desmin^\pm$, $vimentin^+$, $CD68^\pm$, $S100^\pm$, $CD21^\pm$, $CD34^-$, $CD8^-$, $ALK1^-$	Indolent, responsive to splenectomy, incomplete resection may recur as follicular dendritic cell sarcoma

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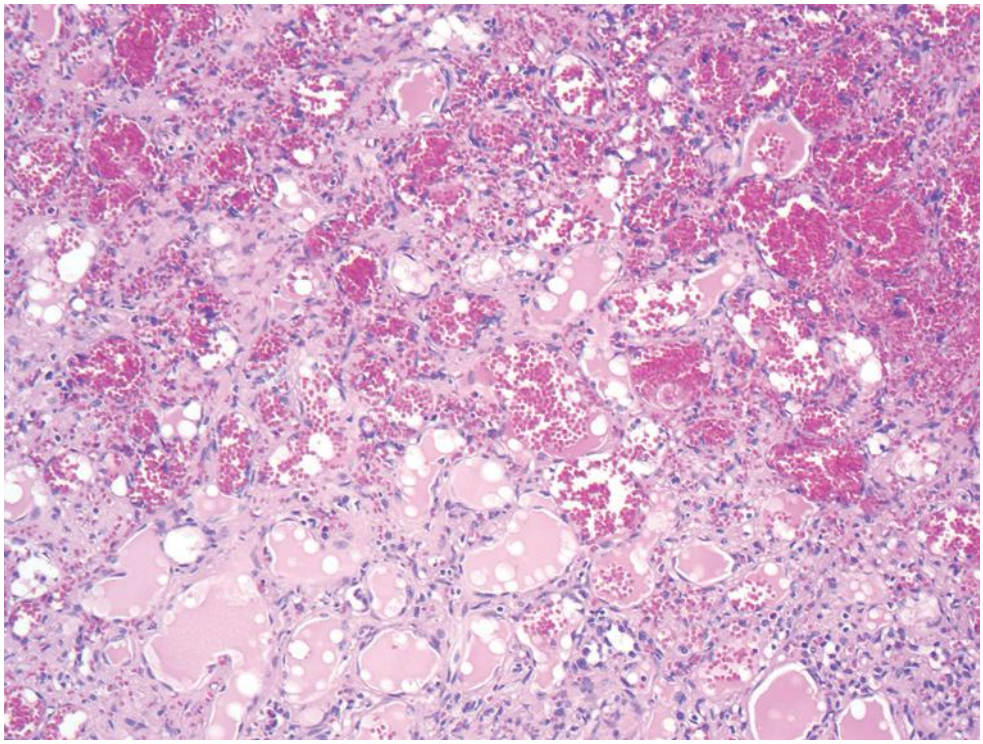


FIG. 22.10
Hemangioma. These proliferations can form a distinct mass or diffusely involve the spleen. They show numerous, variably sized vessels with intervening fibrosis without atypia or necrosis.

LITTORAL CELL ANGIOMA—FACT SHEET

Clinical Features
- Median age 49 years; no gender preference
- Occasionally splenomegaly with thrombocytopenia or anemia
- Reported association with secondary malignancies

Gross Findings
- Splenomegaly with diffuse multinodularity with spongy, dark red nodules

Morphology
- Anastomosing vascular channels with irregular lumina
- Channels lined by plump cells with nuclear enlargement and variable hemophagocytosis

Immunophenotype
- $CD31^+$, $CD163^+$, vWF^+, $CD68^+$, $CD21^{\pm}$, $CD34^-$, $CD8^-$

Prognosis and Therapy
- Splenectomy for therapy
- A benign disease with variable prognosis given the associated possible comorbidities

Differential Diagnosis
- Hemangioma
- Angiosarcoma

SPLENIC ANGIOSARCOMA

Splenic angiosarcoma is a rare tumor that occurs most commonly in adults; fewer than 200 cases have been reported. It is usually associated with splenomegaly, abdominal pain, and cytopenias, and splenic rupture can be seen in up to 30% of cases. Because most angiosarcomas involving the spleen are high-grade sarcomas with dissemination, it is often difficult to determine whether the splenic tumor is primary or secondary. The tumor typically forms an infiltrating mass but can also form a network of anastomosing vascular channels lined by atypical hobnail cells, with high mitotic activity and necrosis. Areas of cystic hemorrhage are sometimes present. Many cases may be difficult to differentiate from other high-grade sarcomas, and immunohistochemical detection of vascular antigen expression (e.g., CD31, CD34, von Willebrand factor) is necessary to diagnose such cases. The differential diagnosis includes littoral cell angioma, cavernous hemangioma, normal splenic sinuses, or other sarcomas such as Kaposi sarcoma. Immunohistochemical studies for CD8, CD34, CD31, CD68, or human herpesvirus 8 can usually distinguish angiosarcoma from littoral cell angioma and Kaposi sarcoma, and the presence of necrosis or mitotic activity essentially excludes littoral cell angioma. High-grade angiosarcomas involving the spleen have a generally poor prognosis, and most patients die of disease within 1 year of diagnosis;

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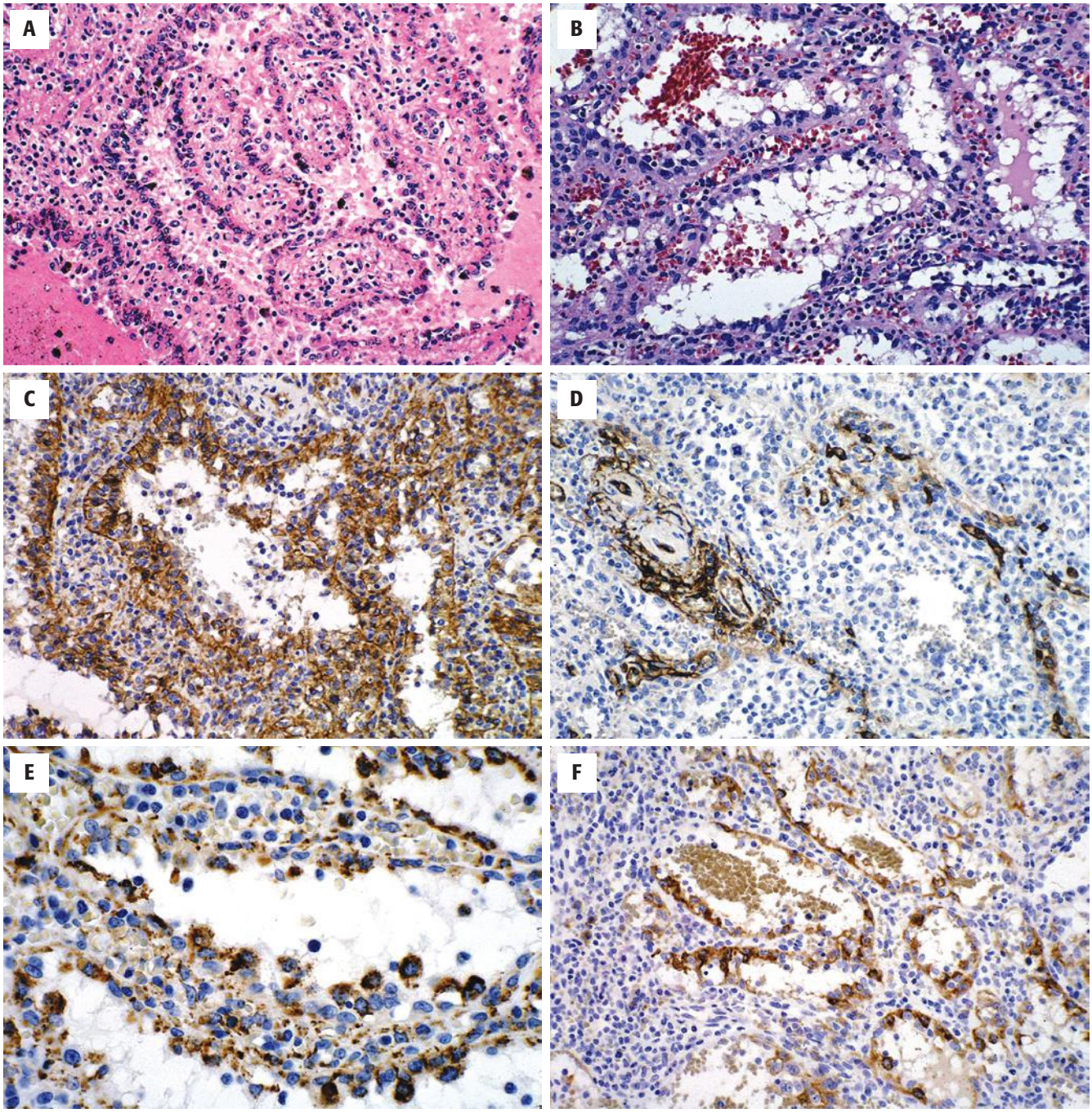


FIG. 22.11

Littoral cell angioma. A, These tumors form hemorrhagic masses with a usually papillary histologic appearance. B, The lining cells show nuclear enlargement, with cells often sloughing into the vascular spaces but do not show necrosis. The lining cells express CD31 (C) but not CD34 (D) and focally express CD68 (E) and CD21 (F).

however, rare cares with long-term survival following splenectomy are reported.

SPLENIC LYMPHANGIOMA

Lymphangiomas of the spleen are uncommon tumors that develop as an isolated nodule or diffusely infiltrate the spleen, often in the setting of a patient with lymphangiomatosis. Localized tumors are subcapsular, whereas diffuse proliferations can involve the entire spleen. Three histologic categories are recognized: cystic, cavernous, and simple or capillary. Cystic lymphangiomas are most common and show thin-walled cysts of variable size filled with serous fluid (Fig. 22.12). The endothelial cells are positive for CD31 and D2-40 and often focally positive for CD34 but negative for CD21 and CD68. These lesions are benign and often are found incidentally and require no treatment.

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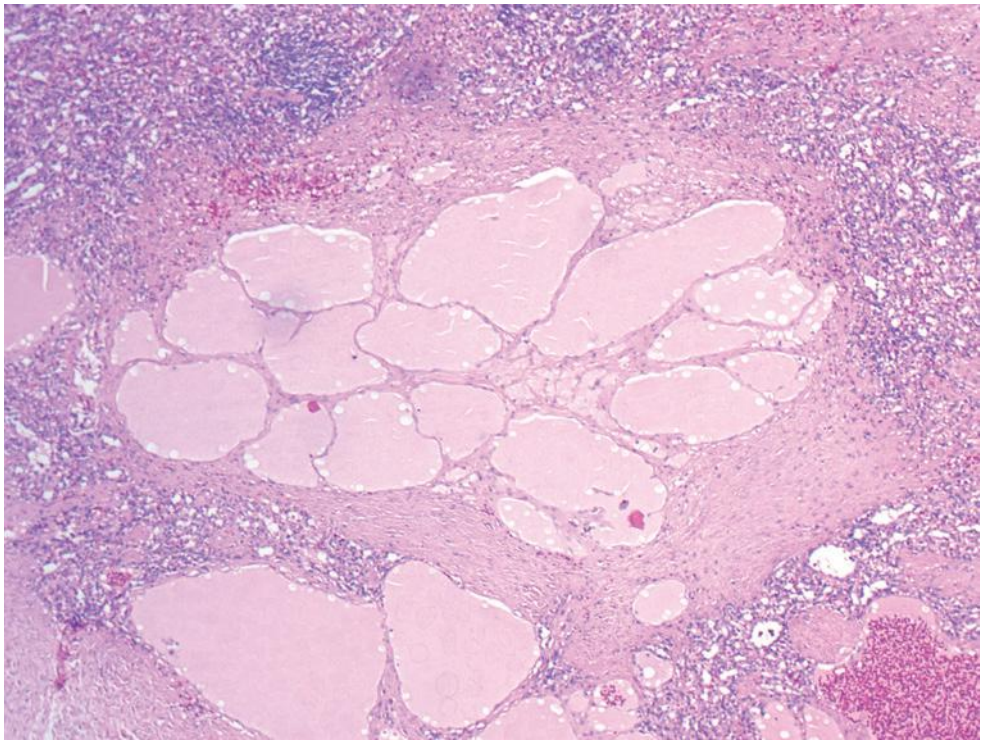


FIG. 22.12
Lymphangioma. These proliferations are often subcapsular and consist of multiple, small, dilated fluid-filled spaces without atypia.

SCLEROSING ANGIOMATOID NODULAR TRANSFORMATION OF THE SPLEEN

Sclerosing angiomatoid nodular transformation (SANT) is a non-neoplastic vascular lesion of the spleen. The median age at presentation is 54 years but ranges from 22 to 74 years, and a female predominance is seen (female-to-male ratio, 2:1). Although most patients are asymptomatic, with a mass found only incidentally on imaging, as many as 16% of patients may complain of abdominal pain. Interestingly, a small subset of patients may have leukocytosis, an elevated erythrocyte sedimentation rate, and a polyclonal gammopathy.

Grossly, the spleen is generally normal to slightly enlarged in size and on cut cross section reveals a single unencapsulated mass composed of multiple red-brown nodules separated by stellate fibrous stroma (Fig. 22.13). Microscopic examination shows that some nodules are surrounded by densely collagenous fibrotic tissue, whereas other nodules are circumscribed by a fibrinoid granulomatous rim. Within the nodules, numerous slit-like round vascular spaces are seen that are lined by plump endothelial cells and pericytes. Rare mitoses can be seen, but cellular atypia should not be prominent. The fibrosclerotic internodular spaces are composed of myofibroblasts and a mixed inflammatory infiltrate of lymphocytes, plasma cells, and macrophages.

SCLEROSING ANGIOMATOID NODULAR TRANSFORMATION OF THE SPLEEN—FACT SHEET

Clinical Features
- Average age 54 years; female predominance (2:1)
- Of patients, 16% present with abdominal pain
- May have leukocytosis, elevated erythrocyte sedimentation rate, and polyclonal gammopathy

Morphology
- Nodules composed of slit-like, round vascular spaces lined by plump endothelial cells and pericytes
- Rim of fibrosclerotic or fibrinous granulomatous tissue
- Myofibroblasts with a mixed inflammatory infiltrate within internodular regions

Immunophenotype
- Three staining patterns
  - Splenic sinusoidal $CD34^{-}CD31^{+}CD8^{+}$ endothelial cells
  - Capillary-like $CD34^{+}CD31^{+}CD8^{-}$ endothelial cells
  - Venous $CD34^{-}CD31^{+}CD8^{-}$ endothelial cells

Differential Diagnosis
- Other vascular tumors of the spleen

Prognosis and Therapy
- Indolent with no tendency for recurrence after splenectomy

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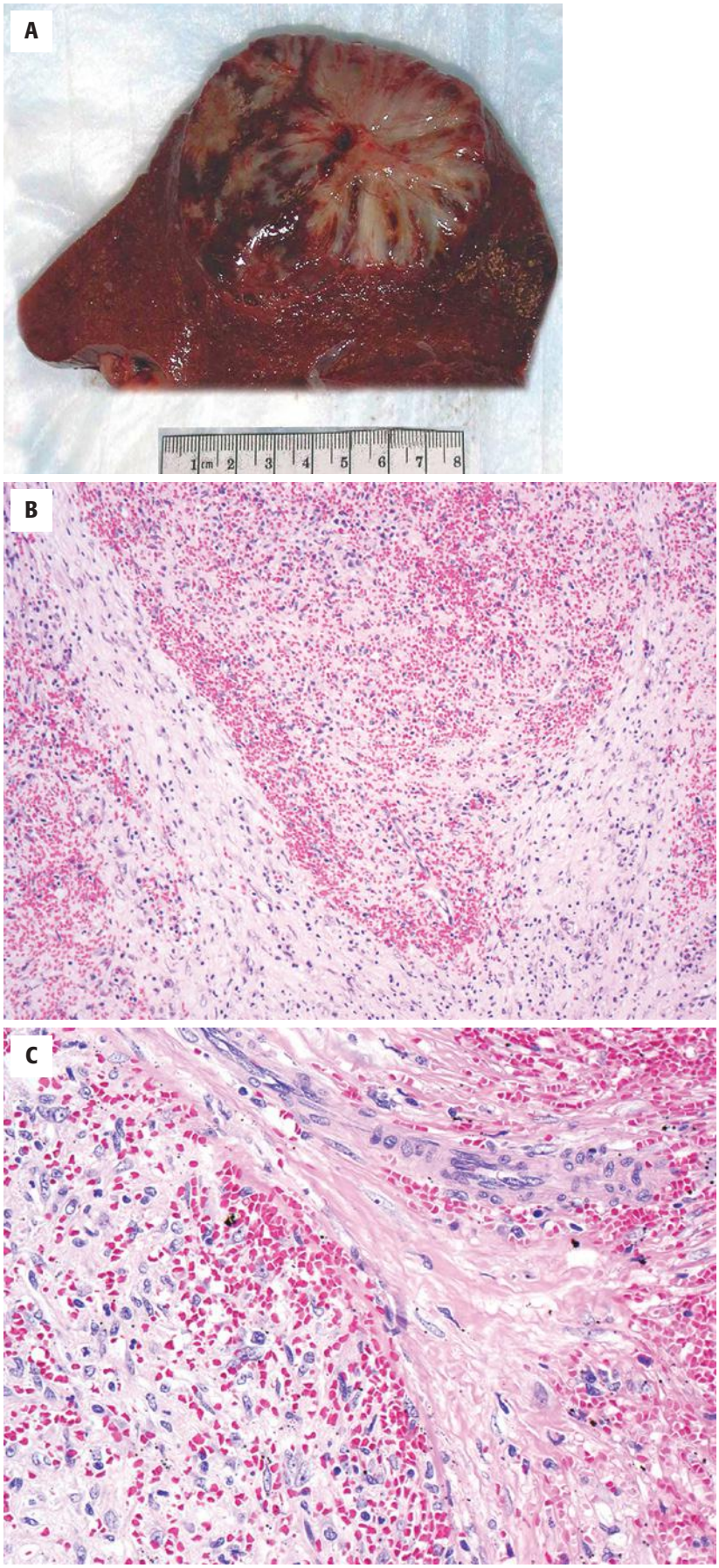


FIG. 22.13

Splenic angiomatoid nodular transformation. A, Grossly, these proliferations are distinct but lobulated. B, Microscopically, there are multiple hemorrhagic nodules with small vessels and intervening fibrosis (C) containing larger vessels. *(A, Courtesy Raj Dewar, MD, Boston, MA.)*

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The vessels of SANT show three staining patterns: a splenic sinusoidal immunophenotype with vessels lined by $CD34^{-}CD31^{+}CD8^{+}$ endothelial cells, a capillary-like immunophenotype with vessels lined by $CD34^{+}CD31^{+}CD8^{-}$ endothelial cells, and a venous immunophenotype with vessels lined by $CD34^{-}CD31^{+}CD8^{-}$ endothelium. Expression of CD68 within nodules can also be demonstrated. These staining patterns are reminiscent of normal vasculature of red pulp vessels. A background of scattered IgG4-positive plasma cells in the fibrosclerotic stroma is also seen, but SANT is not considered part of the spectrum of IgG4-related disease. EBV is not present in these lesions.

The differential diagnosis of SANT includes other vascular lesions of the spleen, nodular transformation of the splenic red pulp in response to metastatic carcinoma, and inflammatory pseudotumor. The nodular pattern with three different vessel types differentiates this proliferation from the others in the differential diagnosis. These proliferations are considered indolent, with no tendency for recurrence after splenectomy.

PELIOSIS OF THE SPLEEN

Peliosis of the spleen is a rare proliferation of dilated blood-filled cavities. It is more common in the liver, but the spleen is occasionally a site of disease. Secondary conditions, such as infections, particularly tuberculosis; malignant conditions, such as lymphomas and leukemias; and drug use as in chemotherapy can be associated with peliosis. Sections of the spleen demonstrate multiple round to oval blood-filled cysts with or without endothelial lining cells. Peliosis differs from hemangiomas or hemangiomatosis by the lack of intervening fibrosis, and in peliosis, the dilated vascular spaces are separated by normal-appearing splenic red and white pulp. Finally, peliosis may be associated with spontaneous splenic rupture.

SPLENIC HAMARTOMA

Hamartoma of the spleen is a rare benign nodular lesion with an incidence of 0.13% and equal occurrence in males and females. It appears most commonly in older patients, but up to 20% of cases can occur in children. Clinically, patients can have splenomegaly, thrombocytopenia, or other symptoms of hypersplenism; however, 50% of patients will be asymptomatic. These lesions are generally less than 3 cm in size but can reach 18 cm and form a red, bulging tumor on cut sections of the gross spleen (Fig. 22.14). On histologic analysis, they are indistinct lesions that mimic normal red pulp; they show numerous slit-like vascular channels lined by plump to flattened endothelial cells with absence of normal red pulp cord, lymphatic or organized white pulp elements. The cells lining the spaces show an immunophenotype of normal sinusoids with expression of CD8 and CD31; CD34 expression is variable. These cells lack CD21 and CD68, and

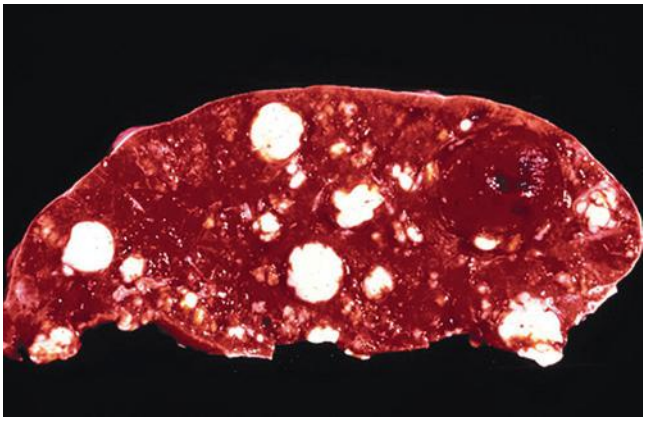


FIG. 22.14

Spleen involved by diffuse large B-cell lymphoma and hamartoma. The multiple tan-white nodules are characteristic gross features of splenic involvement by large B-cell lymphoma and classical Hodgkin lymphoma. The large, red nodule in the upper right with a bulging cut surface is the typical presentation of splenic hamartoma, which may be an incidental finding in spleens removed for other reasons.

$CD21^{+}$ follicular dendritic cell aggregates of the white pulp are typically absent within the hamartoma. The differential diagnosis includes inflammatory pseudotumor-like follicular/fibroblastic dendritic cell sarcoma, SANT, and other vascular tumors, but the lack of a distinct tumor nodule on histologic sections is a characteristic finding of splenic hamartoma. Although most cases are benign in progression, there can be a risk of rupture of large lesions. Splenectomy is the most frequent treatment of symptomatic hamartomas.

SPLENIC HAMARTOMA—FACT SHEET

Clinical Features
- Of patients, 50% are symptomatic with splenomegaly, thrombocytopenia, or other symptoms of hypersplenism

Morphology
- Indistinct lesion that mimics normal red pulp
- Numerous slit-like, vascular channels lined by plump to flattened endothelial cells
- Absence of normal red pulp cord, lymphatic or organized white pulp elements

Immunophenotype
- $CD8^{+}$, $CD31^{+}$, $CD34^{\pm}$, $CD21^{-}$, $CD68^{-}$

Prognosis and Therapy
- Generally benign, although large lesions have risk of rupture
- Splenectomy for symptomatic hamartomas

Differential Diagnosis
- Other vascular tumors of the spleen
- Inflammatory pseudotumor-like follicular dendritic cell sarcoma of the spleen

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INFLAMMATORY PSEUDOTUMOR-LIKE FOLLICULAR/FIBROBLASTIC DENDRITIC CELL SARCOMA

Splenic and hepatic inflammatory pseudotumors specifically known as inflammatory pseudotumor–like follicular/fibroblastic dendritic cell sarcomas, are unique to these locations and differ from inflammatory pseudotumors or inflammatory myofibroblastic tumors at other sites. These tumors develop as fibroinflammatory processes, often with fever and abdominal pain; a subset of cases will be associated with a concomitant malignancy either in association or in isolation. The gross appearance of the spleen demonstrates a well-defined, firm, yellow-tan lesion without capsular extension; occasionally multiple nodules are seen. Histologically, the lesion appears as a bland spindle cell myofibroblastic proliferation with interspersed variable amounts of collagenous stroma and intervening abundant inflammatory cells including lymphocytes, plasma cells, histiocytes, and eosinophils (Fig. 22.15). Foci of necrosis may be seen. Unlike inflammatory

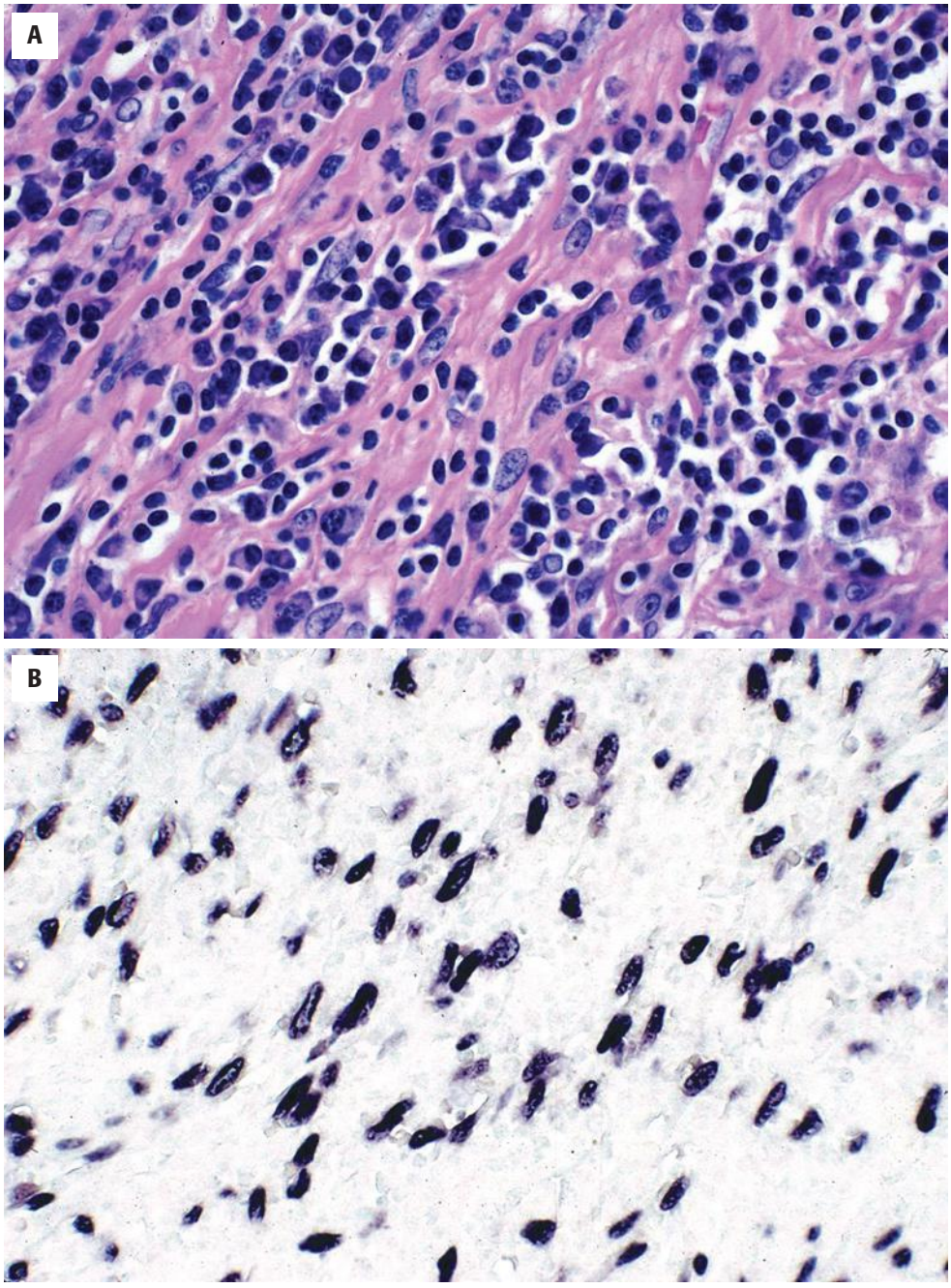


FIG. 22.15

Splenic inflammatory pseudotumor-like follicular/fibroblastic dendritic cell sarcoma. A, These tumors form distinct splenic nodules that contain a mixture of spindled cells with fibrosis, lymphocytes, and plasma cells. They may also contain histiocytes, eosinophils, and neutrophils. B, The spindled cells are positive for Epstein-Barr virus, as shown here by in situ hybridization for Epstein-Barr virus-encoded RNA 1 (EBER1), and usually also express CD21.

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INFLAMMATORY PSEUDOTUMOR–LIKE FOLLICULAR/FIBROBLASTIC DENDRITIC CELL SARCOMA—FACT SHEET

Clinical Features
- Often presents with fever and abdominal pain
- A subset of cases are associated with a concomitant malignancy

Morphology
- Numerous bland myofibroblastic spindle cells
- Interspersed collagenous stroma
- Intervening abundant inflammatory cells

Immunophenotype
- EBV^{+}, CD21^{+}, smooth muscle actin (SMA)$^{\pm}$, vimentin$^{\pm}$, S100$^{\pm}$, CD68$^{\pm}$, CD30$^{\pm}$, CD34^{-}, CD8^{-}, ALK1^{-}

Prognosis and Therapy
- Splenectomy is curative
- Partially resected tumors may recur or metastasize as follicular dendritic cell tumors

Differential Diagnosis
- Vascular tumors of the spleen
- Metastatic carcinoma

pseudotumors in other organs, the spindled cells in the spleen are positive for EBV and may express smooth muscle actin and vimentin. Spindle cells in most tumors express CD21. S100, CD68, or CD30 may be positive in some cells. CD34 and CD8 are not expressed. All cases are negative for ALK1 in contrast to inflammatory myofibroblastic tumor of soft tissue, which is generally positive for ALK1. Complete splenectomy is curative for symptomatic cases, but partial resection can lead to recurrence or metastasis as a follicular dendritic cell sarcoma.


The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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23

Flow Cytometric Principles in Hematopathology

Steven J. Kussick, MD, PhD

INTRODUCTION

The importance of immunophenotyping in diagnostic hematopathology was first emphasized by the Revised European-American Lymphoma classification, and more recently by the 2001, 2008, and 2016 World Health Organization (WHO) classifications of hematolymphoid neoplasms. In these classifications, nearly all myeloid and lymphoid malignancies are defined, at least in part, by the antigenic features of the neoplastic cells. Flow cytometry (FC) is an immunophenotyping technique in which suspensions of living cells are stained with specific, fluorescently labeled antibodies and then analyzed with a flow cytometer. In hematopathology practice, these cell suspensions are derived from blood, bone marrow, body fluids, or fresh solid tissue samples. Parallel advances in our understanding of the basic biology of hematopoietic malignancies, in the technologic attributes of flow cytometers, and in the development of reagents for assessing cellular antigen expression have allowed FC to attain particular prominence in the diagnosis of these malignancies. In addition, FC can identify prognostically relevant antigens and potential therapeutic targets in a number of diseases.

TECHNICAL CONCEPTS AND METHODS IN THE FLOW CYTOMETRIC EVALUATION OF HEMATOLYMPHOID NEOPLASMS

THE FLOW CYTOMETER

The flow cytometer consists of three main components—fluidics, optics, and electronics—in association with a desktop computer. The fluidic system includes the pumps used to aspirate the cell suspension into the cytometer and the tubing through which cells are propelled through the cytometer. After being aspirated into the cytometer, the specimen is surrounded by a stream of buffered saline (sheath fluid) introduced into the instrument at higher pressure than the specimen, such that the cells in the specimen assume a roughly single-file position because of the phenomenon of hydrodynamic focusing. The focused stream then reaches the flow cell, a quartz cuvette where the cells are illuminated by light from one or more lasers. Ultimately, the fluid stream is directed to a waste receptacle.

The optical system includes (1) the lasers used to excite the fluorescent dyes conjugated to the antibodies used in the assay, (2) the system for conveying the laser light to the flow cell, (3) the system for conveying the emitted fluorescent light from the cells to specific detectors, and (4) the detectors themselves. The detectors are usually photomultiplier tubes (PMTs) or photodiode arrays (PDAs) that convert photons to electrical impulses. The light emitted by fluorescently labeled cells is conveyed to the detectors via a combination of dichroic mirrors that allow light of defined wavelengths to pass while reflecting light of other wavelengths and optical filters that further narrow the wavelengths reaching a detector. In newer cytometers, fiber optic cables may help to convey the emitted light to the appropriate detector clusters, whereas the light travels in air in older cytometers.

The electronics system measures the electrical impulses generated by the detectors and converts these measurements to digital information that is gathered and interpreted by the analysis software. The associated computer system directly interfaces with the flow cytometer and controls its functions. In most of the newer cytometer systems, data analysis can be done either on the computer connected to the flow cytometer or on other computers that access the data via a central server (i.e., off-line analysis).

CLINICAL INDICATIONS FOR FLOW CYTOMETRY

There are four major clinical indications for FC in the evaluation of hematolymphoid neoplasia, as defined in the 2006 Bethesda International Consensus Recommendations on the Flow Cytometric Immunophenotypic Analysis of Hematolymphoid Neoplasia. First, FC is central to the diagnosis and classification of hematopoietic neoplasms, by defining cell lineage in the chronic lymphoproliferative disorders and acute leukemias, and by becoming the gold standard for identifying paroxysmal nocturnal hemoglobinuria. The 2006 consensus recommendations also recognized FC as a useful adjunct in

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the workup for myelodysplastic syndromes and myeloproliferative neoplasms, including chronic myelomonocytic leukemia. Second, as antigens associated with disease prognosis in hematopoietic neoplasms are identified (e.g., ZAP-70 and CD49d expression in chronic lymphocytic leukemia/small lymphocytic lymphoma), FC has been used to measure such antigens. Third, with the advent of therapies targeted at specific antigens in hematopoietic neoplasms (e.g., anti-CD20 therapy in most B-cell lymphomas/leukemias, anti-CD52 therapy in some T-cell lymphomas/leukemias), FC has been used to identify potential therapeutic targets. Fourth, FC has been used extensively to look for and quantify residual disease after therapy including minimal residual disease, which usually is defined as less than 0.1% involvement by neoplastic cells.

SPECIMEN REQUIREMENTS FOR FLOW CYTOMETRY

In the clinical laboratory, specimens amenable to FC include fresh, unfixed peripheral blood, bone marrow, body fluids, and finely minced solid tissue such as lymph node biopsy or spleen. For tissue and cerebrospinal fluid (CSF) specimens, immediate immersion into a tissue culture medium, such as RPMI 1640 supplemented with 10% fetal bovine serum, 1% glutamine, and 1% penicillin-streptomycin antibiotics, is required to maximize cell viability. If the tissue or CSF is likely to reach the FC laboratory in 24 hours or less, the cells may be maintained at room temperature in such medium. If delivery to the FC laboratory is likely to take more than 24 hours, or if the specimen may be exposed to relatively high ambient temperatures as during the summer months, the sample container should be shipped with a packet of wet ice inside. Cell suspensions should never be subjected to extremes of temperature (<0°C or >37°C) and should therefore never be transported on dry ice.

Peripheral blood and bone marrow aspirates must be anticoagulated before transport to the FC laboratory. The most common anticoagulants include ethylenediamine tetraacetic acid (EDTA) and heparin, both of which allow high-quality FC evaluation, although other anticoagulants such as acid citrate dextrose (ACD) and citrate do not preclude FC. During the validation of each FC assay, it is important to confirm appropriate assay performance for all anticoagulants anticipated once the assay is brought on-line. Peripheral blood, bone marrow, and non-CSF body fluid specimens are typically transported and stored at room temperature.

SPECIMEN PROCESSING

Universal biohazard precautions should be taken with all specimens in the FC laboratory. Tissue that is minced to create cell suspensions should be filtered through a 40-μM or 60-μM cell strainer to remove large particles that may clog the cytometer tubing and/or bind fluorescently-labeled antibodies non-specifically. After specimens have been incubated with antibodies and washed, but before they are analyzed on the flow cytometer, they should be fixed in a dilute solution of formaldehyde or paraformaldehyde, which stabilizes antigen-antibody interactions by introducing cross-links, and also inactivates infectious agents. Adding bleach to the instrument's waste container at a final concentration of 10% and daily purging of the fluidic system with 10% bleach at the time of instrument shutdown further minimize biohazard risks.

A zero-tolerance policy should exist for specimen mix-ups in the FC laboratory to minimize the chance that incorrect patient data are reported. Procedures to minimize the possibility of mix-ups can include fastidious labeling of specimen containers and associated paperwork, double-checking of all specimens at multiple points during processing, and separate processing of specimens to minimize the risk of cross-contamination.

In any specimen containing a large amount of peripheral blood, the erythrocytes should be removed before introducing the specimen into the flow cytometer. Most clinical FC laboratories lyse the red blood cells at some point during specimen processing, using either a commercially available reagent or a homemade ammonium chloride solution. In many laboratories, antibodies are incubated with the cells in the presence of erythrocytes (the so-called whole blood technique). After incubation, the erythrocytes are lysed and the bound antibodies are fixed to the cells of interest at the end of processing, just before the cell suspension is evaluated on the flow cytometer. An alternative method is up-front bulk lysis of the red blood cells, which is less time efficient than the whole blood method and has the potential to lyse some leukocytes in addition to erythrocytes. Up-front bulk lysis is best avoided in specimens with small numbers of leukocytes, such as CSF and scanty tissue biopsy specimens. Note that erythrocyte lysis techniques, when applied to bone marrow specimens, destroy the great majority of the nucleated erythroid precursors, compromising the ability to evaluate these cells.

The majority of antigens currently evaluated in clinical FC are cell surface associated, but nuclear antigens (e.g., terminal deoxynucleotidyl transferase [TdT] in lymphoblasts) or cytoplasmic antigens (e.g., myeloperoxidase in myeloid blasts) also can be evaluated by FC. Table 23.1 contains a list of antigens commonly evaluated in the workup for hematolymphoid neoplasia. When both cell surface and cytoplasmic antigens are evaluated in the same assay, the surface staining is performed first. The cells are then fixed and permeabilized with reagents for these purposes and then stained for the cytoplasmic antigens.

INSTRUMENT CONFIGURATION AND QUALITY CONTROL

The type of flow cytometer in the laboratory dictates the number of antigens evaluated simultaneously. Single-laser flow cytometers can evaluate three to five antigens simultaneously in addition to the generic light-scatter properties of forward scatter (proportional to cell size) and side scatter

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TABLE 23.1
Antigens Commonly Evaluated in Diagnostic Flow Cytometry

Antigen	Key Normal Hematopoietic Cell Type
CD1a	Immature T cells (common thymocytes), Langerhans cells
CD2	Pan–T cell/NK cell
CD3 (cytoplasmic)	Pan–T cell (mature and immature)/NK cell
CD3 (surface)	Pan–T cell (mainly mature forms)
CD4	Helper/inducer T cells
CD5	Pan–T cell, subset of B cells
CD7	Pan–T cell/NK cell
CD8	Cytotoxic/suppressor T cells
CD9	B cells, platelets, and megakaryocytes
CD10	Immature and germinal center B cells, neutrophils, follicular helper T cells
CD11b	Maturing and mature myelomonocytic cells, NK cells
CD11c	Maturing and mature myelomonocytic cells, myeloid dendritic cells, some B cells
CD13	Pan–myeloid cells
CD14	Maturing and mature monocytes, mature neutrophils
CD15	Maturing and mature myelomonocytic cells
CD16	Later stage neutrophil series cells, NK cells, some monocytes
CD19	Pan–B cell (including immature forms and plasma cells)
CD20	Maturing and mature B cells
CD22	Maturing and mature B cells
CD23	Activated B cells
CD25	Activated T and B cells
CD30	Activated T and B cells
CD33	Pan–myeloid cells
CD34	Myeloid and lymphoid blasts/progenitors and even earlier hematopoietic stem cells
CD36	Maturing and mature monocytes, erythroid precursors, platelets and megakaryocytes
CD38	Plasma cells, blasts, activated B and T cells, NK cells, monocytes
CD41	Platelets and megakaryocytes
CD45	Leukocyte common antigen (all hematopoietic cells except later erythroids)
CD56	NK cells, activated T cells
CD57	Subset of cytotoxic and suppressor T cells
CD61	Platelets and megakaryocytes
CD64	Monocytes, immature granulocytes, activated neutrophils
CD71	Erythroid precursors, most proliferating cells of any type
CD103	Enteric T cells
CD117/c-kit	Myeloid blasts/progenitors, promyelocytes, proerythroblasts, mast cells
CD123	Basophils, plasmacytoid dendritic cells, some blasts
CD133	Immature progenitor cells
CD200	Subsets of mature B and T cells, including almost all CLL/SLL, but only rare mantle cell lymphoma
BCL-2	Most mature B cells except germinal center B cells, most T cells
HLA-DR	Myeloid blasts, all B cells, activated T cells, monocytes, dendritic cells
FMC7	Variety of mature B cells (a CD20 epitope)
κ Light chain	Mature B cells, plasma cells
λ Light chain	Mature B cells, plasma cells
Myeloperoxidase (cytoplasmic)	Mature and immature granulocytes and some myeloid blasts
TdT (nuclear)	Immature B and T cells
Zap-70	T and NK cells

NK, Natural killer; *TdT,* terminal deoxynucleotidyl transferase.

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(also known as *orthogonal* or *90-degree light scatter* and proportional to cytoplasmic abundance or granularity). Two-laser instruments can usually evaluate six to eight antigens simultaneously, depending on the detector configuration, whereas three-laser instruments are typically required to evaluate nine or ten antigens simultaneously. The feasibility of nine- and ten-color FC for leukemia–lymphoma immunophenotyping has been demonstrated, and this technology is currently being used by a growing number of clinical laboratories. The simultaneous assessment of such a large number of antigens minimizes the number of tubes of cells and antibodies that must be set up, and it is of particular benefit for the analysis of scanty specimens. In addition to saving technologist time, which is typically the most valuable commodity in the FC laboratory, nine- and ten-color FC offers additional cost savings by minimizing redundancy of antibody and other reagent usage across tubes and by maximizing the efficiency of flow cytometer use.

Regardless of the type of flow cytometer, quality control (QC) measures must be performed and documented on a daily, weekly, and monthly basis to ensure optimal instrument performance. In our FC laboratory, daily QC usually employs brightly fluorescent, 4- to 6-μM plastic microbeads to help ensure that the voltages allotted to the individual detectors are adequate to detect the expected level of fluorescence and to confirm adequate laser power and alignment. The overriding principle for optimizing detector voltage is to maximize the signal-to-noise ratio for that detector. A second daily QC function in our laboratory is a standard nine-color T-cell analysis assay on a commercially available, well-characterized, stabilized whole blood preparation, and confirmation that the proportions of the various cell populations fall within the published ranges for the preparation. Less frequent QC measures include (1) confirming linearity of detectable fluorescence by using a series of microbeads having known fluorescence properties ranging from negative to very bright; (2) confirming the reproducibility of fluorescence in multiple replicate assays of a single specimen; and (3) confirming an acceptably low level of specimen carryover from one tube to the next. Less than 0.1% carryover should be sought for standard FC assays, and less than 0.01% carryover for minimal residual disease assays, which is usually achievable if an adequate amount of blank sheath fluid is run through the cytometer between the collection of individual tubes of cells and antibodies. Annual or semiannual preventive maintenance by a service representative of the cytometer manufacturer should be performed and documented.

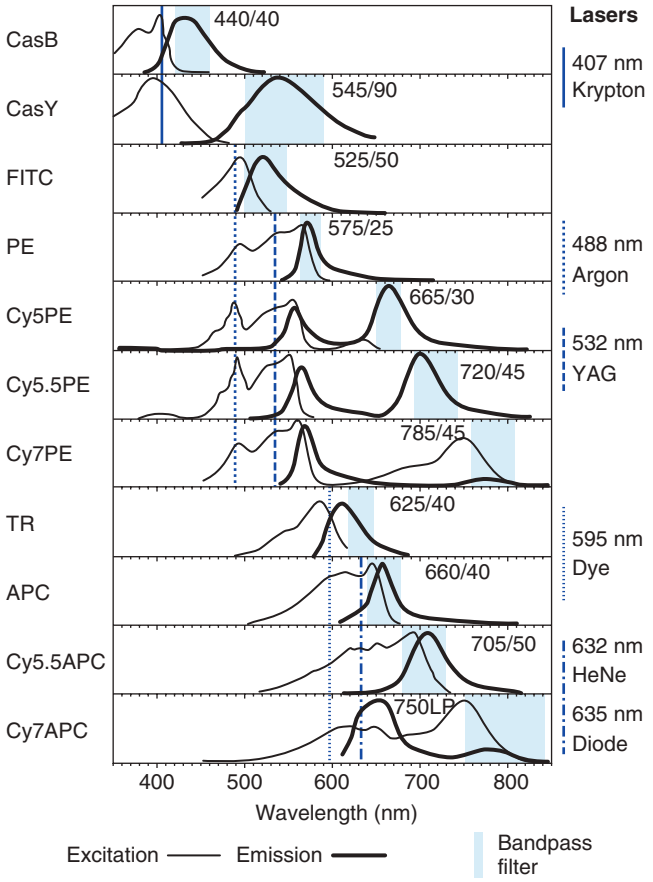


FIG. 23.1

Emission spectra of a range of fluorochromes (fluorophores) excited by violet (407 nm), blue (488 nm), green (532 nm), yellow (595 nm), and red (632 to 635 nm) lasers. The horizontal axis represents wavelength. The vertical lines along the right of the figure depict the wavelengths of commonly used lasers (shown on horizontal axis). For each fluorochrome (shown on vertical axis), the curve on the left represents the excitation/absorption spectrum, the darker and thicker curve on the right represents the emission spectrum, and the blue shaded region represents the width of the emission spectrum commonly detected by the bandpass filter targeting that fluorochrome. The variable overlap of the emission spectra (i.e., spillover), particularly among adjacent fluorochromes, necessitates the analytical technique of compensation. *(From Baumgarth N, Roederer M: A practical approach to multicolor flow cytometry for immunophenotyping,* J Immunol Methods *243:77-97, 2000.)*

ANTIBODIES: COMPENSATION AND PANEL DESIGN

Each of the fluorochromes used in FC has a well-characterized absorption and emission spectrum that extends over a range of wavelengths (Fig. 23.1). The simultaneous use of multiple antibodies conjugated to different fluorochromes therefore results in some degree of spillover, in which a portion of the fluorescence from a given fluorochrome is detected by a detector targeted for a different fluorochrome. As a result of spillover, the fluorescence detected by each detector actually represents the sum of the fluorescence from multiple fluorochromes. The majority of detected fluorescence almost always comes from the fluorochrome the detector was designed to detect, but significant contributions may come from other fluorochromes because of spillover. To adjust for spillover, a mathematical correction known as *compensation* (or *color compensation*) is applied routinely to all multiparametric FC data. As more antibodies are used in individual tubes (e.g., 6- to 10-color analysis), the potential for compensation artifacts increases. A detailed discussion of compensation is beyond the scope of this chapter, but it is important to recognize that proper compensation is another critical QC function in the FC laboratory.

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TABLE 23.2

Useful Antigens in the Evaluation of Specific Hematopoietic Cell Populations by FC

Myeloid Blast/ Progenitor	Granulocyte and Monocyte	Immature B Cell	Mature B Cell	Plasma Cell	Immature T Cell	Mature T/NK Cell
CD13	CD4	CD19[a]	CD19[a]	CD19[a]	CD1a	CD2
CD33[a]	CD10	CD10	CD20[a]	CD20[a]	CD2	CD3
CD34	CD11b	CD20[a]	CD10	CD38[a]	CD3	CD4
CD38[a]	CD13	CD22[a]	CD38[a]	CD45[a]	CD4	CD5
CD45[a]	CD14[b]	CD34	CD45[a]	CD138	CD5	CD7
CD117	CD15	CD38[a]	κ	CD56[c]	CD7	CD8
HLA-DR	CD16[b]	CD45[a]	λ	CD117[c]	CD8	CD10[e]
CD2[c]	CD24[b]	HLA-DR	CD5[c]	Cyκ[d]	CD10	CD16
CD5[c]	CD33[a]	κ	CD11c[e]	Cyλ[d]	CD34	CD45[a]
CD7[c]	CD36	λ	CD22[c,e]		CD38[a]	CD56
CD11b[c]	CD38[a]	TdT[d]	CD23[e]		CD45[a]	TCR-b[e]
CD15[c]	CD45[a]	CD13[c]	CD25[e]		TdT[d]	KIRs[e]
CD19[c]	CD64	CD33[c]	CD103[c,e]		CD13[c]	CD25[a]
CD56[c]	HLA-DR	CyCD79a[d]	CD200[e]		CD33[c]	CD30[a]
CyMPO[d]	CD56[c]		FMC7[e]		CD117[c]	CD52[a]
			CyZAP-70[c,d]		CyCD3[d]	CD279/PD-1[e]
			CyBCL-2[d]			

[a]Denotes antigens of potential therapeutic value.
[b]Denotes glycosylphosphatidylinositol-linked antigens useful in evaluation for paroxysmal nocturnal hemoglobinuria, in addition to CD59 evaluation on erythrocytes.
[c]Denotes aberrantly expressed, nonlineage antigens.
[d]Denotes intracellular antigens assessed in permeabilized cells.
[e]Denotes additional antigens to help classify mature lymphoid neoplasms.

In clinical FC, antibodies are typically used in defined combinations, or panels, to answer specific questions about specific cell populations. Most laboratories performing leukemia–lymphoma immunophenotyping have an acute leukemia panel to distinguish acute myeloid leukemia from acute lymphoid leukemia, and a lymphoma panel to distinguish benign from malignant lymphoid proliferations. At a minimum, antibody panels should measure sufficient antigens to distinguish normal–benign from abnormal–neoplastic cell populations with a high degree of sensitivity and specificity. See Table 23.2 for a list of antigens important in the evaluation of specific hematopoietic cell populations, many of which are included in the published recommendations from the 2006 Bethesda International Consensus Conference. For detailed discussion on FC assay validation, see the 2013 series of consensus documents published jointly by the International Council for Standardization of Haematology and the International Clinical Cytometry Society.

With the expanded utilization of targeted antibody therapy against cell lineage-associated surface antigens, it is important to remember that therapeutic antibodies often prevent diagnostic FC antibodies from recognizing their cognate antigens. For example, is it very common for clinical FC laboratories to receive samples from B cell non-Hodgkin lymphoma patients previously treated with anti-CD20 antibody therapy, such as rituximab. This prior therapy creates the possibility that a neoplastic B cell population will not be identified by an anti-CD20 FC antibody due to masking of the antigen by the therapeutic antigen, or due to selection of a CD20-negative neoplastic subclone. When one designs a new FC assay, it is therefore imperative to be aware of potential targeted therapies that may increase the likelihood of a false-negative result, and to design the assay to minimize the possibility of a false-negative, e.g., by adding anti-CD19 and anti-CD22 antibodies to a B cell-directed assay.

The quality of FC data not only depends on the specific antigens evaluated, but also on the specific fluorochromes conjugated to specific antibodies and on the ways in which antibodies are used together. For example, when a weakly expressed antigen is sought, such as an aberrantly expressed lymphoid antigen on myeloid blasts, conjugation of the relevant antibody to a bright fluorochrome, such as phycoerythrin, can maximize the chance of detecting the antigen. Conversely, it is often unwise to use a bright fluorochrome to detect a strongly expressed antigen, because the bright fluorescence emission from this strategy will likely create compensation problems resulting from spillover.

CLINICAL FC TEST ORDERING

When a specimen is received in the FC laboratory, the pathologist or FC technologist must consider the underlying clinical question in deciding which antibodies and panels to use in the evaluation. When the clinical question is limited, such as whether a bone marrow aspirate from a patient with known B-cell non-Hodgkin lymphoma has evidence of disease, then it is appropriate to perform a limited study focused primarily on the cell population in question, such as the mature B cells. If such a limited evaluation is to be performed, it is

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important to have an antibody to the leukocyte common antigen CD45 in the assay, so that unexpected expansions of cell populations warranting additional FC evaluation, such as blasts or monocytes, will be recognized. When the clinical question is much broader, for example, ruling out a hematolymphoid neoplasm in the bone marrow of a patient with pancytopenia and no known malignancy, then a broader evaluation of the myeloid, lymphoid, and plasmacytic lineages is likely to be appropriate. If there is clinical concern for a lymphoproliferative disorder in a patient without a prior diagnosis, it is prudent to evaluate the B, T, and natural killer (NK) cells whenever possible. However, because B-cell lymphomas are much more common than T-cell or NK-cell lymphomas (particularly in Western patient populations), it is reasonable to rule out a B-cell malignancy before evaluating the T cells if a limited amount of specimen is available.

DATA ACQUISITION

The overall data collection process by which antibody-stained cells are propelled through the flow cytometer, illuminated by the lasers, and detected by the optical system is known as *acquisition*. The number of cells (also known as *events*) required for evaluation depends on both the purpose of the flow cytometric assay and on the nature of the specimen. For example, in the evaluation of a lymph node that is replaced by lymphoma, a relatively low number of viable cells (e.g., 10,000) can be acquired per aliquot (or tube) of cells and antibodies, because this relatively low number of cells will allow adequate characterization of the neoplastic population. In contrast, the characterization of a low-frequency cell population (e.g., myeloid blasts in most bone marrow specimens) requires a much larger number of viable cells (e.g., 100,000) to be collected. In our FC laboratory, if adequate viable leukocytes are available, then we routinely collect at least 100,000 viable leukocytes per tube for initial diagnostic specimens. On the other hand, when we look for minimal residual disease in blood or bone marrow, we make every effort to collect 500,000 to 1,000,000 viable leukocytes depending on the specific assay, to increase the sensitivity of the assay. Assuming that the presence of 50 neoplastic cells in an aliquot will enable confident identification of this population, the evaluation of 500,000 total viable cells offers the ability to detect the neoplastic population at a frequency of 1 in 10,000 cells, or 0.01%.

DATA ANALYSIS

FC data analysis requires specialized computer programs designed for this purpose. Such programs are available for both PC and Macintosh platforms, and they ideally permit adequate evaluation of both QC and specimen data. At a minimum, such programs should be able to perform compensation, generate two-dimensional histograms (also known as *two-parameter dot plots* or *scatter plots*) of the data, and enumerate the various cell populations of interest for reporting purposes.

The process of targeting the analysis to the cell populations of interest is known as *gating*, described in detail in Figure 23.2. In our own laboratory, the gating of FC data always proceeds as follows. First, assess the quality of specimen acquisition via the time parameter (Fig. 23.2A). Second, exclude multicell aggregates (two-cell doublets and higher order aggregates) from the analysis (Fig. 23.2B), because flow cytometric evaluation must be restricted to individual leukocytes (i.e., singlets). Third, exclude nonviable cells from the analysis. Because nonviable cells typically have a marked decrease in forward scatter (FS) because of membrane damage from cellular degeneration, FS-versus–side scatter (SS) gating (see Fig. 23.2C) is a simple and effective way to exclude nonviable cells. FS-versus-SS gating also excludes many nonlysed erythrocytes because of their small size. An alternative method for removing nonviable cells from the analysis is the addition of a DNA-binding dye, such as 7-aminoactinomycin D (i.e., 7-AAD, which is well excited by 488-nm lasers) or 4′,6-diamidino-2-phenylindole, dihydrochloride (i.e., DAPI, which is well excited by 405-nm lasers), to the cell suspension just before acquisition on the flow cytometer. These dyes penetrate the damaged plasma membranes of nonviable cells but are excluded from viable cells. Cells demonstrating the characteristic fluorescence of these dyes are presumed to be dead or dying and are excluded from further evaluation by gating (Fig. 23.2D). Fourth, separate the cells according to broad cell type. Because viable lymphocytes, monocytes, and granulocytes typically show reproducible differences in their combined FS and SS characteristics, FS-versus-SS gating can be used effectively to separate these three cell populations in peripheral blood. This gating strategy becomes less effective when applied to bone marrow, because one particularly important bone marrow population—the blasts/progenitors—has FS-versus-SS characteristics that overlap both the lymphocytes and monocytes. As a result, many laboratories use CD45-versus-SS gating to separate the various bone marrow cell populations, including the blasts/progenitors (see Fig. 23.2E). The fifth gating step, which is particularly useful when looking for a mature B-cell or T-cell lymphoproliferative disorder, is lineage-specific gating (see Fig. 23.2F and G). In this gating strategy, evaluation of a pan–B-cell antigen, such as CD19, or a pan–T-cell antigen, such as CD3, restricts the analysis to these lymphoid populations. An inherent limitation of lineage-specific gating is that B- or T-cell neoplasms with aberrant loss of CD19 or CD3, respectively, will not be identified by this gating procedure. Therefore a more generic gating step, such as CD45-versus-SS gating to identify all the lymphocytes, which can then be evaluated for aberrant antigenic loss, should always be used in conjunction with lineage-specific gating. A sixth potential gating step, which we always perform on the data from our two 10-color FC tubes focussed on myeloid maturation, is to exclude unlysed erythroid precursors from the analysis (Fig. 23.2H). This step is particularly useful in the setting of significant erythroid hyperplasia in the marrow, in which invariably incomplete lysis of erythroid precursors during specimen processing can results in large numbers of early erythroid

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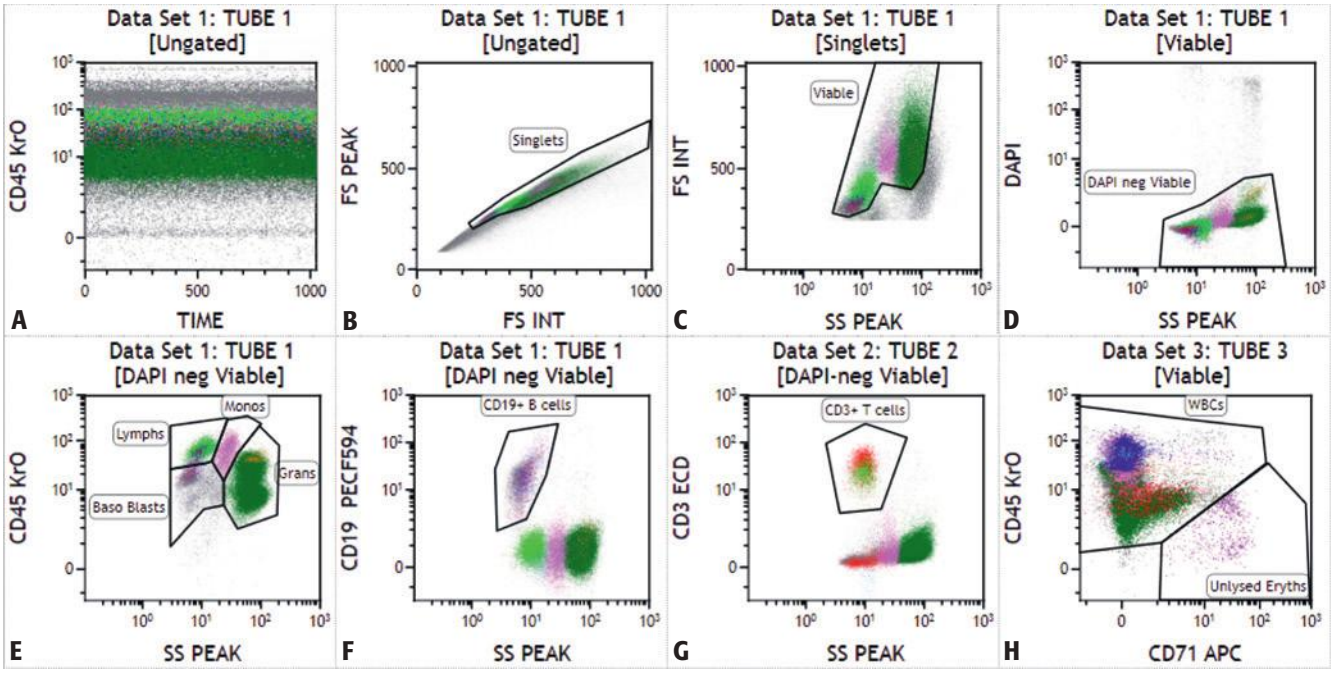


FIG. 23.2

Sample gating strategy for a complex cell mixture such as bone marrow. First, examining the ungated data according to the time of acquisition (A, with CD45 arbitrarily chosen for the vertical axis) enables the analyst to recognize an aberrancy during acquisition—such as a disruptive air bubble or clot in the fluid stream—that might adversely affect data quality; once recognized, the aberrant data can be gated out with the use of the time parameter. Second, if the flow cytometer allows concurrent measurement of both forward scatter height or peak (FS PEAK) and forward scatter area or integral (FS INT), then restricting the analysis to events on the 45-degree line defined by these two parameters, i.e., singlet gating, limits the analysis to single cells (B); for cell doublets and higher-order aggregates, the FSC-A value exceeds the FSC-H value, such that aggregates fall to the right of this 45-degree line when FSC-H and FSC-A are measured on the y and x axes, respectively. Third, after singlets are gated, FS versus side-scatter (SS) gating (C) and/or gating with a dye such as DAPI that is excluded from living cells and therefore associated with no fluorescence among such cells (D) should be used to limit the analysis to viable cells; SS may be measured on either a log or linear scale, with log SS providing a more compressed view of the data than linear SS. Subsequent CD45 vs. SS gating (E) can separate the viable hematopoietic cell populations according to general hematopoietic lineage, e.g., mature lymphocytes (Lymphs), monocytes (Monos), granulocytes (Grans), and cells in the basophil/blast area (Baso/Blasts). Among the lymphocytes, surface CD19 gating (F) can to be used to restrict the analysis to most mature and immature B-lymphoid cells, while surface CD3 gating (G) can to be used to restrict the analysis to most mature T cells and some immature forms. Importantly, neoplastic B or T cells with abnormally decreased expression of gating antigens such as CD19 and CD3, respectively, may not be identified by a single antigen gating strategy, necessitating one or more alternative gating strategies to ensure that such cells will not be overlooked. Finally, if definitive exclusion of unlysed erythroid precursors is desired, then the addition of an antibody to an erythroblast-antigen such as CD71 or CD36 can be used to exclude these precursors and restrict the analysis to the WBCs (H).

precursors, particularly proerythroblasts, contaminating the blast/progenitor gate.

A variety of antigenic abnormalities may be observed during the FC evaluation of malignant hematopoietic cell populations. These abnormalities typically contrast with the highly regular and reproducible patterns of antigen expression seen in benign hematopoietic cell populations and include: (1) abnormal increases or decreases in the levels of expression of antigens normally on the cells of interest, including complete loss of expression (e.g., aberrant loss of CD7 on the neoplastic $CD4^+$ T cells of mycosis fungoides–Sézary syndrome); (2) abnormally homogeneous expression of antigens that normally show coordinate variation in expression in a population of interest (e.g., abnormally homogeneous expression of CD34 and CD38 on myeloid blasts in myelodysplasia or acute myeloid leukemia); (3) asynchronous antigen expression in which the timing of antigen expression during a maturational process is abnormal (e.g., asynchronous expression of CD13 and CD16 during neutrophil maturation in myelodysplasia); and (4) aberrant expression of nonlineage antigens (e.g., aberrant expression of the T-cell–associated antigen CD7 on leukemic myeloid blasts). To appreciate these abnormalities, the flow cytometrist must thoroughly understand the normal patterns of antigen expression in the cell populations of interest.

Thought should be given to the numeric scale used to display each FC parameter. FS is typically presented on a linear scale. SS may be presented on a linear or a log scale, with the latter offering a relatively compressed view of the data. Antigen-associated fluorescence may be presented on a traditional four-logarithm scale or, as some authors have recommended in recent years, on a modified log scale (so-called logicle display) that does not alter the actual data values or summary statistics computed from the data. Functionally, logicle display employs a hyperbolic sine function that transforms the appearance of the compensated FC data by centering antigen-negative and low-positive populations on a linear scale around a "0" point on the relevant axis while retaining logarithmic scaling for antigen-positive populations with moderate- to high-level positivity. In many cases, logicle scaling improves one's certainty about the nature of negative- and low-positive populations.

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DATA REPORTING

Clinical leukemia/lymphoma/myeloma immunophenotyping data are typically reported in one of two ways. The less informative way is to report the percentage of cells in the population of interest that is considered positive for each of the antigens evaluated. Because such lists of antigens do not provide a unifying description of the abnormal cell populations in the specimen, a much more useful way to report FC data is to describe the detailed immunophenotype of each abnormal cell population in a free-text format in the FC report, including the proportion each population represents of the total viable cells. In describing levels of antigen expression associated with abnormal cell populations, our preferred terms are *high-level* and *low-level* instead of *bright* and *dim,* because the latter two terms describe levels of fluorescence associated with bound antibodies, which are only surrogates for actual levels of antigen expression.

In addition to describing the detailed immunophenotype of any abnormal cell populations, it is useful for clinical FC laboratories to report the following basic parameters for each specimen: (1) a CD45-versus-SS-based different count of the relevant viable leukocyte populations in the specimen (i.e., the proportions of mature lymphocytes, monocytes, granulocytes, blasts [including both immature B cells and myeloid blasts], and plasma cells); (2) among the mature lymphocytes, the proportions of B cell, T cells, and NK cells; (3) among the mature B-lymphoid cells, the kappa-to-lambda ratio, which is usually a surface kappa-to-lambda ratio for mature B cells and a cytoplasmic kappa-to-lambda ratio for plasma cells; and (4) among the T cells, the CD4-to-CD8 ratio.

B-LYMPHOID NEOPLASMS

INTRODUCTION

B-lymphoid neoplasms are best understood in the context of normal B-cell maturation in the marrow (Fig. 23.3). Precursor B-cell neoplasms correspond to the pro–B-cell and pre–B-cell, or B-lymphoblast, stages. Mature B-cell neoplasms correspond to the early–naive (pregerminal center), germinal center, and

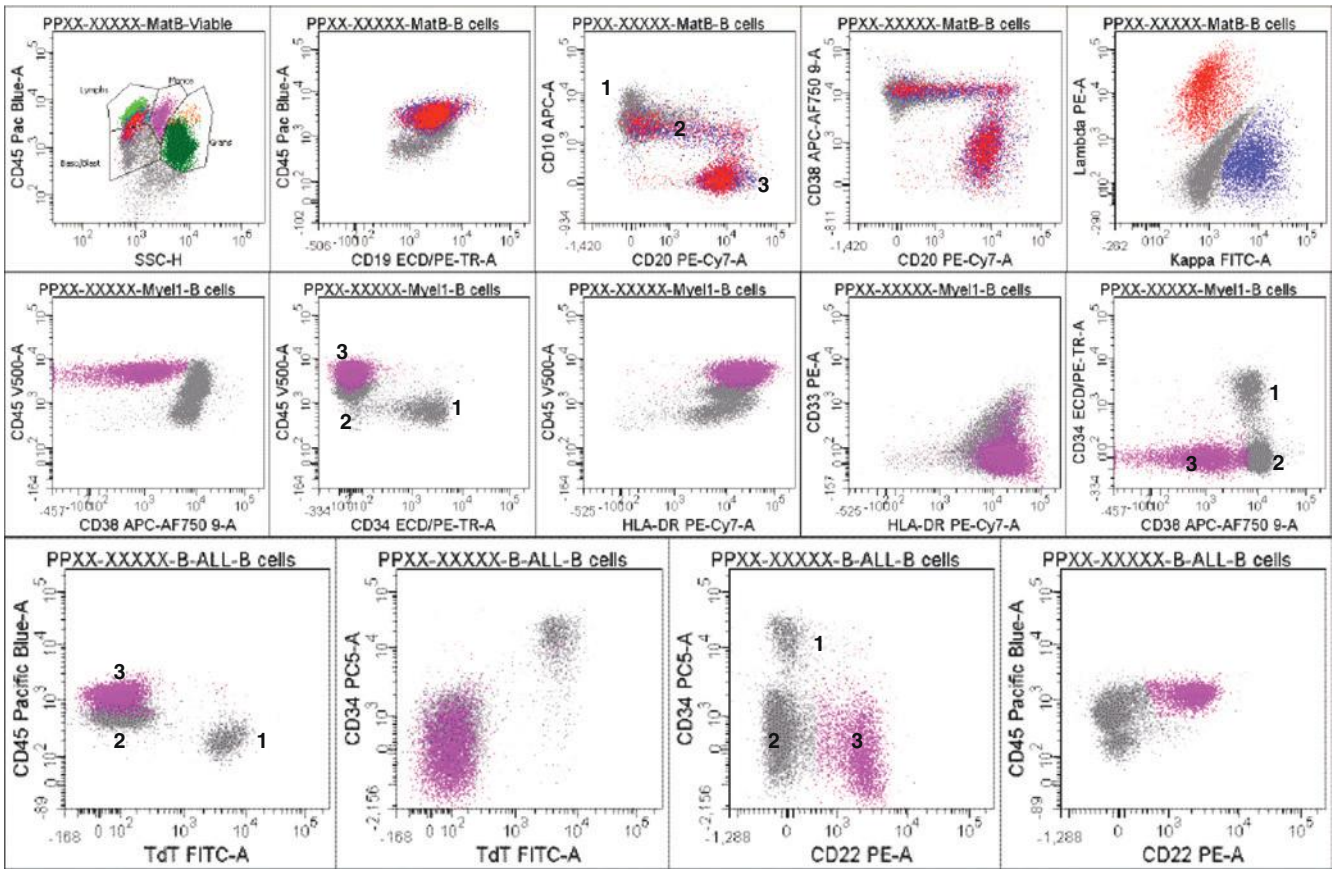


FIG. 23.3

Antigen expression during normal B-cell maturation in the bone marrow. These nine-color FC data show the three distinct phases of normal B-cell maturation. The most immature phase (population 1) is characterized by the lowest level of CD45 and CD19, no CD20, high-level CD10, intermediate-high CD38, coexpression of CD34 and terminal deoxynucleotidyl transferase (TdT), and no surface light chains. The intermediate immature phase (population 2) is characterized by intermediate-level CD45 and CD19, gradual acquisition of CD20 and CD22, intermediate-level CD10, intermediate-high CD38, no CD34 or TdT, and negative to low surface light chains. The mature, naive phase (population 3) is characterized by high-level CD45, intermediate-level CD19, CD20, low to negative CD38, either κ or λ surface light chains (colored blue and red, respectively, when shown as separate κ- or λ-expressing populations, or lavender when all the mature B cells are shown as a single population), and no CD10, CD34, or TdT. Relatively high-level HLA-DR is expressed during all three maturational phases. Maturation proceeds in an orderly fashion from population 1 to population 2 to population 3.

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postgerminal center B-cell stages. Plasma-cell neoplasms correspond to the terminal stage of B-cell maturation.

PRECURSOR B-LYMPHOID NEOPLASMS

According to the 2008 WHO classification (and 2016 update), precursor B-cell neoplasms are referred to generically as *B-lymphoblastic leukemias–lymphomas* (B-LBL); under the 2001 WHO classification, these were called *precursor B-lymphoblastic leukemias–lymphomas*. Most cases develop as leukemias involving the bone marrow and blood, and lymphomatous presentation is rare. B-LBL subtypes with a favorable prognosis include those bearing the t(12;21)(p12;q22) or hyperdiploidy with greater than 50 chromosomes. Subtypes with an unfavorable prognosis include cases having the t(9;22)(q34;q11), the t(1;19)(q23;p13), or the t(4;11)(q21;q23). Virtually all B-LBLs express the B-cell–associated antigen CD19, in addition to human leukocyte antigen (HLA)-DR and the lymphoblast-associated antigen TdT (Fig. 23.4). Most B-LBLs express CD10 (the common acute lymphoblastic leukemia antigen, or CALLA) and the blast-associated antigen CD34, and most lack surface light chains. CD45 expression is typically low to occasionally negative.

In addition to yielding prognostic information, certain karyotypes have characteristic immunophenotypes. The t(12;21) is associated with relatively low-level CD9 and no expression of the mature B-cell antigen CD20. Hyperdiploid cases frequently lack CD45. The t(9;22) is associated with aberrant expression of myeloid antigens, such as CD13 and CD33, and occasional lack of CD45. The t(1;19)(q23;p13) is associated with a lack of CD34. The t(4;11) is associated with loss of CD10 expression (CALLA-negative pre–B-ALL), lack of CD20, and frequent aberrant expression of the myeloid-associated antigen CD15.

MATURE B-CELL NEOPLASMS: GENERAL FEATURES

Almost all mature B-cell neoplasms express CD19 and CD20 at some level. Most cases show restricted cell-surface

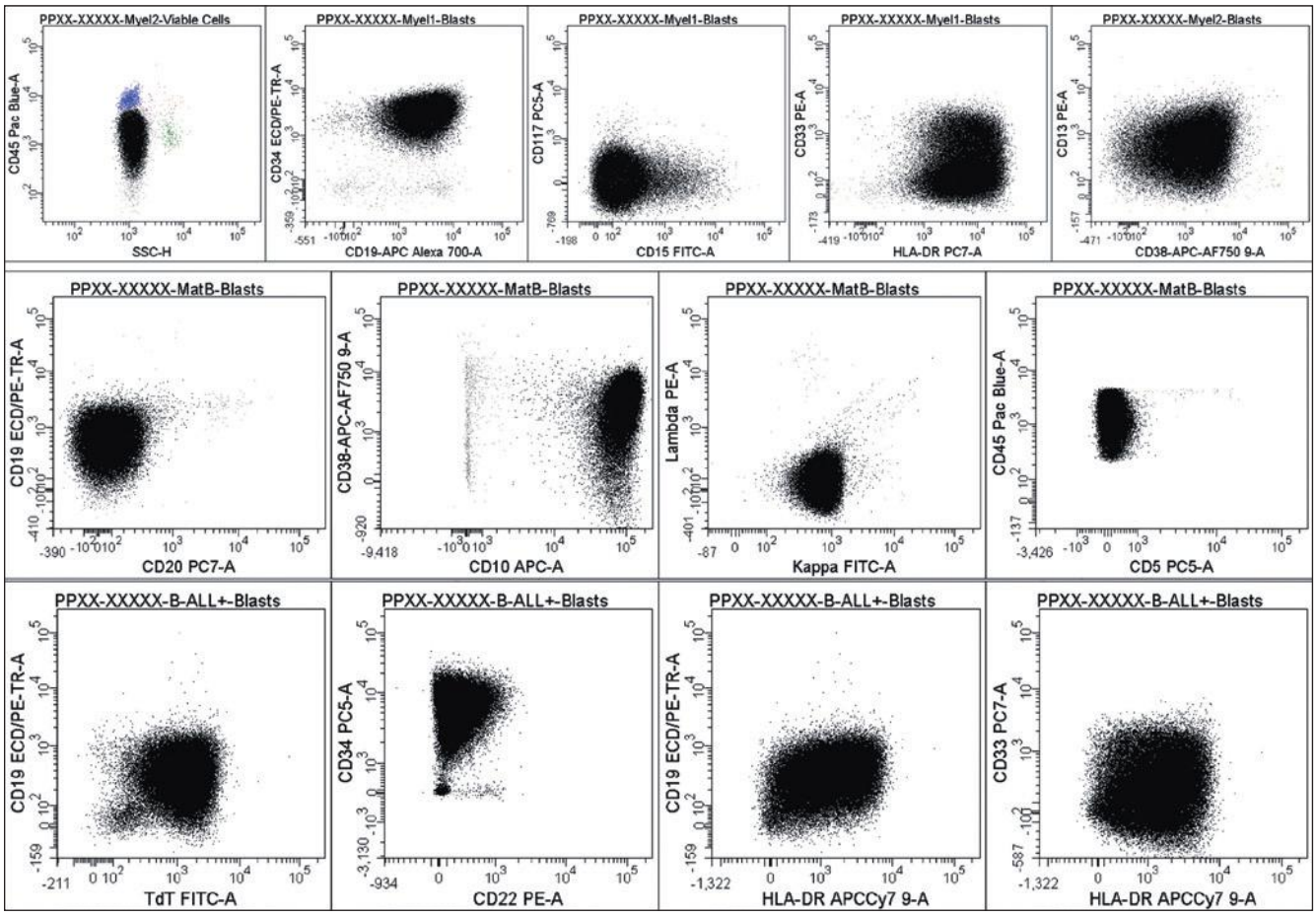


FIG. 23.4

Precursor B-lymphoblastic leukemia/lymphoma (B-ALL). These nine-color FC data show the leukemic B-lymphoblasts (colored black) to express uniform CD34 and terminal deoxynucleotidyl transferase, low CD22, abnormally low-level CD19 and CD38, abnormally high-level CD10, aberrant low-level but uniform expression of the myeloid-associated antigen CD13, aberrant low-level expression of the myeloid-associated antigens CD15 and CD33 on small subsets, and no CD20 or surface light chains. Aberrant myeloid antigen expression is relatively common in B-ALL bearing the Philadelphia chromosome, which was the genotype of this case.

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expression of κ or λ light chains, allowing clonality to be inferred easily by FC. A minority of cases demonstrate a loss of surface light chains, requiring evaluation of cytoplasmic light chains to provide formal evidence of clonality. Cytoplasmic light chain evaluation may not be essential to prove malignancy if the remainder of the surface immunophenotype is unequivocally aberrant. However, in cases with apparent loss of surface light chain expression but an otherwise normal surface immunophenotype, formal evidence of clonality should be obtained from cytoplasmic light chain evaluation, because benign B-cell populations, particularly germinal center B cells, occasionally downregulate surface light chain expression to such an extent that they appear surface light chain–negative.

CD20 is currently such an important therapeutic target in the treatment of B-cell malignancies that evaluation of its expression is required in any FC evaluation for B-cell malignancy. In patients recently treated with anti-CD20 antibodies, FC typically fails to identify a $CD20^+$ cell population, owing to the therapeutic antibody coating of CD20 on B cells. In patients remotely treated with anti-CD20 antibodies, lymphoma recurrences occasionally lack surface CD20, presumably because of the selection of a $CD20^-$ subclone following therapy. Note that rare, mature B-cell neoplasms will appear $CD20^-$ by flow cytometry using antibodies to extracellular epitopes, but $CD20^+$ by immunohistochemistry (IHC), which typically uses clone L26 against an intracellular epitope. Such cases, which likely contain alterations in the extracellular epitopes recognized by the FC antibodies, should be considered $CD20^+$ for therapeutic purposes. Therefore CD20 IHC should be performed on any available paraffin-embedded tissue for all mature B-cell neoplasms that appear $CD20^-$ by FC. Additional potential cell surface therapeutic targets in mature B-cell neoplasms include CD19, CD22, CD38, and CD52, which would mainly be evaluated as therapeutic targets in clinical trials at the present time.

CHRONIC LYMPHOCYTIC LEUKEMIA–SMALL LYMPHOCYTIC LYMPHOMA

A major feature of chronic lymphocytic leukemia–small lymphocytic lymphoma (CLL/SLL) is aberrant coexpression of the T-cell–associated antigen CD5 and T-cell–myeloid-associated antigen CD43 on the neoplastic B cells, along with the B-cell–associated antigens CD19 and CD20 (Fig. 23.5). There is usually diminished expression (compared to normal mature B cells) of a variety of mature

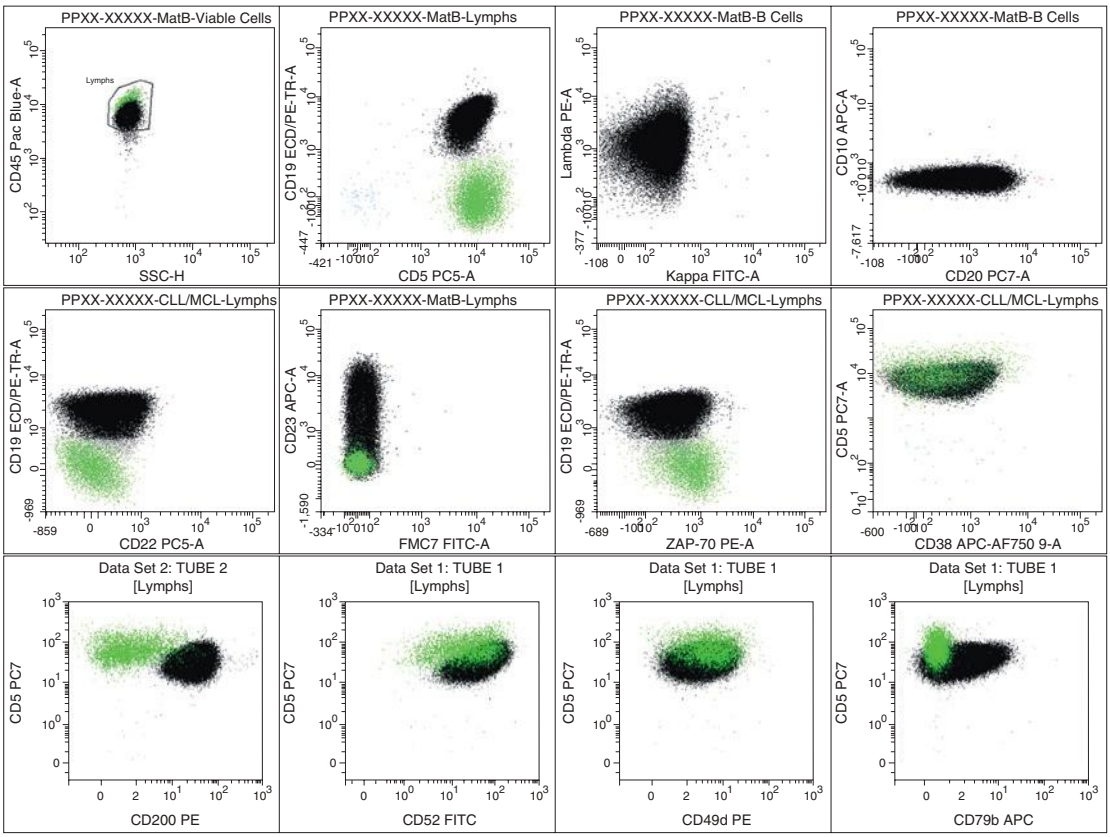


FIG. 23.5

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). *Top two rows:* These nine-color FC data show a typical CLL/SLL population (colored black) coexpressing CD19 and aberrant CD5, with low-level lambda-restricted surface light chain, low-level CD20 and CD22, variable CD23, no FMC7, and coexpression of ZAP-70 and CD38 by >20% and >30% of the population, respectively; these levels of expression of the latter two antigens are adverse prognostic markers. *Third row:* Additional eight-color data, from a different case, showing the characteristic coexpression of CD200, CD52, and low CD79b by CLL/SLL cells, plus CD49d expression by ~50% of the population, which exceeds the 30% level of expression recently associated with an adverse prognosis, independent of CD38 and ZAP-70 expression.

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B-cell–associated antigens—including surface light chains, CD20, CD22, and CD79b—but some cases show an activated phenotype with higher levels of these antigens. Historically, CLL/SLL has been regarded as a CD23-positive, FMC7-negative neoplasm (the FMC7 antigen is believed to be an epitope of CD20), but occasional cases express little or no CD23 and/or some FMC7. In our experience, demonstration of expression of a third antigen—CD200—is more important to confirm CLL/SLL, and to rule out the alternative of mantle cell lymphoma, than is assessing CD23 or FMC7 levels. Cases bearing unmutated immunoglobulin genes, an adverse prognostic feature, have been associated with expression of CD38 (by >30% of the CLL/SLL cells) or cytoplasmic ZAP-70 (by >20% of the CLL/SLL cells), or both. Importantly, recent studies suggest that expression of the cell surface integrin CD49d (by >30% of the CLL/SLL cells) may be a more powerful adverse prognostic factor than either CD38 or ZAP-70 expression. Because CLL/SLL is a common disease, occasional patients show two different CLL/SLL clones. In CLL/SLL progression, the expanded component of larger, prolymphocyte-like cells often shows a relative decrease in CD5 and CD23 expression and a relative increase in surface light chains CD20, CD22, CD79b, and FMC7.

FOLLICULAR LYMPHOMA

The majority of follicular lymphoma (FL) cases coexpress the germinal center–associated antigen CD10 in addition to CD19, CD20, restricted light chains, and cytoplasmic BCL-2 (Fig. 23.6). CD19 and CD38 are often expressed at abnormally low levels compared to normal germinal center B cells. Surface light chain expression is often brighter than in CLL/SLL, although some cases show aberrant loss of surface light chains. Low-grade follicular lymphomas (grades 1 and 2 of 3 in the WHO system) tend to show relatively small cell size by FS and SS light characteristics, whereas grade 3 follicular lymphomas tend to show a spectrum of larger cell size.

HAIRY CELL LEUKEMIA

Hairy cell leukemia (HCL) cells show increased SS because of their relatively abundant cytoplasm, high-level CD19 and CD20 compared with normal B cells, and restricted surface light chains. The key to making the diagnosis of classic HCL is identifying coexpression of CD103, CD25, and relatively high-level CD22 and CD11c (Fig. 23.7). Classic HCL also typically expresses CD123, expresses CD10 in a minority of cases, and is associated with monocytopenia. A distinct subset of HCL-like cases, formerly referred to as *hairy cell variants* and currently referred to as *splenic B-cell lymphoma, not otherwise specified* under the 2008/2016 WHO classification, typically lacks CD25 and CD123, with an otherwise HCL-like phenotype.

MANTLE CELL LYMPHOMA

Mantle cell lymphoma (MCL), like CLL/SLL, is characterized by coexpression of CD5 and CD43 on the neoplastic B cells, along with CD19, CD20, and restricted light chains. However, in contrast to CLL/SLL, MCL typically

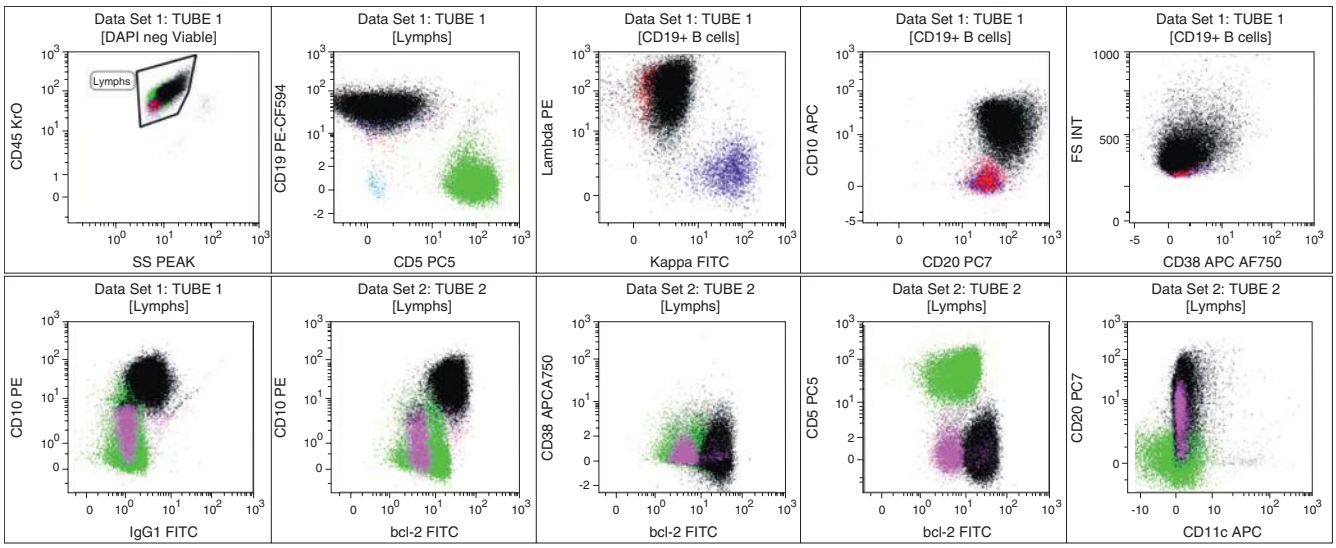


FIG. 23.6

Follicular lymphoma. The *upper row* has nine-color FC data showing coexpression of abnormally increased CD10 and decreased CD38 on the lambda-restricted, neoplastic, follicle center B cells (colored black), plus high-level CD20 and normal CD19 compared to the few background benign B cells (the latter are colored blue or red depending on whether they express kappa or lambda surface light chains, respectively). The *lower row* shows clear cytoplasmic bcl-2 overexpression by the abnormal B cells, compared to both the light green-colored T cells and the lavender-colored normal B cells. Cytoplasmic antigen evaluation is one of the few instances in which we usually run a separate isotype-matched control (an IgG1-FITC control for the bcl-2-FITC antibody, which is represented in the left-most dotplot in the second row). The lack of CD11c expression on the neoplastic B cells rules out the formal alternative of CD10-positive hairy cell leukemia.

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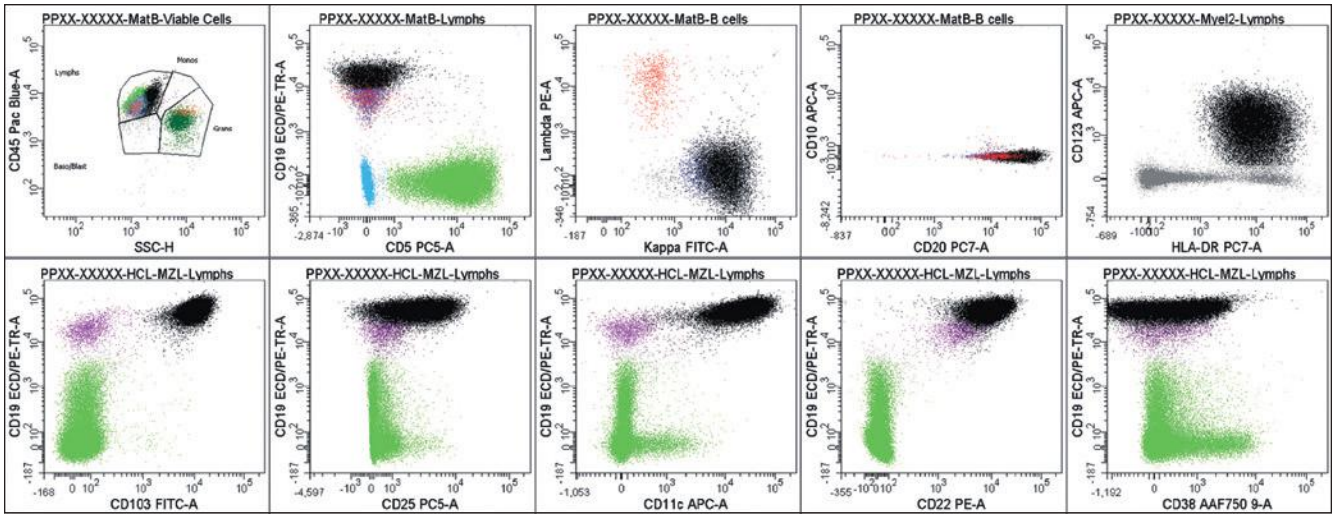


FIG. 23.7

Hairy cell leukemia. Hairy cell leukemia has a distinctive immunophenotype enabling the flow cytometric identification of small populations of these cells (<0.01% in some cases). These nine-color FC data show the neoplastic B cells (colored black) with the characteristic increase in side scatter (corresponding to the increased cytoplasm in the cells), expression of high-level CD19, CD20, CD22, and CD11c, and the disease-defining coexpression of CD103 and CD25. CD123 expression is also characteristic of (classic) hairy cell leukemia. Hairy cell leukemia variant typically lacks expression of both CD25 and CD123.

shows moderate- to high-level expression of surface light chains CD20, CD22, and CD79b, with coexpression of FMC7 and little or no CD23 or CD200. Rare cases express little or no CD5 or CD43 and/or express low CD10. Flow cytometric evaluation of nuclear cyclin D1 expression, the protein overexpressed as a result of the characteristic t(11;14)(q13;q32) of MCL, is not in common clinical usage; therefore, the diagnosis of MCL is usually confirmed by cyclin D1 IHC, t(11;14) fluorescence in situ hybridization, or cytogenetics.

DIFFUSE LARGE B-CELL LYMPHOMA

Diffuse large B-cell lymphoma (DLBCL) generally shows increased cell size based on FS and SS characteristics, although degenerating or partially necrotic specimens may show an artifactual decrease of both light scatter parameters. CD19, CD20, and restricted light chains are generally expressed at moderate to high levels but can be unusually low, as with decreased CD20 in immunoblastic/plasmablastic lymphomas because of plasmacytoid differentiation. When present, CD10 expression suggests a germinal center origin of the neoplastic cells. Certain subtypes of DLBCL recognized by the WHO classification have distinctive immunophenotypes that may be identified by flow cytometry: (1) intravascular large B-cell lymphoma often shows aberrant CD5 coexpression; (2) T-cell/histiocyte–rich large B-cell lymphoma generally shows a prominent benign background T-cell population, few normal small B cells, and few if any detectable neoplastic large B cells; (3) primary mediastinal large B-cell lymphoma often expresses CD200 and/or CD23, with loss of surface HLA-DR and loss of both surface and cytoplasmic light chains; and (4) primary effusion lymphoma generally demonstrates a loss of B-cell–associated antigens, such as CD19 and CD20, but may express CD45, CD38, CD30, or restricted cytoplasmic light chains.

BURKITT LYMPHOMA

Burkitt lymphoma (BL) typically demonstrates intermediate, rather than large, cell size based on the light-scatter properties, often with a prominent nonviable population because of the very high apoptotic rate among the tumor cells. There is moderate to high-level expression of CD19, CD20, CD38, and restricted light chains, along with variable CD10 (Fig. 23.8). CD43 is aberrantly expressed in most cases. In contrast to most FLs and many DLBCLs, cytoplasmic bcl-2 is not expressed in BL.

DOUBLE HIT AGGRESSIVE B-CELL NON-HODGKIN LYMPHOMAS

Among the de novo aggressive B-cell lymphomas, (not representing high-grade transformations of under lower grade follicular lymphomas), the double-hit lymphomas (DHLs) were first recognized by the 2008 WHO classification. In this classification, the DHLs were mainly classified as either DLBCL or B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL. Under the revised 2016 WHO classification, the DHLs will almost all be classified as high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangement. As least one study has suggested that the DHLs frequently coexpress CD19, CD10, CD38, and CD45, with significantly decreased CD20. Therefore, any morphologically aggressive B-cell lymphoma with a

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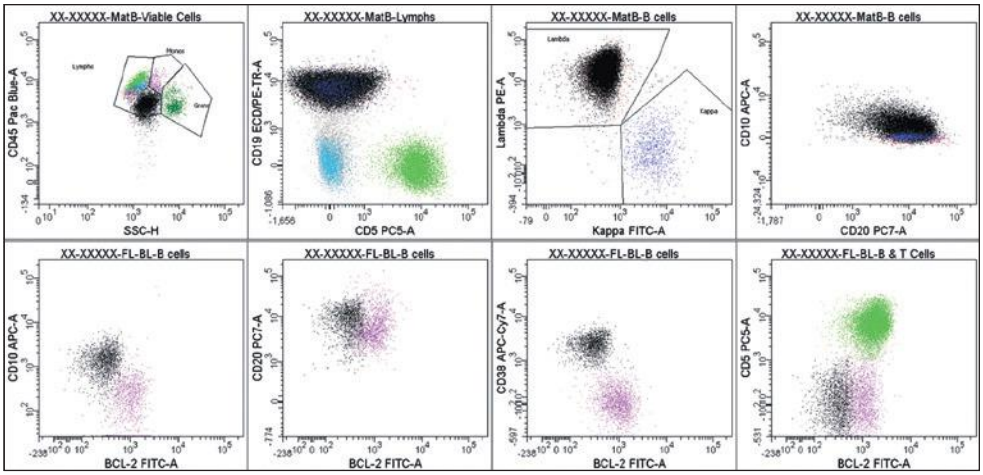


FIG. 23.8

Burkitt lymphoma. These nine-color flow cytometry data show the characteristic features of Burkitt lymphoma (neoplastic cells colored black), including abnormally decreased CD45 (for mature B cells), coexpression of CD19, CD20, CD10, CD38, and restricted surface light chains (λ), and no cytoplasmic BCL-2 in comparison to the normal B cells (colored lavender) and T cells (colored green).

similar immunophenotype should be evaluated for DHL by fluorescence in situ hybridization (FISH).

PLASMA CELL NEOPLASMS

Plasma cells characteristically express higher level CD38 than any other cell in the bone marrow, with at least some CD138 and variably increased cell size, making them relatively easy to identify by FC. In almost all cases, plasma cell neoplasms express restricted cytoplasmic light and heavy chains identifiable by FC in permeabilized specimens. In practice, many clinical flow cytometry laboratories that evaluate plasma cell clonality do so by examining light chain expression alone, relying on serum or urine immunofixation, or both, to identify the heavy chain component. In rare cases, neoplastic plasma cells express restricted surface light chains in addition to cytoplasmic light chains, or they show aberrant loss of both surface and cytoplasmic light chain expression. The majority of true plasma cell neoplasms (rather than the neoplastic plasma cells of B-cell non-Hodgkin lymphomas with plasmacytic differentiation) have abnormal surface immunophenotypes, with decreased to absent CD19, CD27, CD45, and CD81, occasionally decreased CD38, decreased or increased CD138, frequent aberrant CD56, and occasional aberrant CD117/c-kit and/or CD20 (the latter is associated with the t(11;14) involving the *CCND1* and *IGH* genes) (Fig. 23.9, upper two rows).

As anti-CD38 targeted therapy becomes more common in the treatment of plasma cell neoplasms, FC assays designed to detect neoplastic plasma cells in the post-therapy setting must include antibodies to other plasma cell associated antigens, e.g., CD138, CD229, and/or CD319. The lower row in Figure 23.9 shows a bone marrow sample from a different myeloma patient recently treated with anti-CD38 therapy, which blocks the ability of our diagnostic CD38 antibody to recognize its epitope, necessitating reliance on other assay antibodies to enable plasma cell detection without the aid of CD38.

T-LYMPHOID AND NATURAL KILLER–CELL NEOPLASMS

INTRODUCTION

T-lymphoid neoplasms, like B-lymphoid neoplasms, can be understood in the context of normal T-cell maturation, which occurs in the thymus and is illustrated by benign T cells in the setting of thymoma (Fig. 23.10A). Normal mature T cells have a virtually identical immunophenotype in the blood and bone marrow (see Fig. 23.10B). Precursor T-cell neoplasms correspond to the prothymocyte and immature, common, and mature thymocyte stages. Mature T-cell neoplasms usually derive from single-positive (i.e., $CD4^+$ or $CD8^+$) T cells expressing α-β T-cell antigen receptors (TCRs), although rare γ-δ T-cell malignancies occur. NK cells represent a lymphoid lineage distinct from T cells and, unlike T cells, do not have rearranged T-cell antigen receptors.

PRECURSOR T-CELL NEOPLASMS

According to the 2008 WHO classification (and 2016 update), precursor T-cell neoplasms are referred to generically as *T-lymphoblastic leukemias/lymphomas* (T-LBL; under the 2001 WHO classification, these were called *precursor T-lymphoblastic leukemias/lymphomas*). Compared to B-LBL, T-LBL is much more likely to present with soft-tissue involvement, particularly in the anterior mediastinum/thymus. Like B-LBL, T-LBL consists of a number of genetically distinct entities, many of which are thought to derive from different stages of early T-cell maturation; all these stages typically express TdT, CD99, and CD7. The least mature T-LBLs, which are also called early T-cell precursor (ETP) leukemias and are associated with a poor prognosis, are thought to derive from prothymocytes, which express TdT, HLA-DR, CD34, and CD7 (Fig. 23.11); ETP T-LBL is defined as having a CD1a-negative, CD8-negative immunophenotype with low or no CD5. Somewhat more mature T-LBLs may correspond

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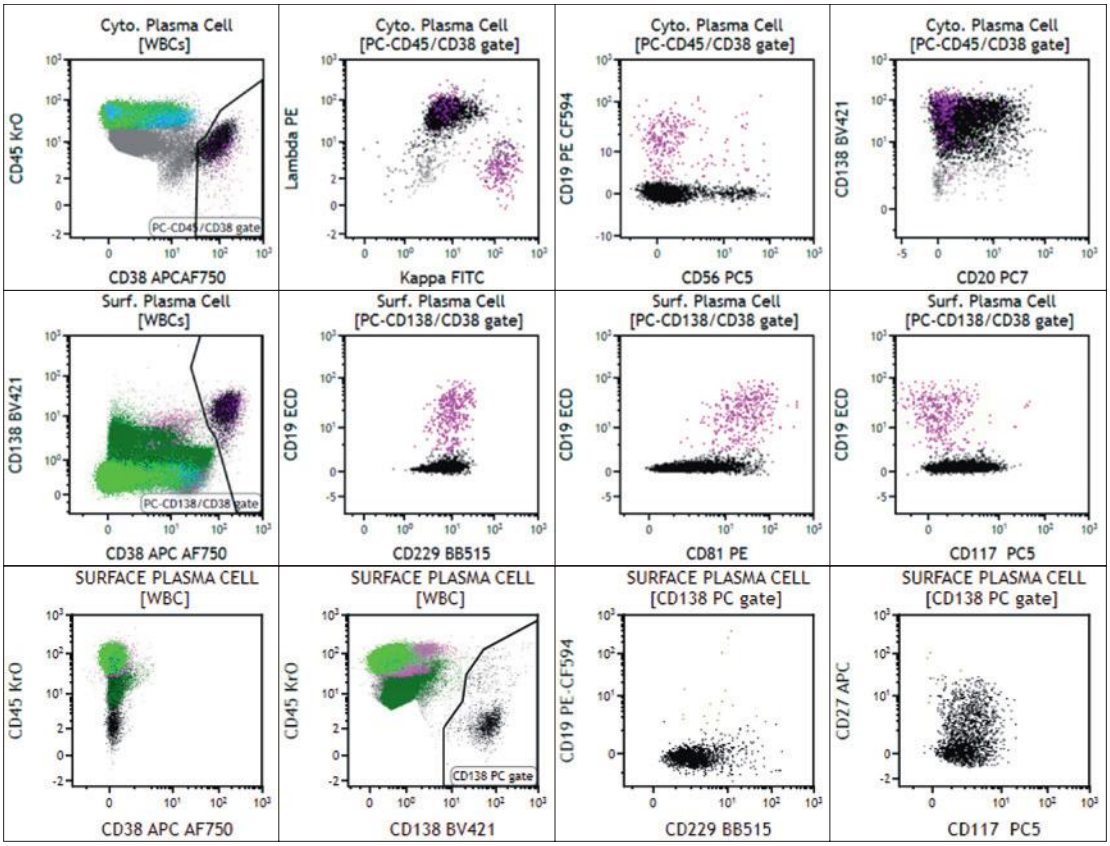


FIG. 23.9

Plasma cell myeloma. These nine-color FC data show a typical case of plasma cell myeloma, in which the high-level CD38$^+$ plasma cells can be identified by either CD45 versus CD38 gating (*upper row)* or CD138 versus CD38 gating *(middle row).* Compared to the small population of normal, polytypic plasma cells in the specimen (colored lavender), the neoplastic plasma cells (colored black) show cytoplasmic lambda light chain restriction, essentially normal/retained expression of CD45, CD38, CD138, and CD229 (the latter is an antigen that can help identify plasma cells in the setting of recent anti-CD38 therapy), abnormally decreased CD19 and CD81, and aberrant coexpression of low CD20 and CD117, plus low CD56 on a small subset. The assay shown in the upper row combines antibodies to surface antigens with antibodies to cytoplasmic kappa and lambda, whereas the assay in the lower row only evaluates surface antigens and is based on recently published consensus antigens for myeloma minimal residual disease detection; this surface-only assay also evaluates CD27 expression, which is often abnormally decreased in plasma cell neoplasms. The *lower row* shows the results of the surface-only assay performed on a low-positive bone marrow aspirate from a different myeloma patient who, unbeknownst to our laboratory, had anti-CD38 therapy shortly before the bone marrow specimen was obtained. The complete absence of CD38 immunoreactivity in the specimen almost certainly reflects antigen masking by the therapeutic antibody, such that our diagnostic anti-CD38 antibody is unable to recognize its epitope. The presence of a CD138 antibody in the surface assay, plus antibodies to both normal plasma cell-associated antigens (CD19, CD27, CD45, CD81, CD229) and aberrant plasma cell antigens (CD56, CD117), enables this assay to detect abnormal plasma cell populations with high sensitivity, even in the setting of anti-CD38 therapy. In the present case, the abnormal plasma cells (colored black in the flow histograms) show abnormally decreased CD45, CD19, CD229, and CD27, and low aberrant CD117.

to the immature thymocyte stage, at which time HLA-DR and CD34 are lost and CD2, CD5, and cytoplasmic CD3 are acquired. T-LBLs of the common thymocyte stage typically show dual expression of CD4 and CD8 and express the common thymocyte antigen CD1a at some level. Finally, occasional T-LBLs have a mature thymocyte immunophenotype, with single positivity for CD4 or CD8 and acquisition of surface CD3. The identification of immunophenotypic aberrancy among immature T cells, such as abnormally homogeneous expression of normal immature T-cell antigens or aberrant expression of non–T-cell–associated antigens, such as CD13, CD33, or CD117, can be helpful in distinguishing mediastinal T-LBL from thymoma or thymic hyperplasia, because the benign immature T cells of thymoma or thymic hyperplasia show a normal maturational spectrum of antigen expression. CD79a expression has been described in both T-LBL and benign thymocytes.

MATURE T-CELL AND NATURAL KILLER–CELL NEOPLASMS: GENERAL FEATURES

With the exception of γ-δ T-cell lymphomas and NK-cell lymphomas, the entities discussed in this section involve α-β T cells. These neoplasms all lack CD34, TdT, and CD1a. Historically, prominent abnormal increases or decreases in levels of expression of T-cell–associated surface antigens have been used as surrogates for clonality in the evaluation of T-cell neoplasms. In this approach, care must be taken to avoid overinterpreting changes in surface antigen expression, because certain benign T-cell populations show characteristic decreases in surface antigen expression. For example, both benign and malignant large granular lymphocytes of the (CD8$^+$) T-cell type characteristically show a decreased level of CD5 compared with other CD8$^+$ T cells, such that mildly decreased CD5 among a subset of the CD8$^+$ T cells is not sufficient

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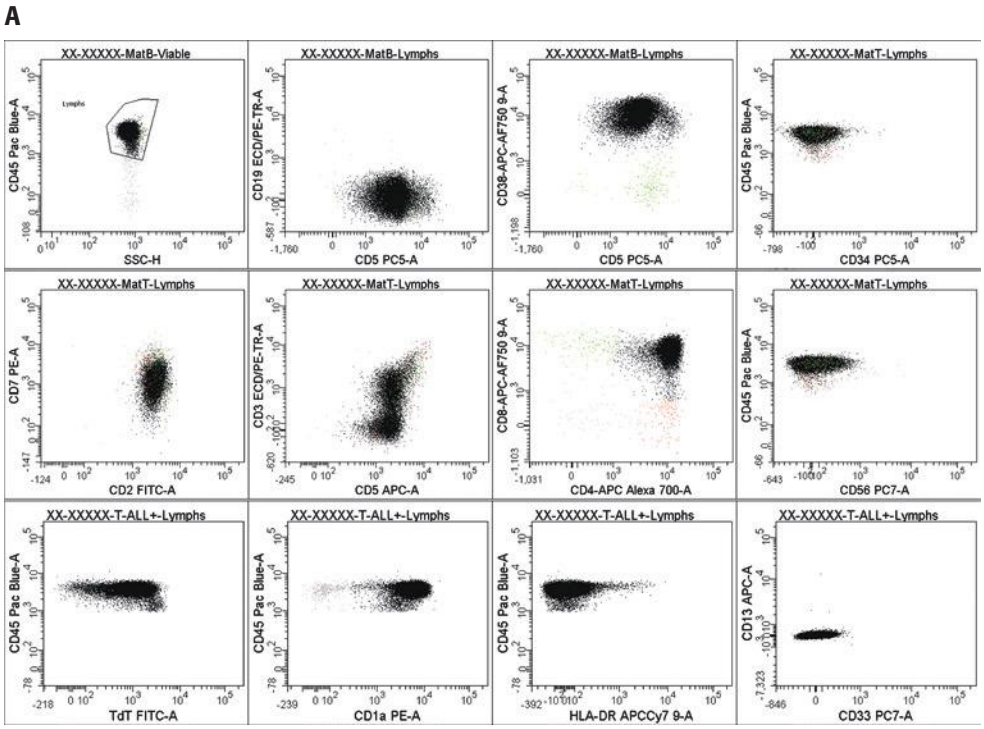


FIG. 23.10

Normal immature T cells in a case of thymoma (A) and normal mature T cells in the bone marrow (B). Although the common lymphoid stem cell arises in the bone marrow, most T-cell maturation occurs in the thymus. The nine-color FC data in A show a predominant population of normal immature T cells of common thymocyte stage (colored black) with characteristic coexpression of CD4, CD8, CD1a, and terminal deoxynucleotidyl transferase. The rare, mature, single $CD4^+$ and single $CD8^+$ T cells are colored red and green, respectively, in the middle panel. Among the normal bone marrow T cells in B, the $CD4^+$ and $CD8^+$ cells are also colored red and green, respectively. The light blue-colored cells are normal natural killer cells, and the few lavender cells are monocytes in the gate. Normal peripheral blood T and natural killer cells have immunophenotypes identical to those of their bone marrow counterparts.

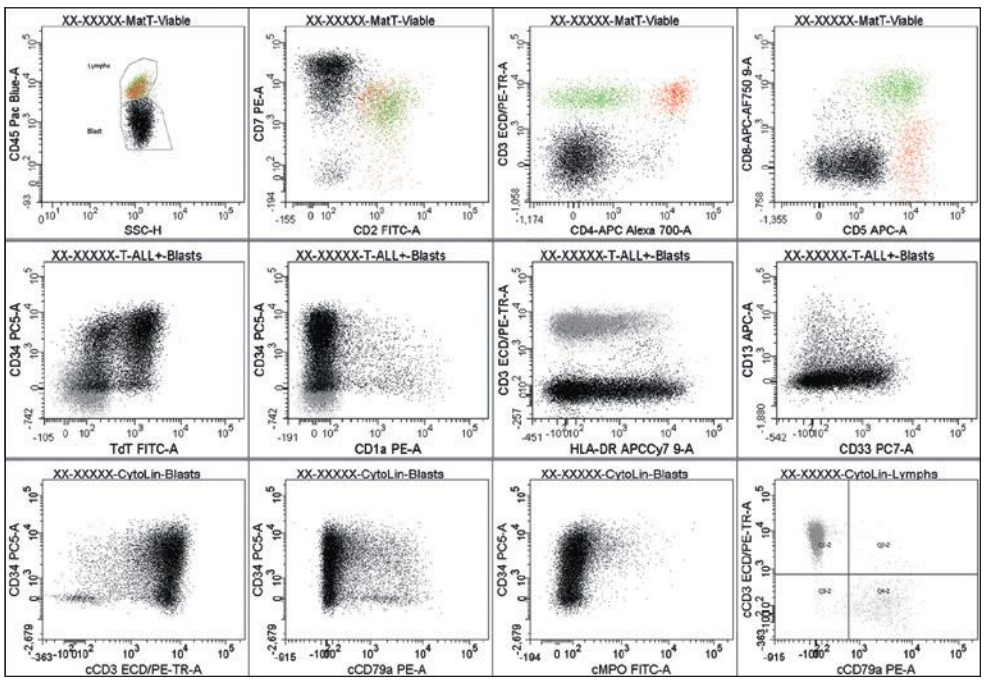


FIG. 23.11

Precursor T-lymphoblastic leukemia–lymphoma of prothymocyte stage (early T-cell precursor leukemia). Precursor T-ALL can show immunophenotypic features reminiscent of any stage during early T-cell development. In this case, the leukemic T lymphoblasts (colored black in these nine-color FC data) display a prothymocyte phenotype, with coexpression of CD34, terminal deoxynucleotidyl transferase (TdT) on a subset, cytoplasmic CD3, high-level CD7, low-level CD5, low-level aberrant CD33 on a subset, and no significant surface CD1a, CD3, CD4, or CD8.

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to make the diagnosis of T-cell large granular lymphocytic leukemia. Similarly, $CD4^+$ memory T cells characteristically show diminished CD7 expression compared with other T cells.

Antibodies to 24 different TCR-β isoforms are commercially available, enabling identification of restricted TCR-β usage in approximately 70% of neoplasms derived from α-β T cells. As a result, clonality now can be proved by FC in the majority of T-cell neoplasms. For the other 30% or so of α-β T-cell neoplasms, a lack of reactivity of the tumor cells with any of the 24 antibodies allows clonality to be inferred. Importantly, because clonal T-cell expansions can be seen in benign immunologic reactions, FC demonstration of T-cell clonality must be interpreted in the overall context of the case before a diagnosis of T-cell malignancy is made. In laboratories not using TCR-β antibodies, proof of T-cell clonality will require TCR gene polymerase chain reaction evaluation.

Note that the large majority of the mature T-cell neoplasms express some level of CD52, a potential target in salvage therapy of these tumors. Expression of CD25 and CD30, which are other potential therapeutic targets, is less common in T-cell neoplasms.

T-CELL PROLYMPHOCYTIC LEUKEMIA

T-cell prolymphocytic leukemia (T-PLL) typically involves clonal T cells expressing CD2, CD3, CD5, and CD7, although one or more of these antigens, or rarely CD45 (Fig. 23.12), may be expressed at abnormal levels. Of cases, 60% are reported to be $CD4^+$ and $CD8^-$, 25% of cases coexpress CD4 and CD8, and 15% of cases are $CD8^+$ and $CD4^-$. Coexpression of CD4 and CD8 is relatively unique to T-PLL among mature T-cell neoplasms, which helps to distinguish it from other mature T-cell neoplasms of small to intermediate cell size. Formal confirmation of the diagnosis of T-PLL can be achieved by cytogenetic or fluorescence in situ hybridization demonstration of the characteristic inv(14) or t(14;14) involving the *TCL1* gene, demonstration of a chromosome 8 abnormality, or both. *TCL1* expression is seen with IHC in more than 80% of cases and can be used to confirm the diagnosis in paraffin-embedded tissue, because *TCL1* expression in T cells is specific for T-PLL.

T-CELL LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

The majority of T-cell large granular lymphocytic leukemia cases are clonal $CD8^+$ T-cell neoplasms showing variable loss of CD5 and often decreased CD7, commonly with CD57 coexpression. Rare T-cell large granular lymphocytic leukemia variants express CD4, occasionally together with CD8. There is commonly a history of prolonged cytopenias in these patients, most commonly neutropenia, but occasionally anemia or thrombocytopenia, or both. Underlying autoimmune disorders are common, particularly rheumatoid arthritis. Occasional cases with marked anemia show pure red cell aplasia in the marrow.

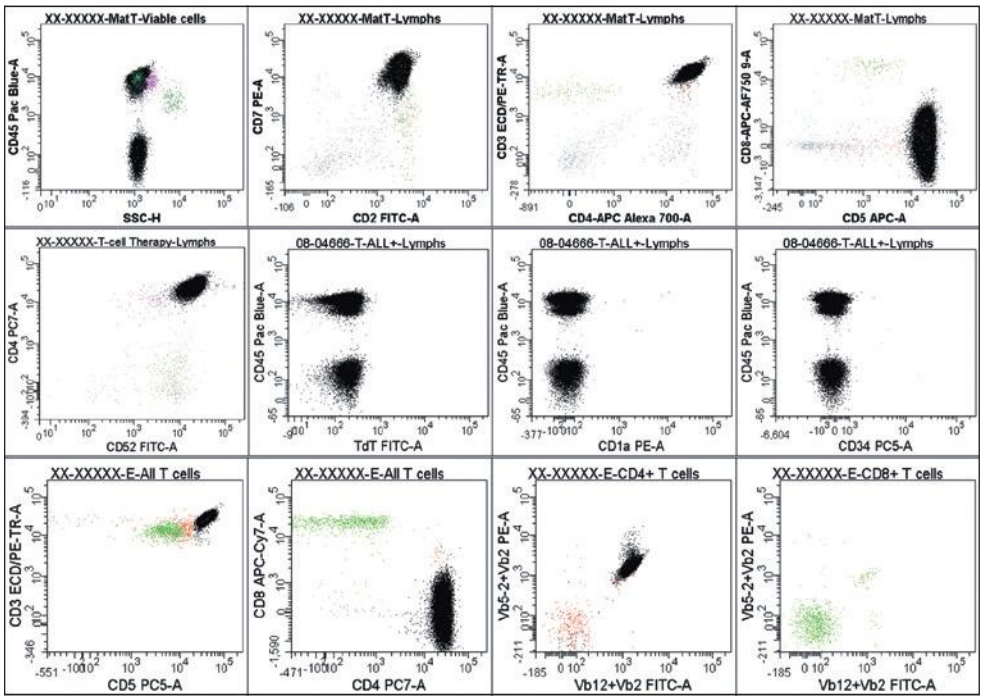


FIG. 23.12

T-cell prolymphocytic leukemia. The neoplastic $CD4^+$ T cells in this nine-color analysis (colored black) show slightly increased expression of CD3, CD5, and CD7 compared to the few normal T cells and include a discrete subset with aberrant loss of CD45. Flow cytometric evaluation of TCR-β expression showed Vβ-2 restriction (lower row of flow histograms), confirming monoclonality.

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MYCOSIS FUNGOIDES (SÉZARY SYNDROME)

The neoplastic cells in mycosis fungoides (Sézary syndrome) are usually clonal $CD4^+$ T cells showing loss of CD7 and CD26 expression and occasional increases or decreases in the levels of other pan–T-cell antigens. Rare cases express CD8. Transforming cases may express CD30.

PERIPHERAL T-CELL LYMPHOMA, UNSPECIFIED

No consistent immunophenotypic pattern exists in peripheral T-cell lymphomas (PTCLs), although most cases are clonal $CD4^+$ T-cell neoplasms, and aberrant loss or gain of one or more T-cell–associated antigens is common. Fewer cases express CD8, and rare cases coexpress CD4 and CD8, or lack expression of both CD4 and CD8. Some peripheral T-cell lymphoma cases express variable CD30.

ADULT T-CELL LEUKEMIA–LYMPHOMA

The neoplastic cells in adult T-cell leukemia–lymphoma (ATLL) are typically clonal $CD4^+$ T cells expressing CD25 (the α chain of the IL2 receptor) and showing aberrant loss of CD7. The expression of CD25, however, is not specific for ATLL. Proteins produced by the causative agent of adult T-cell leukemia–lymphoma, human T-lymphotropic virus-1 (HTLV-1), currently are not being assayed in clinical FC.

ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

The neoplastic cells in angioimmunoblastic T-cell lymphoma (AITL) are typically $CD4^+$ T cells that often demonstrate aberrant loss of surface CD3 or CD7 expression, or both, with retention of surface CD5 (Fig. 23.13). In the majority of cases, the neoplastic cells show uniform CD279/PD-1 and some degree of CD10 expression, a unique feature among T-cell malignancies that reflects the follicular T–helper cell origin of the neoplastic cells in AITL. In a subset of cases, a simultaneous clonal B cell population may be detected, presumably because of underlying immunologic dysregulation and reactivation of Epstein-Barr virus in the clonal B cells.

HEPATOSPLENIC T-CELL LYMPHOMA

The neoplastic cells in this disorder are usually γ-δ T cells, although a minority of cases expresses the α-β TCR. Expression of the γ-δ form of TCR can be demonstrated by FC. The neoplastic cells typically express CD2, CD3, CD7, and CD56, without CD4, CD5, or CD8. In contrast, benign γ-δ T cells usually express variable low-level CD5 and CD8. Because the neoplastic cells do not express the α-β TCR, they cannot be identified as clonal using antibodies to TCR-β isoforms. Formal proof of clonality in these cases requires molecular evaluation of the TCR-γ or δ chains.

ANAPLASTIC LARGE-CELL LYMPHOMA

With the exception of the small-cell variant, the neoplastic cells in anaplastic large-cell lymphoma (ALCL) are typically very large in size as assessed by FS and SS, often requiring gating strategies including the maximal FS values in two-dimensional dot plots. Moderate- to high-level CD30 typically is seen by FC, as is expression of the T-cell activation–associated antigen HLA-DR (Fig. 23.14) and frequently CD25. Expression of CD30 has taken on therapeutic significance with the recent approval of the anti-CD30 monomethyl auristatin E conjugate brentuximab vedotin for Hodgkin lymphoma and ALCL. Surface CD2 and CD4 can be identified with flow cytometry, although the cells are usually negative for surface CD3, CD5, and CD7. Note that the systemic and cutaneous forms of ALCL, although biologically distinct neoplasms, have relatively similar immunophenotypes by FC, such that clinical and immunohistochemical criteria, including evaluation of anaplastic lymphoma kinase (ALK) and epithelial membrane antigen (EMA) expression, are required to distinguish between these entities. Aberrant expression of the myeloid-associated antigens CD13 and/or CD33 is reported to be common in ALK-positive ALCL but not ALK-negative ALCL, creating the potential for misclassification of the former as myeloid sarcoma.

AGGRESSIVE NATURAL KILLER–CELL LEUKEMIA AND NASAL-TYPE EXTRANODAL NATURAL KILLER–CELL LYMPHOMA

The clinical settings of aggressive NK-cell leukemia and nasal-type NK-cell lymphoma are different, but the immunophenotypes of the neoplastic cells are identical in these two aggressive malignancies. The neoplastic cells typically show an aberrant NK-cell immunophenotype, with the characteristic lack of surface CD3 expression and frequent loss of one or more NK-cell–associated antigens, including CD2, CD7, CD8, CD16, and CD56 (Fig. 23.15). Analogous to TCR-β evaluation in T cells, NK-cell clonality can be assessed by flow cytometric evaluation of the killer inhibitory receptor (KIR) antigens. These antigens are expressed in a mutually exclusive manner on NK cells, similar to the TCR genes in T cells. In benign or polyclonal NK-cell populations, flow cytometric evaluation of the three KIRs p158a, p158b, and p158e/KIR p70 typically reveal expression of one of these antigens in 50% to 80% of the NK cells. Therefore, restricted expression of one of these three KIRs, or a complete lack of expression of one of these three KIRs, suggests clonality among an NK-cell population. As with the flow cytometric identification of T cells, the flow cytometric identification of NK-cell clonality does not prove malignancy. Therefore, the flow cytometric findings must be

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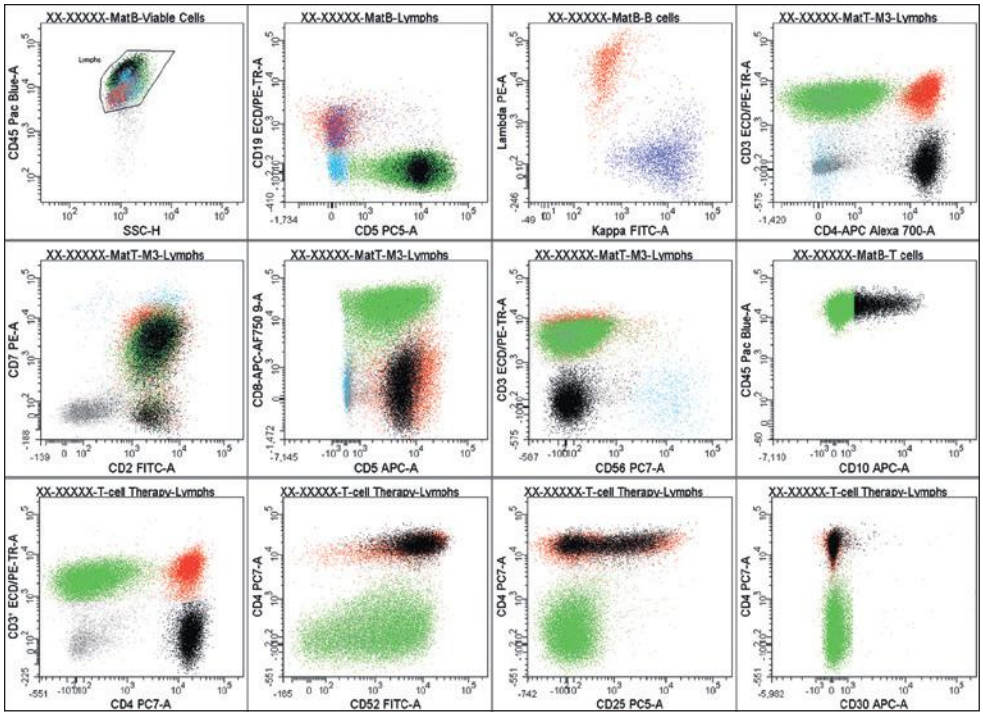


FIG. 23.13

Angioimmunoblastic T-cell lymphoma partially involving a lymph node. The neoplastic $CD4^+$ T cells (colored black in this nine-color analysis) show aberrant loss of surface CD3 expression (seen with two different anti-CD3 antibodies), loss of CD7 on a subset, relatively normal levels of CD2, CD4, and CD5, and low-level CD10 on a subset, which are all common findings. In our laboratory, it is routine to evaluate expression of the potential therapeutic targets CD52 and CD25 in all cases containing a significant abnormal T-cell population of small-medium cell size.

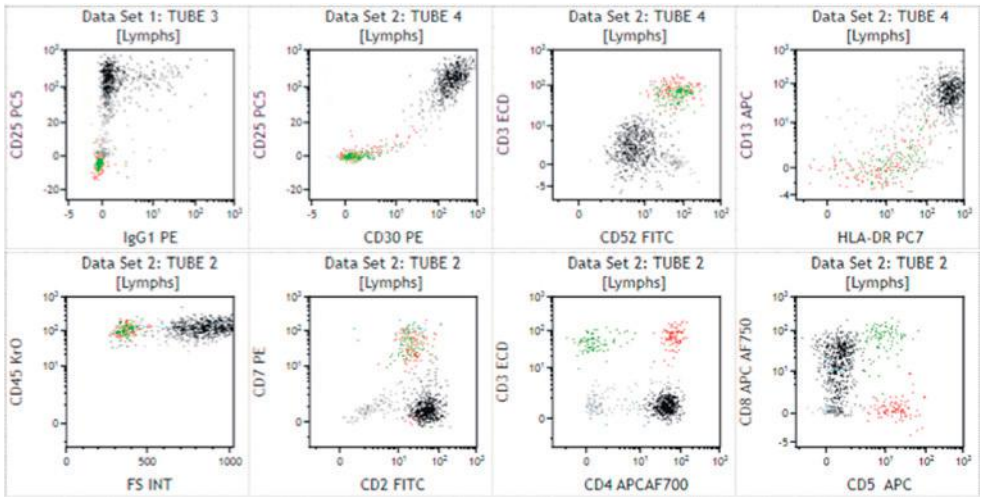


FIG. 23.14

Anaplastic large cell lymphoma (ALCL), anaplastic lymphoma kinase (ALK)-positive, in a paraspinous muscle mass (tumor cells are colored black in this nine-color analysis). The high-level coexpression of CD30, CD25, CD13, and HLA-DR is characteristic of this entity; in contrast, CD13 is not expressed by ALK-negative ALCL. As in this case, CD2 and CD4 expression are relatively common in ALCL, but the variable CD8 coexpression in this case was an unusual feature. Immunohistochemistry on a concurrent lymph node biopsy showed coexpression of CD30 and ALK by the tumor cells, confirming the diagnosis. In our laboratory, it is routine to evaluate CD30 and CD25 expression in all cases containing a significant abnormal T-cell population of large cell size.

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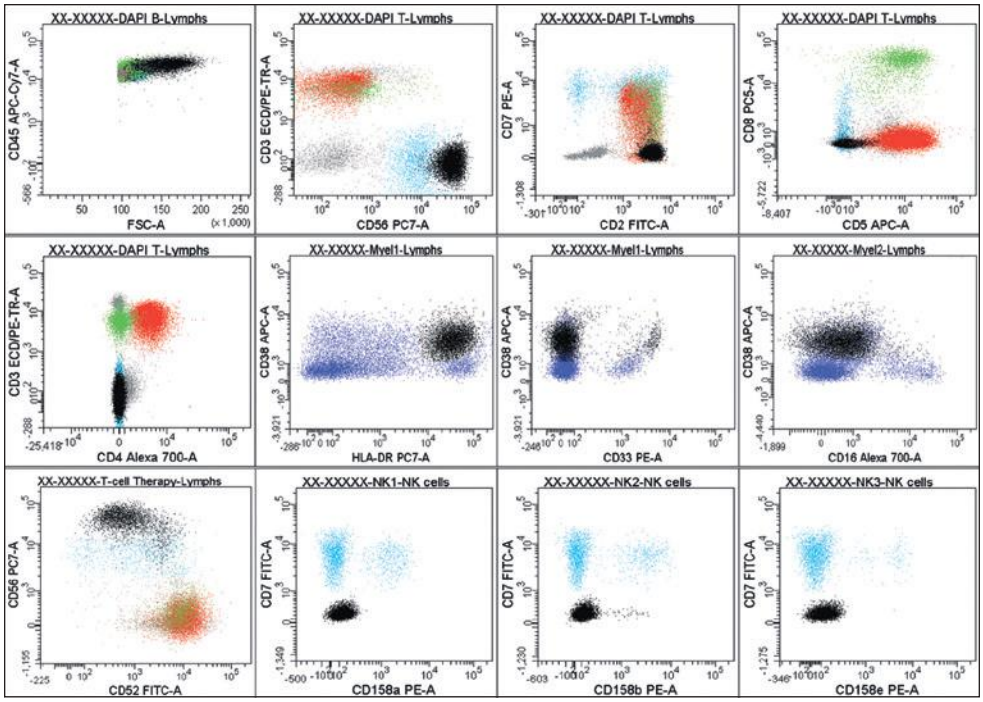


FIG. 23.15

Aggressive natural killer cell leukemia. These nine-color FC data from a bone marrow aspirate show the black-colored neoplastic population to express normal CD2 and CD38, abnormally high-level CD56 and HLA-DR, and aberrant loss of CD7, CD8, and CD16. The neoplastic cells were negative for the three different KIR isoforms examined (CD158a, CD158b, and CD158e, bottom row of images), implying clonality. The increased forward scatter of these cells suggests larger size than residual normal natural killer cells (colored light blue). Epstein-Barr virus–encoded RNA (EBER1) in situ hybridization on the marrow biopsy confirmed that the tumor cells were positive for EBV.

interpreted in the overall context of the case, including evaluation for Epstein-Barr virus infection, before a diagnosis of NK-cell malignancy is made.

MYELOID STEM CELL NEOPLASMS

LOW-GRADE MYELODYSPLASTIC SYNDROMES AND CHRONIC-PHASE MYELOPROLIFERATIVE NEOPLASMS

Multiple studies have demonstrated that myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPNs), and MDS/MPN overlap syndromes such as chronic myelomonocytic leukemia (CMML) frequently manifest abnormal antigen expression on the myeloid blasts or maturing granulocytes and monocytes. In clinical FC laboratories in which the patterns of antigen expression during normal myeloid maturation in the bone marrow (Fig. 23.16A) are well understood, abnormalities in these patterns can be used to support the diagnosis of MDS, MPN, or MDS/MPN in patients suspected of having these diagnoses based on clinical and morphologic grounds. This additional immunophenotypic information can be particularly helpful in morphologically equivocal cases in which cytogenetic studies fail to detect a clonal karyotypic abnormality. In the example shown in Fig. 23.16B, the constellation of antigenic abnormalities among the myeloid blasts and maturing granulocytes and monocytes (described in the figure legend) supported the diagnosis of CMML-1 in the context of a persistent absolute monocytosis and a detectable TET2 gene mutation, despite the normal cytogenetics and normal MDS FISH results. Importantly, in all cases of MDS, MPN, and MDS/MPN, clinical, morphologic, and cytogenetic-molecular correlation are required to render a specific WHO diagnosis.

ACUTE MYELOID LEUKEMIA WITH THE t(8;21)(q22;q22)

In addition to a markedly expanded myeloid blast population by CD45 versus side-scatter gating, this acute myeloid leukemia (AML) (Fig. 23.17) typically shows a relatively prominent population of maturing granulocytes and is considered a form of AML M2 under the French-American-British (FAB) classification. CD34 typically is expressed at a high level, often with aberrant CD15, which is normally a more mature myeloid antigen not expressed on blasts. The pan-myeloid antigen CD33 typically is expressed weakly, whereas HLA-DR and the myeloid-associated antigen CD13 and cytoplasmic myeloperoxidase generally are expressed at a high level. There is frequent aberrant expression of the B-cell antigen CD19 or the T/NK-cell antigen CD56, or both; the lymphoblast antigen TdT also may be aberrantly expressed.

ACUTE MYELOID LEUKEMIA WITH THE t(15;17)(q24;q21)

Acute myeloid leukemia with the t(15;17)(q24;q21), also known as *acute promyelocytic leukemia* (APL; or AML M3 under the FAB classification), is a leukemic proliferation of promyelocytes, characteristically expressing CD13, CD33, CD117/c-kit, CD9, and low CD64 and CD4, with loss of HLA-DR (Fig. 23.18). Most cases lack CD34, but in the hypogranular form of APL (~30% of cases), low-level CD34

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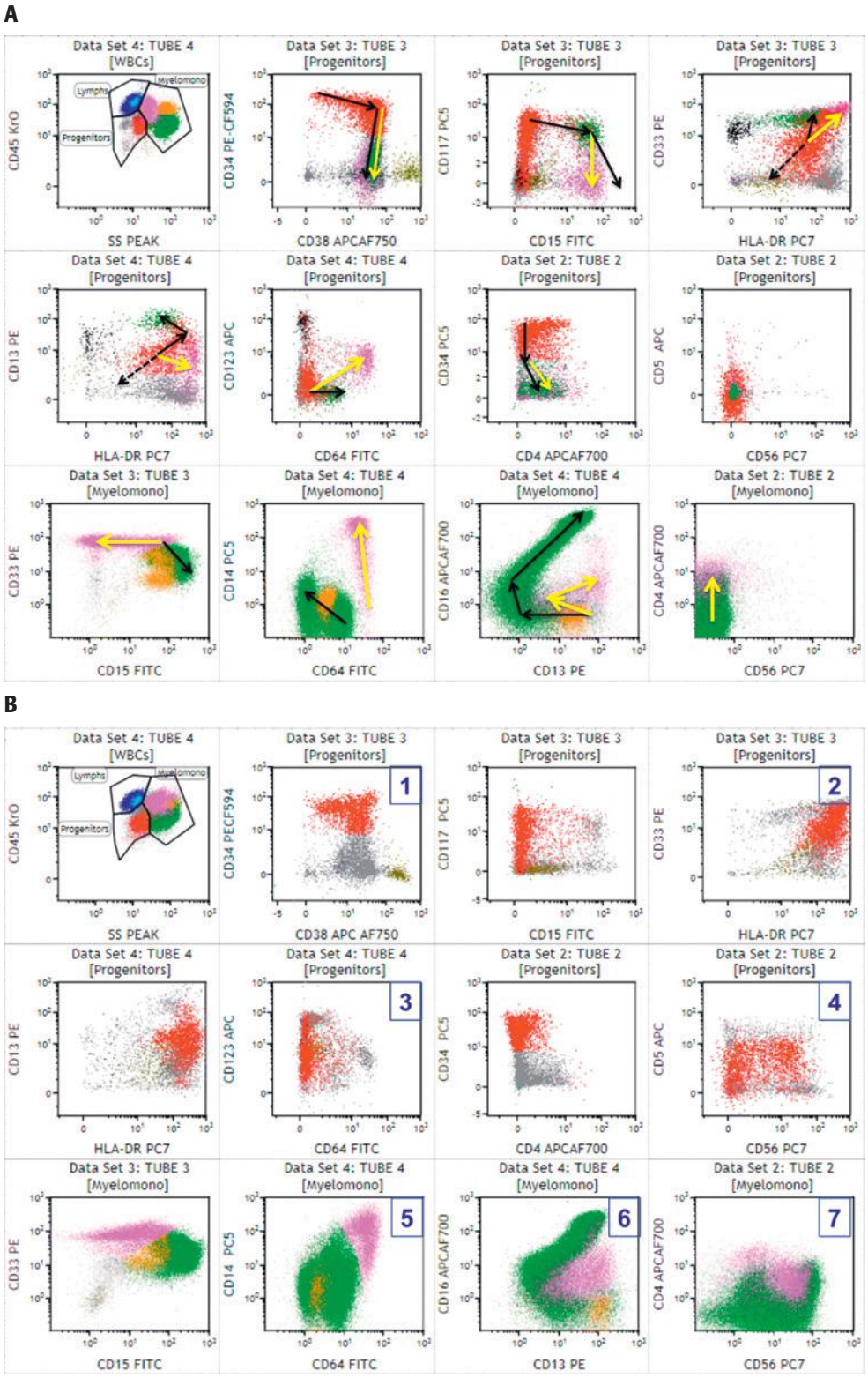


FIG. 23.16

A, Normal maturation of myeloid lineage cells in the bone marrow, by 10-color FC. The following color scheme is used: myeloid blasts/progenitors are *red;* neutrophil series cells are *green;* eosinophils are *orange;* monocyte lineage cells are *lavender;* and mature lymphocytes are *dark blue*. The gated cell population is indicated at the top of each dot plot. Within the Progenitors gate, the *solid black arrows* show the direction of normal maturational progression from early (CD38-negative) to later (CD38-positive) myeloid progenitors, and from the progenitors as a whole (CD34-positive) to early neutrophil precursors (CD34-negative). The *solid yellow arrows* within the Progenitors gate indicate the direction of normal maturational progression from the myeloid progenitors (CD64-negative) to early monocytic precursors (CD64-positive). Within the Myelomono gate, the *solid black arrows* indicate progression from early neutrophil series cells (expressing relatively high CD33 and CD64) to later forms (lower expression of these two antigens, with high CD16), while the *solid yellow arrows* indicate progression from early monocytes (CD14-negative) to late monocytes (high CD14). Within the Progenitors gate, the *dashed lines* denote progression to erythroid precursors from the common myeloid progenitor that gives rise to the entire myeloid lineage. The patterns in this figure are highly reproducible across normal bone marrows. B, Abnormalities of myeloid maturation in a case of CMML-1, by 10-color FC. The color schemes and gated populations in these dotplots are identical to those in Figure 23.16A, to allow direct comparison with the normal patterns of myeloid maturation. Blue numbers denote the seven dotplots showing the most obvious immunophenotypic abnormalities in the CMML-1 case. Among the myeloid progenitors, these abnormalities include dyssynchronous CD34/CD38 expression *(1),* mildly increased CD33 and mildly homogeneous HLA-DR *(2),* low-level aberrant CD123 expression *(3),* and variable aberrant coexpression of the non-myeloid antigens CD5 and CD56 *(4).* Among the maturing myelomonocytic cells, abnormalities include increased CD14 on the least mature neutrophil series cells *(5),* dyssynchronous CD13/CD16 expression by the neutrophil series cells *(6),* and prominent aberrant CD56 expression on both the neutrophil and monocytic series cells *(7).*

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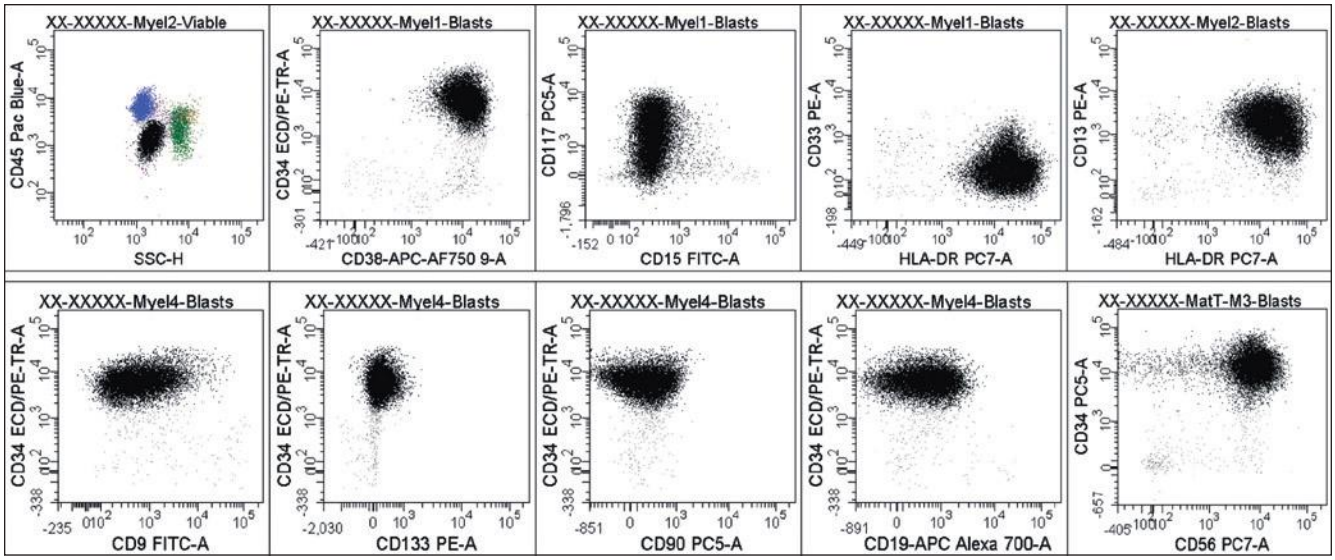


FIG. 23.17

Acute myeloid leukemia with the t(8;21)(q22;q22). The nine-color FC data show many of the characteristic features of the leukemic blasts (colored black), including abnormally increased CD34 and decreased CD33 expression, aberrant low-level CD19, and aberrant CD56 (unusually high level in this case).

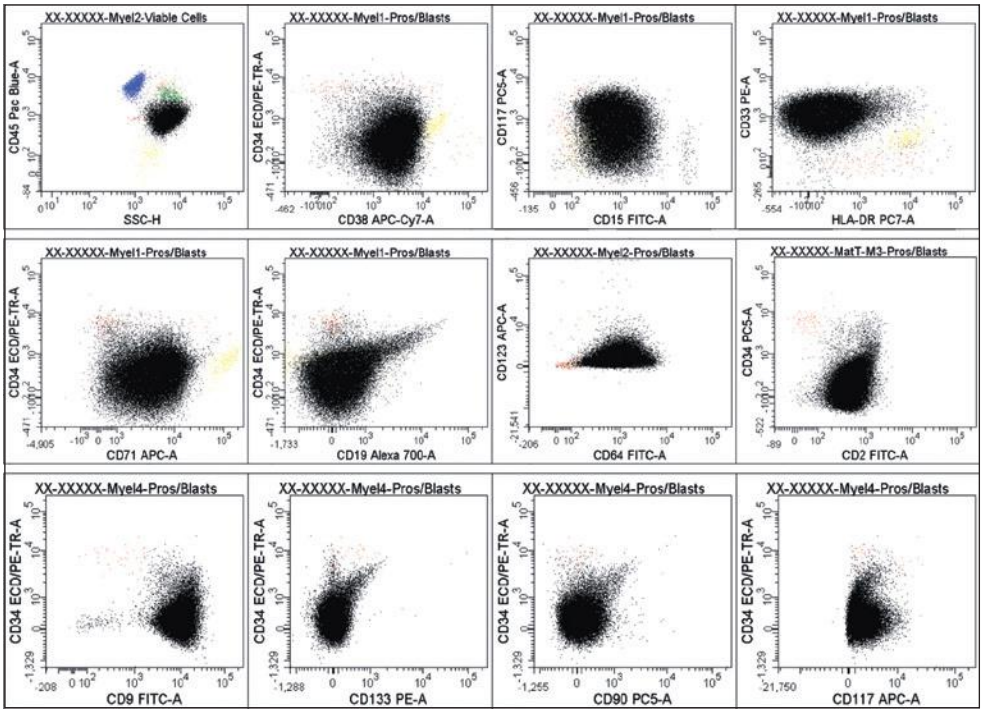


FIG. 23.18

Acute myeloid leukemia with the t(15;17)(q24;q21). The nine-color FC data show the characteristic features of the leukemic promyelocytes (colored black), including minimal CD34 and human leukocyte antigen (HLA)-DR expression, abnormally low-level CD15 (compared to normal promyelocytes) with retention of CD117/c-kit and CD64, high-level CD9, and aberrant low-level CD2. As in most cases and in normal promyelocytes, the leukemic cells in this case also expressed low-level CD4.

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on a minority of the leukemic cells is common. The more mature myeloid-associated antigen CD15 is expressed much more weakly on neoplastic promyelocytes than on normal promyelocytes, which is useful in distinguishing APL from markedly left-shifted benign marrow. Aberrant CD56 and CD2 expression are relatively common in APL.

ACUTE MYELOID LEUKEMIA WITH inv(16)(p13;q22) OR t(16;16)(p13;q22)

This leukemia, which is AML M4eo under the FAB classification, typically shows a prominent $CD34^+$ myeloblast population, and evidence of both monocytic and granulocytic differentiation by CD45 versus SS gating. The myeloid blasts may show aberrant coexpression of CD15 or CD2 and, by evaluation of CD45 versus SS, generally form a maturational continuum with both the monocytes and granulocytes. The monocytic component is invariably CD34-negative and shows relatively strong expression of CD33, CD64, and HLA-DR and acquisition of CD14 on the more mature forms; in this and other AMLs with monocytic differentiation, the CD14-low-to-negative cells in the monocyte gate usually represent the FC correlate of morphologic promonocytes. The neutrophilic component expresses relatively weak CD33 and CD64, without HLA-DR, and acquires both CD13 and CD16 with maturation. A variably expanded eosinophil population, with relatively bright CD45 and increased SS, but without the CD16 expression seen on neutrophils, is characteristic.

ACUTE MYELOID LEUKEMIA, NOT OTHERWISE CATEGORIZED

Flow cytometry can be helpful in subclassifying AMLs without recurrent cytogenetic abnormalities. For example, the diagnosis of AML, minimally differentiated under the WHO classification (AML M0 under the FAB classification), which by definition lacks myeloperoxidase reactivity by cytochemistry, depends on FC to establish myeloid lineage. In those occasional cases of acute myelomonocytic or acute monocytic–monoblastic leukemia showing little nonspecific esterase reactivity by cytochemistry, FC can confirm monocytic differentiation. Finally, FC identification of megakaryocyte-associated antigens, such as CD9, CD36, CD41, and CD61 on leukemic myeloid blasts provides important support for the diagnosis of acute megakaryoblastic leukemia (AML M7 under the FAB classification) and helps to distinguish it from AML M0.

ACUTE MYELOID LEUKEMIA, FLOW CYTOMETRIC CHANGES WITH THERAPY

Clinical flow cytometrists who evaluate hematolymphoid neoplasms invariably encounter post-therapy specimens in their practice—usually from bone marrow or blood—to look for low-level residual disease that will not be discernible by routine microscopic evaluation. The extensive literature on residual disease evaluation by FC in acute leukemia has primarily been focused on acute lymphoblastic leukemia, so a brief consideration of the major issues in evaluating for residual AML after therapy seems warranted. Important points to remember for the analysis of AML post-therapy specimens include the following:

1. Since AML is a bone marrow-based illness, evaluation of bone marrow is more sensitive for residual disease than is peripheral blood evaluation.
2. Among the current AML therapies given with curative intent, all-trans retinoic acid (ATRA)-based acute promyelocytic leukemia therapy is designed to differentiate rather than kill the leukemic cells, while other AML therapies (typically "7+3"-like therapy with 7 days of cytarabine and 3 days of a concurrent anthracycline) are designed to kill the leukemic cells, so APL therapy is associated with a cell-rich bone marrow whereas other AML therapies are associated with a cell-poor bone marrow.
3. The differentiating effect of APL therapy ultimately leads to apoptosis of the leukemic cells, but in the days to weeks immediately after therapy, the flow cytometrist should expect to encounter abnormal maturing myelomonocytic cells with very little if any phenotypic resemblance to either the original leukemic promyelocytes or normal differentiated myeloid cells.
4. Because the initial myeloablation of non-APL therapy is followed by a wave of myeloid regeneration that typically starts in the third week after the initiation of AML therapy, it is important for the flow cytometrist to understand benign bone marrow regeneration in detail, in order to distinguish benign regenerating myeloid progenitors from recurrent AML in the posttherapy setting.
5. Recurrent AML, like other malignancies, may have an altered immunophenotype compared to that seen at diagnosis (so-called antigenic drift, which presumably enhances therapeutic evasion), so the flow cytometrist should be alert to abnormal cell populations that are not entirely identical to the original AML.

Figure 23.19, which follows a single case of monocytic AML from diagnosis, through therapeutic responsiveness, to eventual relapse at about 6 months after the start of therapy, illustrates a number of these points (see legend for details).

BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM (BPDCN)

This aggressive neoplasm, which almost always involves the skin and usually involves the bone marrow, was termed *blastic NK cell lymphoma* in the 2000 WHO classification, and hematodermic $CD4^+CD56^+$ neoplasm in the 2005

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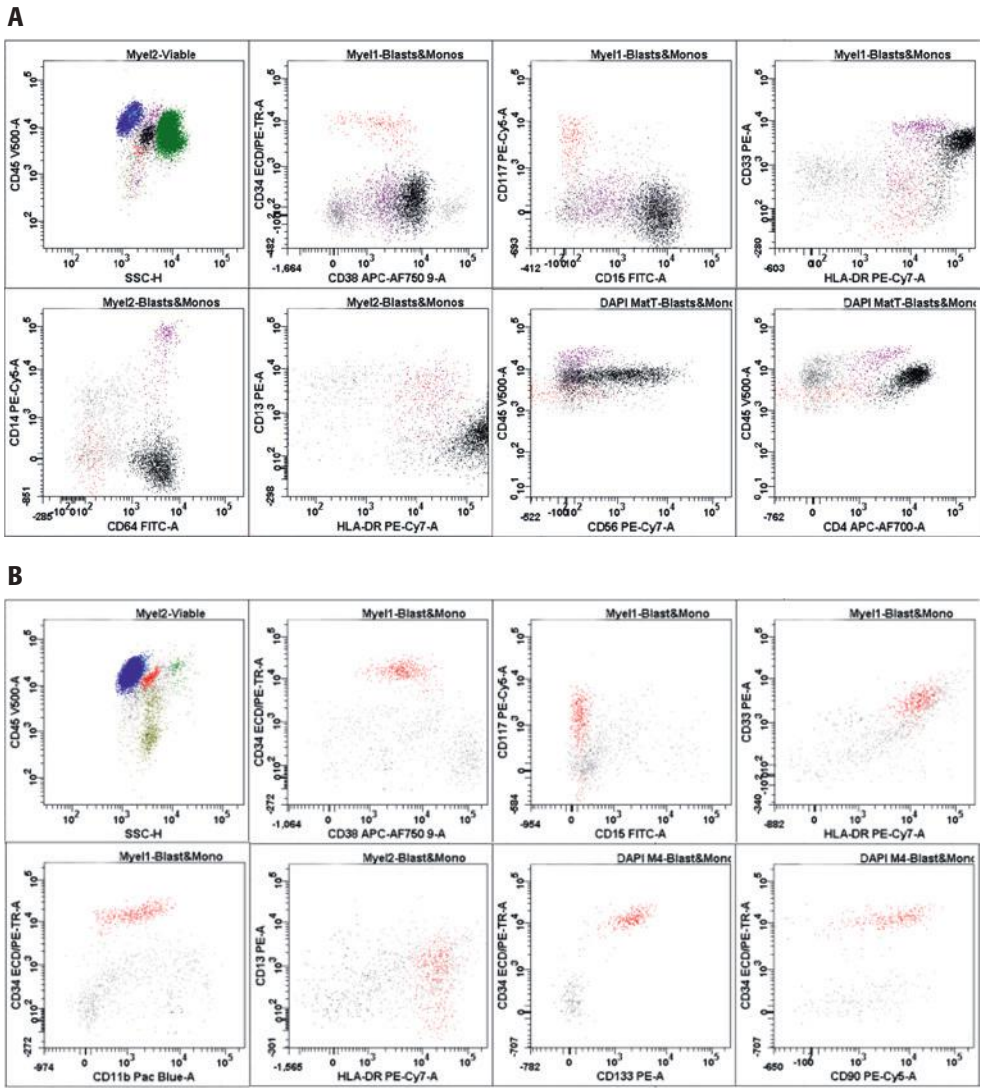


FIG. 23.19

Ten-color FC evaluation of a single case of monocytic AML from four different times during the course of disease, illustrating a number of points about FC in the diagnosis and posttherapy followup of AML. The normal cell lineages are colored as in Fig. 23.16, whereas the leukemic cells are colored *black*. A, At diagnosis, the leukemic population (*black* in the dotplots) is identified in a background of normal hematopoiesis in a hemodilute bone marrow aspirate; the leukemic cells have a characteristic immature monocytic/promonocytic immunophenotype, expressing high-level CD33, HLA-DR, CD64, and CD4, intermediate-level CD45, CD38, and CD15, very low CD13, and variable aberrant CD56. B, The day 14 postinduction bone marrow specimen shows the expected paucity of maturing myelomonocytic cells, with a predominance of mature lymphocytes *(dark blue)* and no cells bearing the leukemic immunophenotype. Importantly, the myeloid blasts in the sample *(red)* show the typical immunophenotype of early regenerating blasts, including relatively homogeneous overall antigen expression, increased CD45 (nearly equivalent to that of the mature lymphocytes), somewhat decreased CD38, low CD11b, and relatively high-level coexpression of the early progenitor-associated antigens CD133 and CD90. C, At 2.5 months postinduction, the bone marrow shows characteristic immunophenotypic features of ongoing marrow regeneration during consolidation chemotherapy, including relatively homogeneous overall antigen expression and lower-level CD133 and CD90 among the myeloid blasts *(red)*, and dyssynchronous expression of CD13 and CD16 among the maturing granulocytes, in which the typical smooth arc of this relationship is replaced by a right-angled relationship (the right-angled CD13/CD16 relationship is routinely seen in any setting of granulocytic regeneration, and is not limited to postchemotherapy recovery). D, Relapse at 5.5 months postinduction, in which the leukemic cells *(black)* are again identified by their aberrant CD56 coexpression, but now show some antigenic drift compared to the immunophenotype at diagnosis, most notably a significant decline in CD45 expression, with a more subtle decrease in HLA-DR expression and an apparent slight increase in CD14 expression.

WHO-European Organisation for Research and Treatment of Cancer (EORTC) classification of cutaneous hematolymphoid neoplasms. BPDCN is believed to derive from precursors of plasmacytoid dendritic cells, and usually shows coexpression of CD4, CD43, CD123, TCL1, and aberrant CD56. Antigens less commonly expressed include CD7, CD33, CD68, and aberrant TdT. CD34, CD117, and Epstein-Barr virus–associated antigens are not expressed.

ACUTE LEUKEMIAS OF AMBIGUOUS LINEAGE

The classification of these rare acute leukemias was significantly revised between the 2000 and 2008 WHO classifications, but initial description of the 2016 classification suggests that the features of these leukemias will not change much compared to 2008. According to the 2008 WHO classification,

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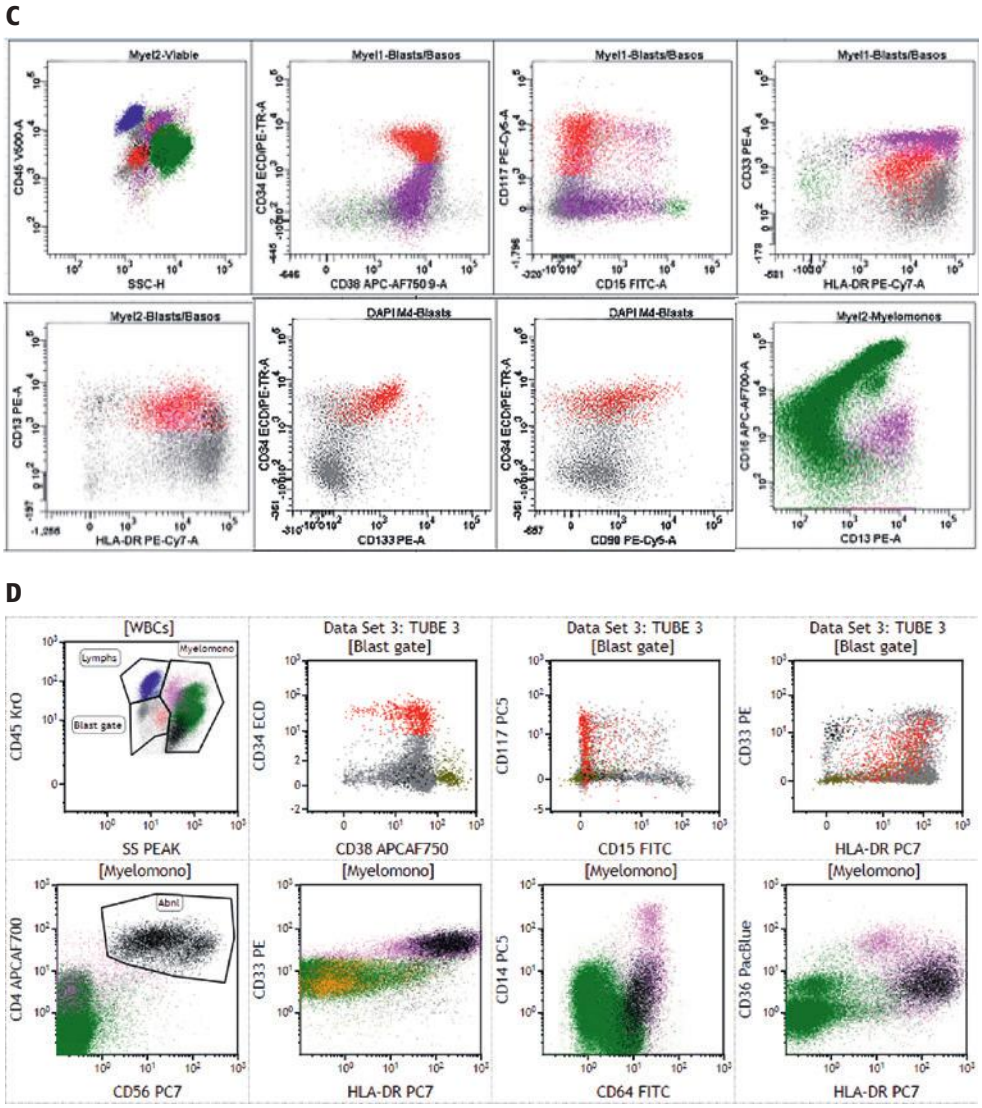


FIG. 23.19, cont'd

acute undifferentiated leukemia lacks antigens specific for B-lymphoid origin (strong CD19 with strong CD10 or cytoplasmic CD79a or CD22, or weak CD19 with at least two of the following: strong CD10 or cytoplasmic CD79a or CD22), T-lymphoid origin (cytoplasmic or surface CD3), myeloid origin (cytoplasmic myeloperoxidase), or monocytic origin (at least two of the following: nonspecific esterase, CD11c, CD14, CD64, or lysozyme). Acute undifferentiated leukemia blasts often express HLA-DR, CD34, or CD38 and may express TdT. Mixed phenotype acute leukemia with the t(9;22) (q34;q11.2) is defined by the presence of a *BCR-ABL1* rearrangement and coexpression of myeloid-associated antigens with either B-lymphoid antigens (most common) or T-lymphoid antigens (less common). Mixed-phenotype acute leukemia with the t(v;11q23) is defined by the presence of a chromosomal translocation involving the *MLL* gene and usually includes a $CD10^-$ B-lymphoblast population with frequent coexpression of CD15, and a separate population of leukemic myeloid cells, commonly with monocytic differentiation. In addition to the latter two genetically defined acute leukemias, the 2008 WHO classification also includes mixed phenotype acute leukemia, not otherwise specified, of either B-myeloid or T-myeloid lineage. Rarely, the blasts in these ambiguous cases coexpress markers for all three lineages.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

This clonal myeloid stem cell abnormality results from the loss of cell surface glycosylphosphatidylinositol (GPI)-linked proteins because of acquired mutation of the *pigA* gene. Loss of GPI linkages leads to abnormal susceptibility to complement-mediated lysis among erythrocytes, plus other features of the disease, including predisposition to thrombosis. The most common GPI-linked proteins assayed to identify affected cells include CD59 on erythrocytes, CD14 on monocytes, and CD16, CD24, and CD66b on neutrophils.

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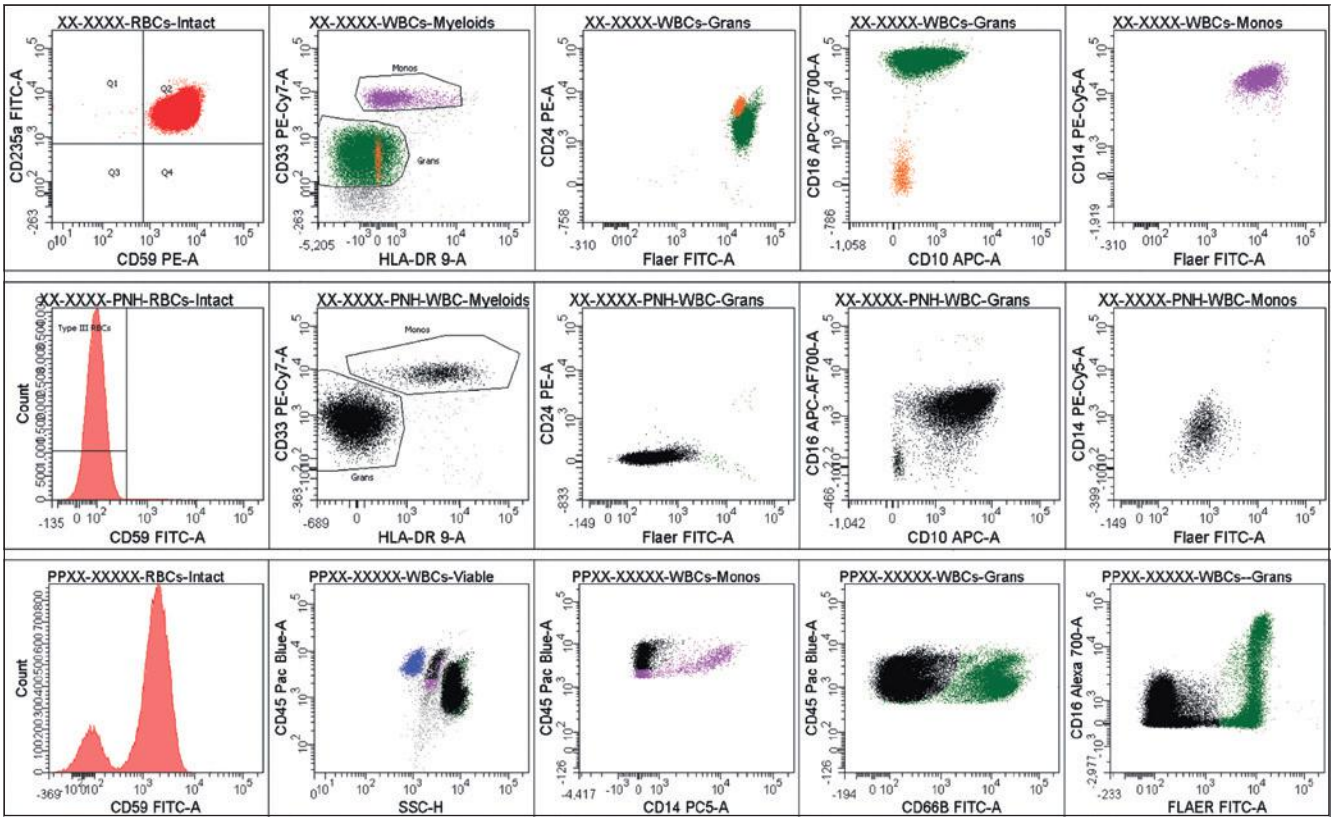


FIG. 23.20

Evaluation for paroxysmal nocturnal hemoglobinuria (PNH). In these images, erythrocytes are red, neutrophil series cells green, eosinophils orange, and monocytes lavender. The *top row* depicts a normal peripheral blood specimen, negative for evidence of PNH. In the two-color erythrocyte evaluation, CD235a (glycophorin A) is a gating reagent used to limit the analysis to intact erythrocytes, whereas CD59 is the glycosyl-phosphatidylinositol (GPI)-linked protein evaluated on these cells. In the seven- or eight-color evaluations of granulocytes and monocytes, CD33 and HLA-DR are gating reagents used to restrict the analysis to intact cells of these two lineages, although an alternative approach is to use CD15 and side scatter to separate these lineages. Among the granulocytes, CD45, CD10, and side scatter can be used to separate neutrophils from eosinophils. Leukocyte GPI-linked proteins that we routinely evaluate include CD24, CD16, and CD66b on neutrophils and CD14 on monocytes. The FLAER reagent (fluorescently labeled aerolysin, a bacterial toxin that binds to GPI linkages on all leukocytes) is included in all leukocyte PNH assays. The *middle row* shows peripheral blood from a patient with an extremely large PNH clone representing greater than 98% of the erythrocytes (CD59-negative "type III" cells), neutrophils (negative for FLAER and CD24, with the characteristically decreased, but not absent, CD16), and monocytes (negative for FLAER and CD14); the GPI-negative leukocytes are colored black in the images. The *bottom row* shows a bone marrow aspirate from a different patient with prolonged anemia, submitted to rule out myelodysplasia. The diagnosis of PNH was strongly suggested by the loss of CD59 on a significant minority of the erythrocytes and the prominently expanded mature (high-level $CD45^+$) neutrophil and monocyte populations with loss of CD16/CD66b/FLAER binding and CD14/FLAER binding, respectively (abnormal populations both colored black in the images). This case shows that PNH clones can be readily identified in bone marrow aspirates, but note that peripheral blood evaluation is required to assess baseline PNH clone size for the purpose of following the disease.

Recent studies have suggested CD157, a GPI-linked protein expressed by both monocytes and granulocytes, can replace more monocyte-specific and granulocyte-specific markers in paroxysmal nocturnal hemoglobinuria (PNH) assessment. Because of the heterogeneity of CD14 and CD16 expression among immature monocytes and neutrophil lineage cells, respectively, evaluation for PNH is best performed on peripheral blood, where only mature forms of these cell lineages are expected to occur. Over the past 15 years, a number of studies have shown that fluorescently labeled aerolysin (FLAER) protein from the bacterium *Pseudomonas aeruginosa* is a sensitive and specific reagent for detecting GPI-linked proteins on leukocytes (but not erythrocytes), such that PNH monocytes and granulocytes show abnormally decreased ability to bind FLAER. Serial evaluation of the size of the PNH monocyte and neutrophil populations in the peripheral blood is used to provide surrogate information about marrow involvement by the PNH clone. Evaluation of $CD59^-$ erythrocyte populations is less useful for this purpose, because affected erythrocytes are more sensitive to complement-mediated lysis than are leukocytes; therefore, the size of the $CD59^-$ erythrocyte population in the blood typically underestimates the size of the PNH clone in the bone marrow. Flow cytometric evaluation of GPI-linked proteins in peripheral blood from a healthy individual, and in blood and bone marrow from two different patients with PNH, is shown in Fig. 23.20. It is important to remember that detection of a PNH-type clone is not diagnostic of clinical PNH, and small PNH clones can be seen in settings such as myelodysplasia and bone marrow failure disorders such as aplastic anemia. Indeed, PNH-type clones can be detected in healthy individuals (on the order of 1 in 10^6 cells when extremely sensitive assays are used). A PNH FC assay sensitivity of 1% is sufficient for most clinical purposes, although

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high-sensitivity assays with the ability to detect 1 in 10^{-4} cells are becoming more common. Suggested guidelines for performing PNH assays by flow cytometry have been published and may serve to help standardize testing in clinical laboratories.

CONCLUSION

The central importance of immunophenotyping in the current diagnosis and classification of hematolymphoid diseases makes an understanding of antigen expression patterns in benign and neoplastic settings imperative for any pathologist who diagnoses these diseases. Familiarity with antigen expression patterns in benign and neoplastic hematolymphoid processes is also useful for any hematologist or oncologist who treats these diseases. An understanding of both the advantages and the potential pitfalls of FC evaluation is likewise important for all physicians involved in the diagnosis and treatment of these diseases. Although FC is used primarily in diagnosis and disease monitoring, it will assume greater importance in therapeutic decision making as more potential therapeutic targets are identified by basic research on hematopoietic disease.


The references for this chapter can be found online by accessing the accompanying Expert Consult website.

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Molecular Hematopathology

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■ INTRODUCTION

Molecular investigation of the hematopoietic neoplasms has contributed significantly to our understanding of the "genetic basis" of cancer, and progress in this dynamic field continues unabated. Accordingly, new knowledge concerning recurrent genetic abnormalities in myeloid and lymphoid cancers continues to be integrated within the most recent World Health Organization (WHO) classification of the hematopoietic and lymphoid tumors. This chapter summarizes key technical considerations, essential molecular alterations observed in specific tumor associations, and the application of molecular diagnostic approaches to the classification and monitoring of lymphoid and hematologic neoplasms.

■ ASSAY SENSITIVITY AND SPECIFICITY IN MOLECULAR DIAGNOSTICS

Clinical application of laboratory methods to detect chromosomal or genetic aberrations in the hematolymphoid disorders requires a clear understanding of assay sensitivity and specificity, to determine the relative use and limitations of various seemingly complementary techniques in routine practice. Most commonly, reference is made to the concept of analytic sensitivity. This is a very important measurement, defined as the ability of a given assay to detect the lowest amount or concentration of a substance when it is present in a test sample. For molecular (DNA or RNA) analytes, typical analytic sensitivity can attain very low limits of detection (e.g., 10^{-3} to 10^{-6}), in contrast to microscopic-based platforms such as fluorescence in situ hybridization (FISH) and conventional karyotyping (Table 24.1). Analytic test sensitivity is further determined in the context of the lowest *reproducible* level of the intended target, which constitutes the limit of detection (LOD) of the assay. For quantitative assays, the LOD is established along with stringent precision measurements (e.g., mean value with standard deviation and coefficient of variation, 95% confidence intervals); numeric values obtained below this threshold are considered potentially inaccurate despite being detectable owing to stochastic reaction effects. An analytic measurement range (AMR) is also applied to quantitative tests to detail the linearity of the system across the clinically useful biologic range of target concentration or copy number.

Good analytic sensitivity is often associated with diagnostic (or clinical) sensitivity, the latter being the ability of an assay to detect a particular clinical condition (signified by the presence of the target analyte) when it is present in a general population of subjects. However, analytic and diagnostic sensitivity can diverge if, for example, the analyte in question is not uniformly represented (e.g., due to cell/tissue distribution effects, temporal or ethnic variations) or if the underlying genetic alteration exhibits significant heterogeneity (e.g., multiple possible mRNA transcripts arising from a single gene rearrangement event, such as the *BCR-ABL1* abnormality). This can lead to the situation wherein a clinical test may have exquisite detection sensitivity yet may miss some patients with the condition of interest (i.e., clinical false negativity). Analytic specificity concerns the ability of an assay to identify only the target analyte, even in heterogeneous mixtures with other molecules having potentially overlapping features; evaluating and eliminating potential analytic interferences is also an important component of test validation. For most molecular assays, high analytic specificity can be readily achieved by relying on the highly complementary nature of DNA fragment binding, as well as additional technical maneuvers to enhance high-affinity nucleic acid interactions or amplify positive signals. Clinical or diagnostic specificity refers to the ability of a test to accurately exclude individuals who do not have the disease or condition in question. Once again, these two parameters are linked but can show a significant lack of concordance if, for instance, a truly negative specimen is affected by external contamination with the nucleic acid of interest or if related molecules in the sample are not reliably discriminated by the assay (i.e., analytic false positivity). Amplification-based assays are especially prone to false-positive results, which are an unfortunate potential by-product of high analytic sensitivity; therefore, scrupulous control of contamination is essential in any molecular diagnostic laboratory performing polymerase chain reaction (PCR)-based analyses. Related to the issue of clinical specificity, an understanding of pathologic context is required because a given molecular abnormality may occur in different disease entities. Finally the clinical

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TABLE 24.1

Relative Sensitivity of Standard Methods Used to Detect Genetic Abnormalities in Leukemias and Lymphomas

Method	Analytic Sensitivity (%)	Notes
Cytogenetics	5–10	Global genomic assessment of numerical and large structural chromosome defects including partial or whole gains, losses, duplications, translocations Laborious; requires fresh, sterile cells for metaphase analysis Difficult or impossible to detect smaller scale alterations in DNA (i.e., less than several megabases)
Fluorescence in situ hybridization	1–5	Targeted assessment of large regional genomic abnormalities (sub-megabase range) Technically straightforward and rapid; can frequently be performed on interphase nuclei Applicable to a variety of tissue sources including paraffin-embedded biopsies Requires rigorous quality standards to avoid false-positive results at low levels of the abnormality in question
Chromosome arrays	20–30	Genome-wide assessment of structural variation (copy number changes, CNV) Main platforms include array competitive genomic hybridization (A-CGH) and single-nucleotide polymorphism (SNP) array (SNP-A) Resolution is better than FISH (100s kb–Mb range) Both platforms detect CNV alterations of loss of heterozygosity (LOH) or genomic region amplification; however SNP-A also detects copy neutral LOH (congenital or acquired uniparental disomy, UPD) Cannot detect balanced translocations or nucleotide base alterations
Southern blot hybridization	5–10	Targeted assessment of structural changes in genomic DNA (kilobase range) Technically laborious and time-intensive Requires samples that preserve high-molecular-weight DNA (e.g., fresh or frozen cells)
Polymerase chain reaction (PCR)	1×10^{-4} to 10^{-6}	Ubiquitous molecular analytic method for rapid qualitative or quantitative applications using DNA or mRNA templates (hundreds of bases range) Requires post-PCR product detection procedures with variable analytic sensitivities (e.g., DNA sequencing, electrophoretic sizing) Applicable to a broad range of sample types, including fresh and fixed paraffin-embedded tissues Common method for high-sensitivity minimal residual disease (MRD) monitoring (real-time quantitative PCR technique)
Digital PCR (D-PCR)	1×10^{-3} to 10^{-6}	Quantitative PCR method based on enumeration of end-point detection of reactions in numerous individual nanoliter reactions (direct measurement of target copy number/unit volume) Potential for highly accurate and sensitive MRD detection of DNA or mRNA targets without post-PCR analytic steps Sensitivity is dependent on size of partition space (total number of individual micro-reactors on the platform)
Sanger sequencing	~20	Commonly used to detect base-level DNA sequence alterations Automated systems perform sequencing of PCR-amplified templates using fluorescent dye terminators Dedicated analysis software required Single target/reaction Maximum sequence length ~500 bp Difficult to resolve more complex insertion/deletion events and cannot detect large deletions or amplifications Cannot reliably distinguish allelic nature of heterozygous changes (e.g., biallelic versus compound heterozygote) without specialized techniques
Next-generation sequencing (NGS)	~5–10	Massively parallel sequencing of DNA with applications spanning targeted gene panels to whole exome and genome sequencing; efficient detection of single base changes and small insertion/deletion events Extended uses: RNA sequencing (transcriptome profiling), mate pair sequencing (translocation detection), epigenetic profiling Common platforms include Ion Torrent (semiconductor) and Illumina (solid support with fluorescent reversible dye terminator method) Ability to multiplex many samples per run with sample indexing Generates very large amounts of sequence data from millions of "short reads" (e.g., 100 or 150 bases), but collectively can identify alterations over many hundreds to thousands of bases of DNA Can resolve complex small insertion/deletion events and often distinguish precise nature of heterozygous mutation variants Requires complex bioinformatics algorithms and extensive quality assessments for analysis Web application interfaces allow direct visualization of analyzed data and sequence variants (BAM or VCF file formats) Requires integrated approach involving technical, medical/scientific, genetic counseling, and informatics expertise to generate high-quality clinical reports Nominal sensitivity is ~5% in most applications, better than Sanger method

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validity of a biomarker or analyte is an important factor in test development. Validity is determined by several measures, including but not limited to (1) published consensus guidelines from professional bodies; (2) well-established use of an analyte to support a specific disease diagnosis or to monitor a specific therapy; and (3) substantial clinical trial or other peer-reviewed data indicating the required or suggested utility of a marker for diagnostic, prognostic, or predictive purposes. Clinical validity is a major consideration related to obtaining reimbursement for often costly molecular diagnostic tests. All of these facets combine to produce a strong test that must then fit into a framework of clinical practice utility—how and when to use a certain molecular diagnostic assay and, as important, when not to do so. Therefore, when designing molecular genetic diagnostic tests with high analytic and potential clinical utility, the hematopathologist must understand these concepts thoroughly and include sufficient data in validation studies to mitigate potential concerns regarding accuracy, sensitivity, and specificity.

COMMON AND EMERGING METHODS USED IN THE MOLECULAR DIAGNOSIS OF HEMATOLYMPHOID NEOPLASMS

To best understand diagnostic assay design and methodologic advantages and limitations, a basic understanding of the technical aspects of molecular diagnostic methods is required. Although a complete primer on molecular diagnostic techniques is beyond the scope of this chapter, a description of important techniques such as quantitative PCR analysis, digital PCR, next-generation sequencing (NGS), DNA hybridization technologies, microarray, and immune-receptor clonality assays is available as an on-line supplement. Table 24.1 summarizes important sensitivity characteristics of standard methodologies used in hematopathology. The reader is encouraged to review the online supplemental material that provides some technical details on molecular diagnostic methods before proceeding.

MOLECULAR GENETIC EVALUATION OF NON-HODGKIN LYMPHOMAS FOR DIAGNOSIS AND CLASSIFICATION

Understanding the pathogenesis of the non-Hodgkin lymphomas (NHL) has been greatly aided by the recognition and characterization of recurrent, non-random chromosomal translocation events (Table 24.2). For B-cell NHL, such translocations typically result in the placement of a proto-oncogene adjacent to one of the transcriptionally active immunoglobulin genes, most commonly *IGH*. In turn, the normal regulation of the oncogene becomes disrupted, leading to aberrant activity resulting in unchecked cellular proliferation or protection from normal cellular apoptosis thresholds. In certain other subtypes of lymphoma (e.g., some extranodal marginal zone lymphomas, anaplastic lymphoma kinase (ALK)-positive anaplastic large-cell lymphoma), translocations produce a chimeric fusion gene, expressed mRNA transcript, and novel oncoprotein. In this situation, pathophysiologic effects arise in part from disruption of key cellular signaling pathways, mediated by aberrant activity of the chimeric protein. In B-cell lymphomas, various mechanisms have been proposed to explain the occurrence of translocation-associated gene rearrangements. These include (1) abnormal targeting of the RAG recombinase machinery during physiologic immunoglobulin gene rearrangements; (2) aberrant resolution of DNA double-strand breaks during germinal center processes (e.g., somatic hypermutation, class switch recombination) mediated in part by the enzyme activation-induced cytidine deaminase; and (3) fragile DNA intermediates prone to breakage and errant recombination.

The identification of characteristic cytogenetic abnormalities is not always essential for establishing a diagnosis or specific subclassification of NHL; most often, a correct diagnosis is arrived at by a combination of morphology and immunophenotypic studies. Furthermore, the detection of expressed oncoproteins by immunohistochemistry provides a simple alternative method of confirming the presence of an underlying specific molecular genetic event in some types of NHLs (e.g., cyclin D1 expression in mantle cell lymphoma and ALK expression in anaplastic large-cell lymphoma). Nonetheless, there are many circumstances in which the presence or absence of these genetic anomalies can be critical for correct disease diagnosis. The detection of lymphoma-associated genetic abnormalities can be achieved by cytogenetics, FISH analysis, or PCR-based methods. Cytogenetics is often limited by the relative unavailability of fresh tissue for sterile culture. PCR assays relying on clustering of genomic breakpoints have been developed to detect some gene fusion abnormalities in NHL, but this approach in general suffers from suboptimal clinical sensitivity because of the large and inconstant breaksite regions in respective chromosome locations precluding the use of standard short-range PCR techniques. FISH methods, in contrast, are very robust in this regard and are most often applied to reveal the presence of translocation events in NHL. The emergence of NGS is also providing new opportunities to potentially evaluate tumor DNA for the presence of chromosome translocations and simultaneously screen for nucleotide base-level mutations. Targeted small NGS panels can be cost-effective solutions to interrogate many disease-related recurrent genetic abnormalities in B-cell neoplasms.

SMALL B-LYMPHOCYTE LYMPHOMAS AND CHRONIC B-CELL LEUKEMIAS

FOLLICULAR LYMPHOMAS AND THE T(14;18)/*BCL2-IGH* ABNORMALITY

The t(14;18)(q32;q21)/*BCL2-IGH* abnormality, which is prototypic of lymphoma translocations, occurs in approximately 90% of follicular lymphomas (FL) and up to 30% of

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TABLE 24.2

Common Genetic Abnormalities in the Non-Hodgkin Lymphomas and Chronic Leukemias Detected by Molecular Diagnostic Methods

Disease Associations	Major Genetic Abnormalities	Molecular Pathogenesis[a]	Molecular Diagnostic Detection[b]	Notes
Follicular lymphoma (FL) Diffuse large B-cell lymphoma (DLBCL)	t(14;18)/*BCL2-IGH*	Overexpression of antiapoptotic BCL2 protein	FISH (DNA PCR)	90% of FL and ~20% to 30% DLBCL PCR for *BCL2* MBR locus rearrangements detects only 60% to 70% of cases; multiple primer sets required to increase overall detection
Mantle cell lymphoma (MCL)	t(11;14)/*CCND1-IGH*	Overexpression of G1-phase cell cycle protein cyclin D1	FISH (DNA PCR)	>95% of MCL; very rare cases with MCL-like gene expression have rearrangements of the *CCND2* gene (~50% of *CCND1*-negative MCL) PCR for *BCL1* MTC locus rearrangements detects <50% of cases Cyclin D1 overexpression by immunohistochemistry is also diagnostic in correct pathologic setting
Hairy cell leukemia (HCL) Langerhans cell histiocytosis (LCH) and Erdheim-Chester disease (ECD)	*BRAF* Val600Glu	Constitutive MEK-MAP kinase pathway signaling	Allele-specific PCR (including RQ-PCR), sequencing	*BRAF* mutations present in >90% classical HCL but not seen in cases of HCL-variant A small number of *BRAF*-negative HCL have mutations in the downstream *MAP2K1* gene ~50% of LCH and ECD harbor *BRAF* V600E mutations
Lymphoplasmacytic lymphoma (LPL) Splenic marginal zone lymphoma (SMZL) Diffuse large B-cell lymphoma (DLBCL)	*MYD88* Leu265Pro	Activation of NF-κB pathway signaling	Allele-specific PCR, sequencing	>90% association with LPL; present in ~10% SMZL and rarely in other low-grade B-cell lymphoma types A subset of *MYD88* L265P-positive LPL patients have concurrent truncation-type mutations in *CXCR4* *MYD88* L265P is absent in rare presentations of IgM myeloma Present in ~30% of nodal DLBCL and in majority (70% to 90%) of cases involving the central nervous system (CNS), orbit, or testis
Extranodal marginal zone lymphomas (ENMZL)	t(11;18)/*BIRC3-MALT1* and t(14;18)/*MALT1-IGH*	Upregulation of NF-κB signal transduction pathway activity	FISH (RT-PCR for BIRC3-MALT1 mRNA)	~20% to 30% of ENMZL Frequency of specific translocations varies with different anatomic sites
Chronic lymphocytic leukemia (CLL)	del17p/*TP53*, del11q, IGH-V region somatic hypermutation status	Deletion of tumor suppressor loci (i.e., *TP53* and *ATM*); IGH-V mutation status <2% (close to germline) associated with poor prognosis	FISH for chromosome deletion events; PCR and sequencing (Sanger or NGS) for IGH-V mutation status	Gene mutations in *SF3B1*, *ATM*, *TP53*, *BIRC3*, *NOTCH1* may also have prognostic value and can be collectively evaluated by targeted NGS

Continued

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TABLE 24.2

Common Genetic Abnormalities in the Non-Hodgkin Lymphomas and Chronic Leukemias Detected by Molecular Diagnostic Methods—cont'd

Disease Associations	Major Genetic Abnormalities	Molecular Pathogenesis[a]	Molecular Diagnostic Detection[b]	Notes
Burkitt lymphoma (BL) Diffuse large B-cell lymphoma (DLBCL) High-grade B-cell lymphoma with rearrangements of *MYC* and *BCL2* or *BCL6* ("double-hit" lymphoma)	t(8;14)/*MYC-IGH*, *MYC-IGK*, or *MYC-IGL* and variant *MYC* translocations	Overexpression of potent early response mitogenic transcriptional factor MYC	FISH	*MYC* rearrangements present in ~100% BL and 5% to 10% typical DLBCL *MYC* rearrangement with a non-immunoglobulin gene may have less adverse clinical outcome in DLBCL *MYC* translocations also occur in presentations of other aggressive B-cell lymphomas (e.g., large-cell transformation of low-grade lymphoma, HIV-related, posttransplant-related)
ALK+ T-cell anaplastic large-cell lymphoma (ALK+ ALCL)	t(2;5)/*NPM1-ALK* and other *ALK* translocation variants	Constitutive activation and abnormal localization of Alk tyrosine kinase	FISH (RT-PCR for NPM1-ALK mRNA)	~60% of ALCL *ALK* protein detection by immunohistochemistry is also diagnostic (staining patterns vary according to translocation type) Rare large B-cell lymphomas with *CLTC-ALK* gene fusion
ALK-negative anaplastic large-cell lymphoma	Rearrangements of *DUSP22/IRF4* locus on 6p25 or *TP63* locus on 3q28	Current pathogenic effects are uncertain	FISH	Subset of ALK-ALCL ALK-ALCL with *DUSP22* rearrangement is relatively favorable versus poor prognosis if *TP63* present *DUSP22* also rearranged in some cutaneous ALCL cases
Peripheral T-cell lymphomas (PTCL) with T-follicular helper (TFH) phenotype	*ITK-SYK* and *CTLA4-CD28* gene fusions; recurrent gene mutations (e.g., *IDH2*, *RHOA*, *DNMT3A*, *CD28*)	Abnormal T-cell receptor and cell signaling activation; epigenetic alterations	FISH (RT-PCR) for gene fusions; targeted NGS panel for gene mutations	Detection of these abnormalities is not required for a diagnosis of TFH PTCL but may be helpful to identify prognostic features Gene mutations are not specific for TFH PTCL *CTLA4-CD28* fusions are also described in some cases of PTCL, unspecified and NK/T-cell lymphomas
T-cell prolymphocytic leukemia (T-PLL)	inv14 or t(14;14)/*TCL1A* gene rearrangement	Overexpression of oncogenic transcription factor	FISH	80% of T-PLL *TCL1A* protein detected by immunohistochemistry in most cases Rare variant translocations involving *MTCP1* also seen in T-PLL
T-cell large granular lymphocytic leukemia and chronic NK-cell lymphoproliferative disorders	*STAT3* and *STAT5B* gene mutations	Activation of STAT signaling	Allele-specific PCR, sequencing	*STAT3* mutations occur in 30% to 40% of LGL disease; *STAT5B* is rare (<5%) Helpful to distinguish some cases of clonal from reactive T-LGL disease

[a]Major pathophysiologic effect resulting from genetic lesion.
[b]Detection methods in parentheses indicate less clinically sensitive technique.
FISH, Fluorescence in situ hybridization; *MBR,* major breakpoint region; *MTC,* major translocation cluster; *NF-κB*, nuclear factor κ B; *RT-PCR,* reverse transcriptase polymerase chain reaction.

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diffuse large B-cell lymphomas (DLBCL) typically arising from preexisting FL. The juxtaposition of *BCL2* with transcriptionally active *IGH* leads to overexpression of BCL2 anti-apopotic protein, which supports neoplastic cell survival in the face of proliferative stress or DNA damage. At the genomic level, breakpoints in *BCL2* are most often highly clustered in the distal part of the third non-coding exon, known as the major breakpoint region (MBR), whereas the break-fusion sites in *IGH* are in the vicinity of the J_H exons (Fig. 24.1). Additional *BCL2* breakpoint sites have also been identified loosely grouped downstream of the MBR and have been designated as 3′ MBR, intermediate cluster region (icr), minor cluster region (mcr), and 5′ mcr. PCR approaches using extracted tumor DNA can detect nearly 65% of *BCL2-IGH* translocation rearrangements using a primer situated 5′ to the MBR and a consensus J-region *IGH* primer (see Fig. 24.1). Additional primer sets targeting the mcr and icr can identify these additional breakpoint sites, raising the clinical sensitivity of PCR technique above 80%; however, a significant minority of *BCL2* breakpoints remain undetectable even with these more comprehensive protocols. Notably, as is the case for any PCR assay involving fixed, paraffin-embedded material,

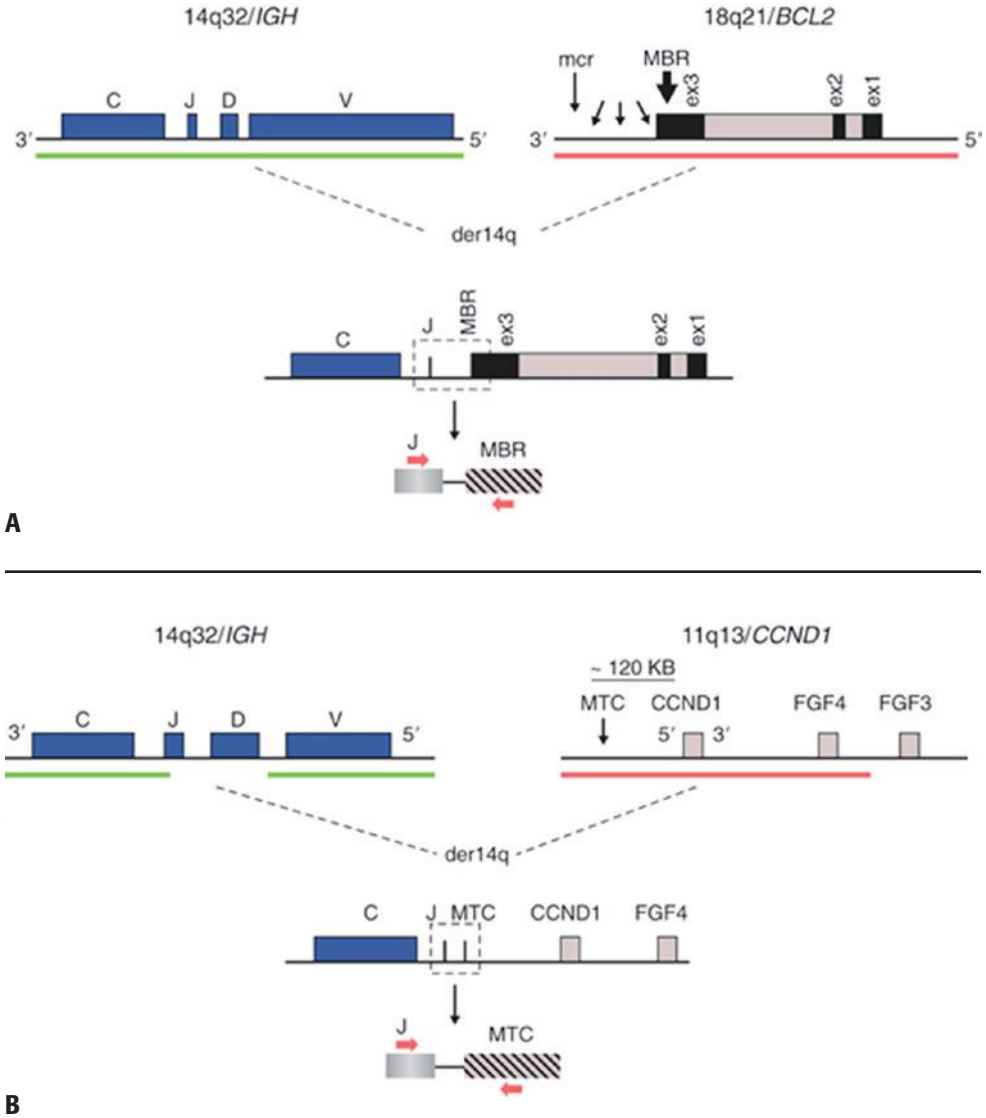


FIG. 24.1

The t(14;18)/*BCL2-IGH* and *CCND1-IGH* abnormalities. Panel A depicts the structures of the *IGH* and *BCL2* genes on their respective chromosomes. Most genomic breakpoints are tightly grouped in the major breakpoint region (MBR) of *BCL2*, situated in the terminal aspect of the non-coding exon 3. A small number of breaksites are distributed in the minor cluster region (mcr). Additional loose cluster areas for breakpoints include the 3′ MBR, 5′ mcr, and the intermediate cluster region (icr); these loci are indicated by *small arrows* between the MBR and mcr. Breakpoints in the *IGH* gene are located within the J_H region. FISH probes for *IGH (green bar)* typically span the entire locus, whereas probes for *BCL2* region *(orange bar)* span the entire gene as well as several hundred kilobases in both 5′ and 3′ directions, thus covering the MBR through the mcr. Panel B depicts the structures of *IGH* and *CCND1* (cyclin D1 gene) loci on their respective chromosomes. *CCND1*-related breakpoints occur over a very large genomic region of 11(q13) referred to as the *BCL1* locus. Approximately 50% of *BCL1* breakage abnormalities occur at the major translocation cluster (MTC); however, the remainder show wide distribution without significant additional clustering. *CCND1* is located more than 100 kb away from the MTC and becomes deregulated via fusion with *IGH* gene J_H region on 14(q32). FISH probes for *IGH (green bars)* typically hybridize to areas on either side of the *IGH* breakpoint region, whereas probes for *CCND1 (orange bar)* span several hundred kilobases of the genomic locus, including the MTC.

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both the analytic and clinical sensitivity will be adversely affected owing to the effects of DNA degradation. Dual color/dual fusion FISH analysis (D-FISH) using probes spanning the constant through variable regions of the *IGH* locus on 14(q32) and the entire *BCL2* gene on 18(q21) is highly sensitive and specific for detecting this translocation. FISH is also reliable using tissue sections or nuclei extracted from paraffin-embedded samples.

Detection of the *BCL2-IGH* can be useful to differentiate follicular lymphoma from atypical reactive follicular hyperplasia in challenging biopsies, especially when immunophenotyping and immunoglobulin gene rearrangement studies are not informative. More commonly, the presence of this genetic abnormality is used to establish the diagnosis of follicular lymphoma and exclude other "small B-lymphocyte" neoplasms with potentially overlapping morphologic features. In this regard it is important to keep in mind that BCL2 protein overexpression is commonly observed in many other types of non-Hodgkin lymphomas, resulting from genetic or epigenetic mechanisms distinct from the *BCL2-IGH*; the presence of Bcl2 protein overexpression per se is therefore not specific for lymphoma subclassification. Some cases of nodal FL in adults lack the t(14;18)/*BCL2-IGH*, and these are usually associated with greater centroblastic (i.e., grade 2 or 3) cytology and a relatively distinct molecular pathogenesis, including *BCL6* translocations. *BCL2* rearrangements are notably also not observed in the subgroup of pediatric-type FL and in rare patients presenting with high-cytologic-grade FL involving Waldeyer's ring tonsillar tissue with rearrangements of *IRF4/MUM1*.

MANTLE CELL LYMPHOMA AND THE t(11;14)/*CCND1-IGH* ABNORMALITY

The t(11;14)(q13;q32)/*CCND1-IGH* abnormality is observed in mantle cell lymphoma (MCL), as well as a subset of multiple myelomas. Past studies identified the t(11;14) in occasional cases of chronic lymphocytic leukemia (CLL), B-cell prolymphocytic leukemia (B-PLL), and splenic marginal zone lymphoma (SMZL); however, most, if not all such occurrences are now considered to represent clinical and morphologic variations of MCL. Thus, the presence of the t(11;14) can be considered essentially pathognomonic of MCL in the differential diagnosis of mature B-cell NHL. This translocation places the *CCND1* locus in proximity to the *IGH* (see Fig. 24.1). *CCND1* encodes the G1-phase cell cycle protein cyclin D1, which becomes highly overexpressed as a result of the t(11;14). The three D-type cyclins (D1, D2, and D3) promote early G1-phase entry to the cell cycle by binding to specific cyclin-dependent kinases (cdk4, cdk6), leading to inactivation of retinoblastoma (Rb) protein, transition to S-phase, and subsequent commitment to mitosis. Cyclin D1 (in contrast to its related counterparts) is notable for not being expressed in normal proliferating B cells; thus, overexpression of cyclin D1 via *CCND1* translocation is a hallmark of nearly all cases of MCL. Consequently, *CCND1* deregulation is thought to result in unchecked cell cycle entry, leading to increased cellular proliferation in MCL. Data from gene expression microarray analysis confirm a prominent proliferation and anti-apoptosis signature that seems to underlie both the pathogenesis of this disease and the observation that patients with MCL have a distinctly adverse outlook compared to other small B-cell neoplasms generally.

From the molecular diagnostic standpoint, approximately 50% of the breakpoints in the 11(q13)/*BCL1* locus aggregate in a region known as the major translocation cluster (MTC) located approximately 120 kilobases (kb) centromeric to *CCND1* (see Fig. 24.1). The remaining breakpoints can occur broadly on either side of the MTC, including even further centromeric or immediately 5′ of *CCND1*. Given the substantial breakpoint heterogeneity in this area of 11(q13) and lack of significant breakpoint clustering outside of the MTC, DNA PCR-based methods to detect the t(11;14) cannot achieve high detection sensitivity. The use of a *BCL1* MTC region primer with a consensus J_H-region *IGH* primer detects slightly less than half of locus rearrangements overall, and this is further diminished in paraffin tissue samples. In contrast, D-FISH employing two *IGH* region probes and a large *CCND1/BCL1* region probe can detect virtually all t(11;14) events in MCL (see Fig. 24.1). Along with the general advantages of the dual fusion strategy, the 11(q13) probe can simultaneously identify *CCND1* gene locus amplification in addition to the presence of the t(11;14). *CCND1* amplification has been associated with the more aggressive "blastoid" variant of MCL. Immunohistochemistry using antibodies directed to cyclin D1 has become a widespread ancillary test in establishing a diagnosis of MCL. Regardless, FISH analysis for *CCND1* gene rearrangements retains an important function for the specificity of MCL diagnosis, given that aberrant cyclin D1 expression can be seen in other types of B-cell neoplasms (e.g., hairy cell leukemia, rare cases of DLBCL) in the absence of the t(11;14) or *CCND1* alteration. Finally, very rare examples of MCL with characteristic morphologic and phenotypic features have been described lacking the *CCND1* gene rearrangement. These unusual MCL cases share a similar gene expression profile to *CCND1*+ MCL, but nearly half demonstrate alternate translocations involving the *CCND2* gene, which can be identified by specific FISH probe analysis.

HAIRY CELL LEUKEMIA AND ABNORMALITIES OF THE MAP KINASE PATHWAY

Classic presentations of hairy cell leukemia (HCL) are associated with well-recognized clinical and pathologic features. Nevertheless, the usual presentation of splenomegaly and bone marrow involvement and the cellular immunophenotype associated with HCL can overlap with other low-grade B-cell chronic neoplasms, notably splenic marginal zone lymphoma. In this regard, the discovery of a recurrent Val600Glu mutation (V600E) in the *BRAF* gene now provides an additional molecular marker to support the diagnosis of HCL, since this abnormality is present in more than 90% of HCL but

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very rarely seen or not observed in other low-grade B-cell tumors. The same *BRAF* mutation is also present in approximately 50% to 60% of Langerhans cell histiocytosis and is similarly found in the rare presentations of Erdheim-Chester disease. In a significant subset (16%) of HCL with *BRAF* V600E, cooperating mutations in the *CDKN1B* gene encoding the p27 cell cycle regulator are also present. Detection of the *BRAF* V600E is not strictly necessary for the diagnosis of HCL but can be a highly useful adjunct in situations where morphologic and immunophenotypic features are conflicting or unclear. In relapsed or refractory disease, the presence of the mutation provides a rationale for targeted therapy with B-Raf inhibitors. The *BRAF* V600E mutation can be detected using PCR and Sanger sequencing or more sensitively by allele-specific or quantitative PCR methods. Bone marrow aspirate preparations from patients with HCL are usually hemodilute secondary to associated reticulin fibrosis, and most individuals have significant pancytopenia at presentation; thus, a relatively sensitive molecular diagnostic approach is preferred. However, highly sensitive assays for *BRAF* V600E must also be constructed carefully to ensure accurate mutant allele discrimination and optimal specificity. Notably, the *BRAF* V600E mutation is not identified in a small proportion of classic HCL associated with *IGH* VH4-34 rearrangements or in cases of hairy cell leukemia–variant (HCL-V); however, mutations in the downstream *MAP2K1* gene have been described in these tumors, again highlighting the importance of deregulation of the mitogen activated protein (MAP) kinase pathway in this B-cell tumor and underscoring the potential for drugable therapeutic targets using Raf and MEK1 inhibitors. Neither *BRAF* nor *MAP2K1* mutations have been described recurrently in SMZL, providing useful molecular markers in this differential diagnostic situation.

LYMPHOPLASMACYTIC LYMPHOMA/ WALDENSTRÖM'S MACROGLOBULINEMIA: *MYD88* AND *CXCR4* GENE MUTATIONS

Low-grade B-cell lymphomas with plasmacytic differentiation include lymphoplasmacytic lymphoma (LPL) and marginal zone lymphoma. Differentiation of these entities based on morphologic and immunophenotypic features can be difficult, particularly in the bone marrow, at extranodal sites, or in small tissue biopsies. Even though both types of lymphomas exhibit indolent disease biology, the clinical manifestations of LPL (Waldenström's macroglobulinemia) can create distinct challenges for patient treatment, underlying the importance of accurate diagnostic classification. LPL is now much better defined by the presence of a recurrent somatic mutation in the *MYD88* gene, causing a leucine-to-proline substitution at amino acid 265 (i.e., p.Leu265Pro, or L265P), which is present in approximately 90% of cases. In the setting of characteristic morphologic features and IgM paraproteinemia, the finding of *MYD88* L265P is therefore a highly specific molecular marker for LPL. This mutation is also present in approximately 50% of IgM monoclonal gammopathy of uncertain significance (MGUS) but absent from true cases of IgM-positive multiple myeloma, suggesting that patients with IgM MGUS share a common pathogenesis more akin to LPL than plasma cell myeloma. Notably, IgG or IgA expressing lymphoplasmacytic lymphomas may harbor the *MYD88* L265P mutation in approximately 40% of cases. Although *MYD88* L265P is highly associated with LPL, it has also been noted to occur in approximately 10% of splenic marginal zone lymphomas, requiring careful attention to clinical and pathologic features to establish a correct diagnosis. The same mutation is also identified in nearly one third of diffuse large B-cell lymphomas (typically of non-germinal center or activated B-cell origin) and up to 90% of large B-cell lymphomas occurring in "immune privilege" sites such as testis, brain, or vitreoretinal tissue.

The *MYD88* L265P mutation can be detected by several techniques, including Sanger sequencing of the exon 5 region or more sensitive allele-specific or quantitative PCR approaches. Relatively high sensitivity (e.g., at least to 1% mutation level detection) is required because of the patchy nature of LPL infiltrates in bone marrow aspirate samples and the lack of significant peripheral lymphocytosis in many cases. Design of a short amplicon PCR method is also important to achieve equivalent clinical and analytic sensitivity from fixed paraffin-embedded bone marrow clot or tissue samples. In nearly 30% of LPL cases with *MYD88* L265P, a concurrent mutation is also present in the *CXCR4* gene. CXCR4 is the receptor for the chemokine CXCL12/SDF-1, acting to increase intracellular calcium levels and promote cellular signaling through MAPK1/3. Germline mutations of *CXCR4* are also found in WHIM (warts, hypogammaglobulinemia, infections, myelokathexis) syndrome and unusually, somatic *CXCR4* mutations in LPL show a similar pattern of C-terminal truncation (e.g., p.Ser338Ter) or frameshift alterations; these mutations can be detected by PCR and Sanger sequencing of the involved region of *CXCR4* exon 2. The co-occurrence of *CXCR4* mutations and *MYD88* L265P in LPL is associated with decreased blood counts, higher disease burden, and poorer response to targeted ibrutinib therapy, although overall survival is not significantly different from patients with LPL without *CXCR4* alterations. Nonetheless, the identification of *CXCR4* mutations can provide additional prognostic information to the clinician and enhance the diagnostic specificity for LPL.

EXTRANODAL MARGINAL ZONE LYMPHOMAS: ABNORMALITIES OF *MALT1, BCL10,* AND *FOXP1* GENES

The extranodal marginal zone (B-cell) lymphomas (ENMZL) or mucosa-associated lymphoid tissue lymphomas ("MALTomas") are a group of indolent B-cell tumors encompassing a variety of anatomic sites and several tumor genetic abnormalities. Although numeric chromosome imbalances (e.g., trisomies of 3, 18) are often observed in ENMZL, several recurrent chromosomal translocations have been identified

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in these lymphomas. The t(11;18)(q21;q21) is most frequently observed in gastric and pulmonary ENMZL, with a much lower incidence in orbital and salivary gland sites. This translocation results in the formation of the *BIRC3-MALT1* gene fusion. *BIRC3*, also called *API2* (or *IAP2*) encodes an apoptosis inhibitor protein, whereas the *MALT1* (or *MLT1*) gene produces a "paracaspase" protein. The chimeric *BIRC3-MALT1* is transcribed and creates a novel oncogenic protein. A second, less frequent translocation in ENMZL is the t(14;18)(q32;q21) generating the *MALT1-IGH* abnormality, with resultant deregulation of *MALT1* gene expression. The *MALT1-IGH* has been associated mainly with orbital, salivary gland, lung, and cutaneous ENMZL but is very infrequently seen in gastrointestinal primary sites. The genomic breakpoints on chromosome 18q21 are only slightly centromeric to the *BCL2* locus, such that classic karyotyping studies cannot differentiate between the *BCL2* translocation of follicular lymphoma and the *MALT1* rearrangement in ENMZL; FISH technique can, however, accurately distinguish these different rearrangements. A third rare t(1;14)(p22;q32) event has been found in gastric and pulmonary ENMZL and is characterized by the *BCL10-IGH* genetic fusion with overexpression of BCL10 protein. Remarkably, although substantial diversity is present in the anatomic location and genetic features of ENMZL, a common molecular pathogenesis has emerged regarding these translocation events. Both BCL10 and MALT1 are cytosolic adaptor proteins involved in a ternary complex with the CARD11 protein, which together mediate antigen receptor-induced activation of the nuclear factor kappa B (NF-κB) signal transduction pathway in normal B cells. In ENMZL, the respective translocations effectively deregulate either *MALT1* or *BCL10* expression, inducing constitutive activation of the NF-κB pathway. In contrast, the BIRC3-MALT1 fusion protein directly stimulates NF-κB activation and concurrently mediates an anti-apoptotic effect. Altered NF-κB effects on cellular proliferation and survival are considered critical to promoting tumor cell growth. The *FOXP1-IGH* abnormality arises from the t(3;14)(p14.1;q32) and has been identified in a small number of ENMZL and rare large B-cell lymphomas often in extranodal (e.g., gastric) sites. FOXP1 is a forkhead transcription factor that associates with nuclear corepressor proteins or modifiers to silence transcription at target-specific genes and is an important regulator of normal B-cell development.

Overall, FISH methods are best suited to identify the common translocations in ENMZL. *MALT1* gene abnormalities derived from the t(11;18) or t(14;18) abnormalities can be ascertained by D-FISH strategies. The chimeric BIRC3-MALT1 mRNA is expressed and can also be detected by RT-PCR technique; however, owing to substantial breakpoint heterogeneity in both of these genes, one of several fusion transcript isoforms could be present in any given case of ENMZL, requiring the use of multiple PCR primers. Molecular diagnostic evaluation is not routinely sought in ENMZL, although some situations may require specific investigations. For gastric ENMZL, identification of the t(11;18)/*BIRC3-MALT1* abnormality can be of clinical importance because translocation-positive tumors do not respond to antibiotic-mediated eradication of *Helicobacter pylori* infection, a therapeutic option which is otherwise often successful in a significant proportion of translocation-negative cases. Small extranodal tissue biopsies involved by low-grade B-cell NHL (B-NHL) can sometimes pose a difficult differential diagnosis, and FISH studies in this setting can be helpful for subclassification. Given that the FISH probes for *BCL2* and *MALT1* rearrangements are non-overlapping, this approach can differentiate between these t(14;18)-related abnormalities, providing an important clinical and pathologic distinction between follicular lymphoma and ENMZL with colonization of germinal centers. Extranodal tissue specimens with suspicious lymphoid populations are often also assessed for monoclonal immunoglobulin gene rearrangements by PCR to support the diagnosis of B-cell lymphoma or to check for minimal persistence of lymphoma following treatment. Positive molecular clonality studies in these scenarios require careful clinical and morphologic correlation in light of reports describing "pseudoclonal" B-cell populations in some reactive lymphoid hyperplasias or in cases with histologically negative post-therapy biopsies showing delayed clearance of true molecular disease.

CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA: MOLECULAR AND CYTOGENETIC PROGNOSTIC MARKERS

Several molecular genetic assays are becoming more frequently incorporated in the initial assessment of chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL). FISH analysis is commonly performed to detect changes in specific chromosomes: -13 or 13q-, 11q-, +12, and 17p-. Of these, 11q- and 17p- are associated with adverse outcomes, characterized by risk of early disease progression or therapeutic failure. Trisomy 12 is considered an intermediate-risk prognostic marker, and sole abnormalities of chromosome 13 appear to connote a more indolent disease course. The significance of 11q- and 17p- is related in part to monoallelic loss of the tumor suppressor genes *ATM* and *TP53*, respectively, in the regions of chromosomal deletion. For 17p-, which occurs in approximately 5% of untreated CLL/SLL patients, a deleterious point mutation of the remaining *TP53* allele is almost always present, indicating biallelic loss of p53 function. Patients undergoing therapy or who experience a transition to more aggressive disease have an even higher prevalence of 17p-/*TP53* mutations. Patients with 17p- disease tend to progress earlier, with poor responses to purine nucleoside analog drugs, and thus require alternative treatment options. Detection of the 17p- abnormality and *TP53* mutations in CLL/SLL is of critical importance clinically. Complementing FISH analysis for the 17p- abnormality (often performed as part of a CLL FISH prognostic panel), *TP53* mutation testing can be accomplished using tumor DNA by PCR and Sanger sequencing of exons 4 to 9, which encompasses the DNA-binding domain.

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Approximately half of patients with CLL demonstrate nucleotide alterations relative to reference germline *IGH* sequences, which are indicative of somatic hypermutation (SHM) following B-cell transit through the germinal center environment and antigen exposure. Extensive studies have shown that an *IGH* V_H mutation load greater than 2% from germline is associated with significantly better outcome (designated "mutated" CLL) versus those cases with SHM 2% or less ("unmutated" CLL). Of note, a subset of CLL cases with V_H3-21 rearrangements is associated with poor outcome regardless of SHM status. Determination of SHM requires an optimized PCR assay spanning the entire leader (or 5′ FR1) through J_H region of the clonal *IGH* rearrangement, followed by Sanger sequencing and database comparison to a reference human *IGH* germline sequence library (e.g., http://www.imgt.org; https://www.ncbi.nlm.nih.gov/igblast). Either DNA or reverse-transcribed mRNA can be used as the starting material, and the PCR strategy can employ leader region consensus primers, FR1 family-specific primers, and J_H primers in a multiplex or multiple single reaction approach. Recommendations for CLL *IGH* V_H SHM analysis have been put forth by the European Initiative in CLL (ERIC). Database analysis to determine clonal identity and SHM percentage from assay-generated sequencing data is now very robust and rapid. Newer developments utilize NGS methods for directly analyzing PCR products and identifying the exact V_H DNA sequence, presence of SHM, percent mutation from germline, and the translational status of the predicted rearrangement (e.g., in-frame versus unproductive) (see Fig. e24.3). NGS techniques also demonstrate very high analytic sensitivity and specificity because of the ability to identify many thousands of individual B-cell rearrangements at great sequencing depth, in turn also providing a basis for ongoing MRD monitoring. Clonal identity evaluation using PCR and Sanger sequencing or possibly NGS has been recognized as a prognostically important factor in patients with CLL who experience transformation to large B-cell lymphoma (Richter transformation). Patients exhibiting the same clonal immunoglobulin gene rearrangement sequence in both the original CLL and large B-cell lymphoma (i.e., true-transformation) appear to have a significantly worse outcome compared to individuals with distinct CLL and large B-cell clones (i.e., de novo large B-cell lymphoma). Broader application of NGS methods in the study of CLL genomics has identified additional recurrent gene mutations of interest, most commonly including somatic alterations in *SF3B1*, *NOTCH1*, *ATM*, and *BIRC3*. Mutations in these genes have been associated with adverse clinical behavior, although currently the optimal use of this information relative to established prognostic factors is not entirely clear in the era of new immunotherapy agents and tyrosine kinase inhibitor use (e.g., Bruton's tyrosine kinase [BTK] targeting by ibrutinib). With the increased use of small-molecule signaling pathway inhibition, acquired resistance mutations are now known to occur in target genes, including *BTK* and phospholipase C-gamma 2 (*PLCG2*). The mutation hotspots are recurrent, allowing for detection by allele-specific (AS)-PCR, melting curve, or sequencing analyses in patients who become refractory to these treatments.

AGGRESSIVE B-CELL LYMPHOMAS

DIFFUSE LARGE B-CELL LYMPHOMAS AND HIGH-GRADE B-CELL LYMPHOMAS: CELL OF ORIGIN CLASSIFICATION AND ASSOCIATED MOLECULAR ABNORMALITIES AND REARRANGEMENTS OF BCL2, BCL6, AND MYC

The diffuse large B-cell lymphomas share similar morphologic features but exhibit substantial tumor genetic heterogeneity underlying the variability in clinical outcomes observed among patients. Gene expression profiling (GEP) of DLBCL has revealed a general cell of origin (COO) dichotomy between lymphomas with "germinal center" (GCB) and non-germinal center or "activated" B-cell (ABC) signatures. GCB DLBCL has been shown to have significantly better overall survival and treatment response compared to ABC DLBCL in the era of routine chemo-immunotherapy protocols. A standard gene expression-based clinical molecular diagnostic method using a limited gene set has been difficult to reproducibly establish. Most lymphoma diagnostic practices therefore rely on immunohistochemical algorithms as a surrogate for GEP. Although immunohistochemistry provides fairly good correlations when applied rigorously, a substantial subset (nearly 20%) of cases is difficult to categorize confidently as GCB or ABC. Recent commercial developments for GEP, such as the Nanostring nCounter technology (Nanostring Technologies, Seattle, WA), appear promising for widespread diagnostic adoption to support COO classification for DLBCL and also enable GEP analysis from paraffin-embedded fixed tissues and small biopsy specimens. This platform employs hybrid capture and fluorescent reporter probes to bind to many specific target mRNA molecules directly, allowing the resolution, digital counting, and precise quantification of transcripts that define either GCB or ABC profiles. Along with improvements to COO classification methods, the genomics of DLBCL continue to be defined using NGS. Important examples include the identification of deregulated toll-like receptor (TLR) and NF-κB pathways, or aberrant ("chronic active") B-cell receptor signaling in many ABC-type DLBCL related to mutations in *MYD88*, *CARD11*, or *CD79A* among others. Some gene mutations are also typically observed almost exclusively in GCB-type lymphomas (e.g., gain of function mutations in the epigenetic modifier *EZH2*). Somatic *TP53* gene alterations are also seen at low frequency in DLBCL regardless of subtype, and this finding has been associated with poorer prognosis. Although NGS testing is currently not in widespread use for DLBCL evaluation, COO classification and comprehensive sequencing analysis of gene mutations represent complementary aspects of integrated genomic profiling for DLBCL; the COO information is directly used for assessing patient prognosis, whereas the detection of specific tumor-associated genetic abnormalities can potentially identify targets of cellular pathway deregulation for novel therapeutic interventions.

Several recurrent cytogenetic abnormalities have been identified in DLBCL, including the t(14;18)/*BCL2-IGH* (20%

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to 30% of cases with GCB phenotype) and rearrangements of the *BCL6*, *MYC*, and *ALK* genes. *BCL6* is located on chromosome 3(q27) and can become deregulated by translocation to the *IGH* gene in a t(3;14)(q27;q32) event. However, *BCL6* also becomes aberrantly activated by promoter substitutions arising from translocations with many other non-immunoglobulin genes. Overall, approximately one third of DLBCL show the presence of a *BCL6* rearrangement; an even higher proportion reveal somatic mutations involving the 5′ regulatory region of *BCL6*, sometimes concurrent with gene translocation. The BCL6 gene product is a critical transcription factor responsible for normal secondary follicle formation during the germinal center reaction in lymphoid tissues and for proper T-cell–dependent antibody responses on exposure to antigen. Accordingly, Bcl6 is strongly expressed by normal germinal center (GC) B cells but is not present in naive and post-germinal center B cells. BCL6 is thought to protect normal GC B cells during the affinity maturation process (i.e., somatic hypermutation) in part by downregulating pro-apoptotic stimuli elicited by physiologic DNA double-strand breakage. BCL6 is overexpressed in a subgroup of B-cell lymphomas, most notably those tumors that derive from normal GC counterparts (e.g., FL, many DLBCL, Burkitt lymphoma). *BCL6* rearrangements in DLBCL can be detected using break-apart probe (BAP) FISH technique. *MYC* gene rearrangements occur in approximately 5% to 10% of typical centroblastic DLBCL via translocations to the *IGH* or light chain (*IGK*, *IGL*) genes, or occasionally to non-immunoglobulin genes. The presence of an *MYC* translocation in general confers a significantly worse outcome, although some data have suggested a less adverse prognosis for non-immunoglobulin partners in this context. Because the morphologic and immunophenotypic features of *MYC*-rearranged DLBCL are not otherwise distinctive, routine FISH evaluation of the *MYC* locus in de novo cases has been advocated, although this remains controversial. Importantly, because some classic DLBCL cases and a subset of B-cell lymphomas with high-grade (e.g., blastoid, "Burkitt-like") morphologic features and GCB phenotype also have gene rearrangements of *MYC* concurrent with *BCL2* and/or *BCL6* (i.e., "double- or triple-hit" lymphomas), many practices combine COO classification, Myc protein expression, and a reflex FISH strategy to recognize these highly aggressive tumors at diagnosis. One such approach could involve initial screening for *MYC* abnormalities by BAP FISH and, if positive, D-FISH to identify the immunoglobulin partner, followed by *BCL2* and/or *BCL6* BAP FISH.

BURKITT LYMPHOMA AND *MYC* GENE REARRANGEMENTS

Burkitt lymphoma (BL) is an uncommon, yet distinctive and highly aggressive, B-cell NHL. BL is classically associated with the t(8;14)(q24;q32) cytogenetic anomaly resulting in the fusion of the *MYC* and *IGH* genes, but less commonly, *MYC* can be involved with translocations to the *IGK* and *IGL* immunoglobulin light chain genes on 2(p12) or 22(q11), respectively. The normal MYC gene product is a highly regulated early response transcription factor that coordinates mitogenic stimuli with nuclear responses for DNA synthesis and replication. *MYC* gene translocation thereby leads to marked overexpression of Myc protein and upregulation of many genes driving cell proliferation. The break-sites in *MYC* are variable and occur over a large genomic span. The so-called endemic (or African) type of BL is characterized by a strong association with chronic Epstein-Barr virus (EBV) infection and demonstrates a molecular pattern of *MYC* breakpoints far upstream of the gene and corresponding *IGH* disruption within the J_H-region. Sporadic BL cases show genomic breaks involving the immediate 5′ region of *MYC* and the heavy chain switch region of *IGH*; sporadic BL is less frequently EBV-positive. The observed variation in *IGH* locus breakpoints suggests that the translocation occurs in B cells at different maturational stages in the endemic versus sporadic types, although there is no apparent clinical relevance to this phenomenon. Recent data implicate activation-induced cytidine deaminase in the pathogenesis of a significant subset of *MYC-IGH* translocations. Mutations in the transcription factor gene *TCF3* (a regulator of CCND3/cyclin D3) or its negative regulator *ID3* are present in a substantial fraction of both sporadic and endemic variants. The definitive diagnosis of BL requires the demonstration of a solitary *MYC* gene rearrangement and, more specifically, the presence of a translocation to the *IGH*, *IGK*, or *IGL* loci in the correct morphologic and immunophenotypic context. The breakpoints occurring in *MYC* and *IGH* are not amenable to standard DNA PCR methods, because of the large intervening DNA regions and lack of significant break-site clustering, but are readily detected by FISH technique. BAP FISH technique can be used to rapidly identify *MYC* gene disruption, in conjunction with a D-FISH approach to specifically identify the *MYC-IGH* or light chain gene fusion (Fig. 24.2). Expedient detection of *MYC* gene rearrangement is critical in BL because the tumor doubling time is rapid and chemotherapy can be curative in many younger patients. As already noted, *MYC* gene rearrangements are also found in several other examples of aggressive B-NHL, including a minority of de novo diffuse large B-cell lymphomas, many "double-hit" high-grade B-cell lymphomas (in conjunction with *BCL2* and/or *BCL6* rearrangements), some large-cell or blastoid transformations of indolent B-cell lymphomas, HIV/AIDS-associated lymphomas, and in some monomorphic posttransplant lymphoproliferative disorders (PTLD).

T-CELL LYMPHOMAS AND CHRONIC LYMPHOID LEUKEMIAS: COMMON TUMOR GENETIC ABNORMALITIES

ANAPLASTIC LARGE-CELL LYMPHOMA: ALK GENE REARRANGEMENT POSITIVE AND NEGATIVE

Among the peripheral T-cell lymphomas, anaplastic large-cell lymphoma (ALCL) is a distinctive pathologic entity.

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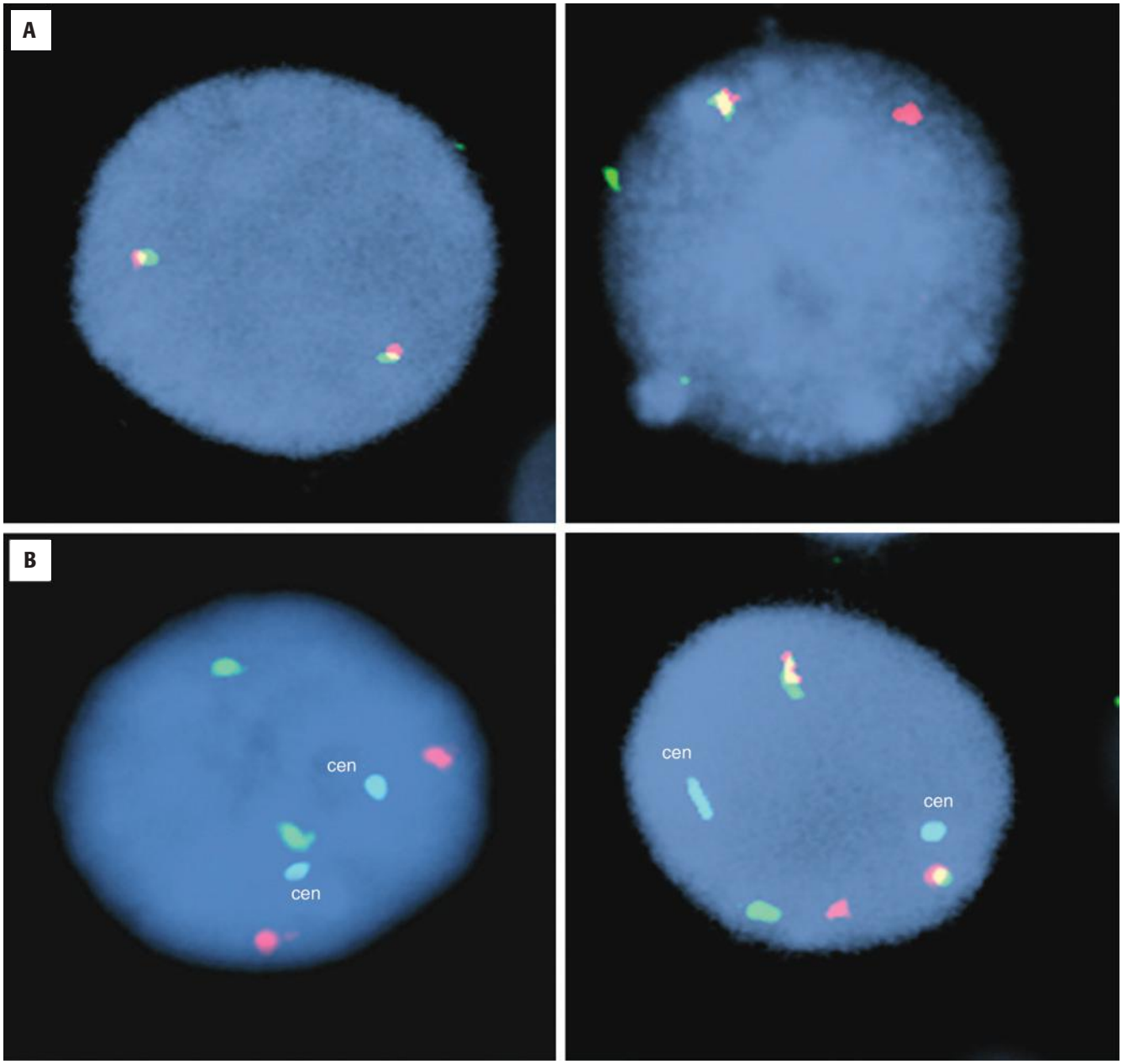


FIG. 24.2

Detection of *MYC* gene rearrangements. *MYC* gene locus abnormalities on chromosome 8(q24) can be detected as disruption of intact *MYC* or as specific translocation events. Panel A (two top images) shows *MYC* break-apart probe (BAP) interphase FISH analysis. Left image displays intact dual color probes directed at 5′ and 3′ regions of the *MYC* locus, generating two distinct yellow fusion signals in a normal cell. Right image of a Burkitt lymphoma cell shows one intact fusion signal (normal) and separate single red and green fluorescence dots, indicating that one locus of the *MYC* has been rearranged, implying *MYC* gene translocation; however, the nature of the underlying translocation is not revealed by BAP-FISH. Panel B demonstrates a D-FISH method to detect *MYC-IGH* gene fusion arising from the t(8;14) abnormality. Left image shows a normal cell pattern with two red and two green signals (normal *IGH* and *MYC* loci, respectively). The aqua color dots labeled "cen" represent chromosome 8 centromeric probes. Right image of a Burkitt lymphoma cell displays two yellow fusion signals indicating reciprocally translocated *IGH* and *MYC* genes on the respective der(14) and der(8) chromosomes; single red and green signals represent the remaining normal genomic regions. *(FISH images courtesy of Dr. Rhett P. Ketterling, MD, Division of Cytogenetics, Mayo Clinic, Rochester, MN.)*

Recognized primarily from its distinctive morphology and uniform expression of CD30 antigen, a substantial subset of systemic ALCL is also genetically defined by the presence of anaplastic lymphoma kinase (*ALK*) gene rearrangements. The most commonly characterized abnormality is the t(2;5)(p23;q35), which couples the nucleophosmin gene (*NPM1*) to *ALK*. NPM1 is a multifunctional protein involved in nuclear-cytoplasmic shuttling of ribosomal units, cell cycle regulation through stabilization of p53 protein, and maintaining proper chromosome alignment during mitosis. ALK is a tyrosine kinase protein that is not expressed in normal lymphocytes. The resultant *NPM1-ALK* fusion gene produces a chimeric protein causing constitutive deregulation of ALK and consequent aberrant phosphorylation of downstream

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signaling target proteins. Other translocations involving *ALK* also occur in this group of ALCL, including the t(1;2)(q25;p23)/*TPM3-ALK*, t(2;3)(p23;q35)/*TFG-ALK*, and inv(2)(p23q35)/*ATIC-ALK* abnormalities. A key feature of *ALK* gene fusion products in ALCL is that the partner gene in each case dictates the intracellular distribution and activity of the aberrantly expressed ALK kinase. Determination of *ALK* gene rearrangement status in a case of morphologic and phenotypic ALCL is critical, since *ALK*-positive neoplasms are characterized by relatively favorable treatment responses and outcome. *ALK* gene rearrangements are best identified using a BAP FISH strategy, one advantage of which is the applicability to paraffin-embedded fixed tissue biopsies. Identification of the specific involved partner gene or chromosome is not necessary, as no particular prognostic significance has been ascribed to the different translocation variants. The technically simple application of ALK immunohistochemistry largely obviates the need for molecular cytogenetic evaluation in many cases, and the cellular pattern of ALK distribution can provide some information concerning the nature of the *ALK* translocation.

The group of *ALK* rearrangement-negative ALCL has also become better defined and now includes two subsets characterized by chromosomal rearrangements of 6p25.3 involving the locus encompassing the dual specificity phosphatase (*DUSP22*) and interferon regulatory factor-4 (*IRF4*) genes, and a small group of tumors with rearrangements of the p53 family *TP63* gene locus on chromosome 3q28. *ALK*-negative ALCL with *DUSP22* rearrangements are associated with a relatively better prognosis compared with CD30-positive peripheral T-cell lymphomas generally, whereas *TP63* rearranged cases have a very aggressive course. Both of these genetic abnormalities are best identified by interphase FISH strategies. A subset of primary cutaneous ALCL also has rearrangements of the *DUSP22/IRF4* locus. Of note, rare cases of CD30-negative large B-cell lymphoma with an unusual immunoblastic (plasmablastic) morphology and cytoplasmic IgA positivity are described with rearrangements of the *ALK* gene arising from t(2;17)(p23;q23)/*CLTC-ALK* or, occasionally, t(2;5)(p23;q35)/*NPM1-ALK* abnormalities. The pattern of ALK protein expression in *CLTC-ALK*–positive B-cell tumors shows a distinctive punctate membranous distribution reflecting localization in clathrin-coated pits.

PERIPHERAL T-CELL LYMPHOMAS: RELEVANT GENETIC ABNORMALITIES

Although the genomic pathology of peripheral T-cell lymphoma (PTCL) remains incompletely understood, some entities, in addition to ALCL, have become more distinctly defined. The group of nodal T-cell lymphomas derived from T-follicular helper (TFH) cells is one category, which include angioimmunoblastic T-cell lymphoma (AITL). Certain translocations, such as the *ITK-SYK* and *CTLA4-CD28* gene fusions, have been associated with this group, although the latter abnormality has also been reported in other PTCL subtypes and extranodal NK/T-cell lymphomas. Detection of the *ITK-SYK* fusion is accomplished by specific D-FISH analysis, and both FISH and RT-PCR techniques can detect the *CTLA4-CD28* anomaly. These gene fusions may represent therapeutic targets with the goal of blocking aberrant intracellular and T-cell receptor signaling, respectively. More extensive analysis by NGS methodology has disclosed recurrent gene mutations involving *IDH2*, *TET2*, *RHOA*, *DNMT3A*, and *CD28*, among others, in the TFH category. *IDH2* R172 mutations, for example, appear to define a unique subset of AITL. Molecular diagnostic evaluation of TFH lymphomas typically involves the supportive assessment of clonal T-cell receptor gene rearrangements when indicated; however, with the recognition of recurrent gene mutations involving metabolic, cell division, and epigenetic and immune processes, focused NGS panels may represent an attractive approach to identify alterations that may be amenable to rational small-molecule inhibitor or immunologic therapies. Despite advances in ALCL and TFH categories, the large group of PTCL unspecified remains genetically complex, and further insights from gene expression profiling and NGS mutation profiling will be central to improving the classification and outcome for these patients.

T-CELL PROLYMPHOCYTIC LEUKEMIA

T-cell prolymphocytic leukemia (T-PLL) is characterized by rearrangements of the *TCL1A* gene. In 80% of T-PLL, *TCL1A* is altered and abnormally activated by an inv14(q11q32) or t(14;14)(q11;q32) event. In either case, the *TCL1A* proto-oncogene is brought in proximity to the T-cell receptor alpha locus (*TRA*) on 14(q11). This pathogenic process leads to overexpression of TCL1A, a transcription factor that is not normally present in mature T cells. *TCL1A* gene rearrangement is also not observed in other types of peripheral T-cell lymphomas, constituting a relatively specific genetic marker for T-PLL. FISH analysis can detect rearrangements of this gene arising from either the inversion or translocation anomalies. Immunohistochemistry can also be used to identify overexpression of the protein. Of note, a small group of T-PLL demonstrates intact *TCL1A* alleles but instead harbor the alternate translocation events t(X;14)(q28;q11) or t(X;7)(q28;q35), which involve fusion of a *TCL1A*-related gene *MTCP1*, to either the *TRA* or T-cell receptor beta (*TRB*) loci, respectively.

T-CELL LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

Recent data have identified deregulation of the JAK/STAT pathway, with the finding of mutations involving *STAT3* (in approximately one third of cases) and less frequently *STAT5B* (<5% of cases) in T-LGL disease. *STAT3* gene mutations are also observed in a subset of chronic lymphoproliferative disorders of natural killer (NK) cells. Mutations in these genes are considered activating and can be detected by PCR

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and sequencing or by target allele-specific PCR methods. Given the difficulty in resolving a reactive subset-restricted T-LGL expansion from true chronic leukemia in many patients, identification of these gene mutations can be a valuable molecular diagnostic adjunct to support the latter diagnosis.

MOLECULAR DIAGNOSTIC EVALUATION OF LYMPHOBLASTIC LEUKEMIAS

DETECTION OF COMMON GENETIC ABNORMALITIES IN B-CELL LYMPHOBLASTIC LEUKEMIA/LYMPHOMA

The molecular genetics of B-cell lymphoblastic leukemia (precursor B-cell acute lymphoblastic leukemia, B-LBL) represent a paradigm for linking tumor genetics with clinical outcomes, especially in the pediatric population. In childhood B-LBL, a wide spectrum of genomic alterations has been identified, including numeric and structural chromosome alterations, activating chimeric kinase gene fusions, gene amplifications, and pathogenic gene mutations or deletions. These events have distinct prognostic value and help to classify patients according to relapse risk (e.g., standard, low, or high risk) and in turn provide the basis of current risk-adjusted therapy (Table 24.3). Some of the same genetic changes are also found in a subset of adult patients with B-LBL, whereas others are exclusive to childhood presentations. This section is primarily focused on the utility of cytogenetic and molecular diagnostic assessment of B-LBL. Genetic abnormalities in T-cell lymphoblastic leukemia/lymphoma (T-LBL) continue to become better defined and include translocations of *HOX* genes with the T-cell receptor gene loci, mutations of *NOTCH1*, *PHF6*, or *JAK1* genes, *TAL1* oncogene activation, and gene fusions involving *ABL1*. Cases of early T-cell precursor (ETP) acute lymphoblastic leukemia have been characterized by gene mutations involving cell signaling (e.g., *RAS*, *FLT3*, *JAK1*/*3*), transcription factors (*RUNX1*, *ETV6*, etc.), and epigenetic modification (e.g., *EZH2*, *SUZ12*) that surprisingly resemble abnormalities observed in myeloid malignancies. Application of molecular diagnostic testing is currently less frequently applied to T-LBL beyond FISH analysis for specific genomic rearrangements and is therefore not further detailed in this section.

Numeric chromosome aneuploidies and a set of recurrent translocations are identified in approximately half of pediatric patients with B-LBL. A hyperdiploid chromosome complement is present in nearly one third of pediatric B-LBL at diagnosis; this is defined as a tumor chromosome number greater than 52 associated with trisomies of chromosomes 4, 10, and 17, and these patients have a "good" risk profile with a favorable long-term outlook. Hyperdiploid cases can be identified by cytogenetics or a combination of flow cytometric assessment of relative DNA content analysis of lymphoblast cell nuclei (DNA index >1.16) and targeted FISH probe analysis. Conversely, the presence of a hypodiploid chromosome number (<44) defines a rare group of individuals with aggressive disease and early treatment failure. These patients may be considered as early candidates for allogeneic stem cell transplantation. Tumor hypodiploidy can similarly be ascertained by standard cytogenetics and FISH analysis, or it can be inferred from flow cytometric DNA index. An intrachromosomal amplification of chromosome 21 ("iAMP21") has also recently been associated with poor outcome, arising from complex break-fusion events (chromothripsis) mainly involving the long (q) arm and leading to amplification of transcription factor genes such as *RUNX1* and *ERG*. Cases of iAMP21 can be identified by FISH analysis of specific loci (e.g., *RUNX1*) or chromosome array (e.g., SNP-A or A-CGH) to detect the variable copy number alterations.

In approximately 25% of pediatric B-LBL, common recurrent chromosome translocations are identified, including the t(12;21)(p13;q22)/*ETV6-RUNX1*, t(9;22)(p34;q11)/*BCR-ABL1*, t(1;19)(q23;p13)/*TCF3-PBX1*, and rearrangements of the *KMT2A* (*MLL*) gene. The t(12;21)/*ETV6-RUNX1* fusion is the most common recurrent gene fusion in pediatric B-LBL (20% of cases), although it is very rarely identified in adult patients. *ETV6-RUNX1*–positive patients have an excellent clinical outcome following chemotherapy, similar to the group with chromosome hyperdiploidy and associated "good prognosis" trisomies. The occurrence of late relapses in *ETV6-RUNX1* B-LBL is a concern for a subset of these individuals, although secondary treatment responses are often successful. The *ETV6* gene, which encodes an Ets family transcription factor, is joined with *RUNX1* to form the chimeric fusion gene on chromosome 12p. The hybrid ETV6-RUNX1 protein disrupts the normal function of core binding factor (CBF) transcriptional activity and, similar to the *RUNX1-RUNX1T1* in acute myeloid leukemia (AML), this leads to profound alterations affecting progenitor cell proliferation and normal differentiation. In addition, a large proportion of *ETV6-RUNX1* lymphoblastic leukemias also have deletions of the remaining *ETV6* allele on 12p, indicating that complete inactivation of ETV6 function may also be pathogenically important. In the majority of t(12;21)/*ETV6-RUNX1*–positive B-LBL, the translocation is cytogenetically cryptic, requiring either RT-PCR or FISH molecular methods for detection. Breakpoints in the *ETV6* gene are generally invariant within intron 5; however, the *RUNX1* gene can show heterogeneity both in genomic break-site location, as well as by alternative splicing of RUNX1 exons in the chimeric mRNA. This situation leads to as few as one and potentially as many as four ETV6-RUNX1 fusion transcripts that can be amplified and detected by RT-PCR technique in any given patient; the most frequent types, present in nearly all cases, involve fusion of ETV6 exon 5 with exons 2 or 3 of RUNX1. FISH technique also readily detects the *ETV6-RUNX1* translocation in pediatric B-LBL, and this approach further enables the simultaneous identification of an accompanying deletion of the non-translocated *ETV6* allele, if present.

The t(9;22)(q34;q11)/*BCR-ABL1* abnormality (Philadelphia, or Ph, chromosome) is present in 3% of B-LBL in children and 20% to 25% of adult patients and is associated with a

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TABLE 24.3
Genetics of B-Lymphoblastic Leukemia/Lymphoma (B-LBL)

FAVORABLE PROGNOSIS ABNORMALITIES		
Genetic Abnormality	**Molecular Diagnostic Detection**	**Notes**
t(12;21)/*ETV6-RUNX1*	FISH, RT-PCR	20% of childhood B-LBL, rare in adult patients
Hyperdiploid chromosome content (>52) with trisomies of 4, 10, and 17	Cytogenetics, DNA index (flow cytometry), and FISH	25% to 30% childhood B-LBL
UNFAVORABLE PROGNOSIS ABNORMALITIES		
Genetic Abnormality	**Molecular Diagnostic Detection**	**Notes**
Hypodiploid chromosome content (<44)	Cytogenetics, DNA index (flow cytometry), and FISH	Rare (<5%) childhood B-LBL
t(9;22)/*BCR-ABL1*	Cytogenetics, FISH (RT-PCR)	3% of childhood and 20% to 25% of adult B-LBL Quantitative p190 or p210 RT-PCR useful for sensitive MRD detection
iAMP21	FISH chromosome microarray (A-CGH, SNP-A)	Rare (2%) of childhood B-LBL Complex rearrangements of 21q region resulting in multiple extra copies encompassing *RUNX1* and other gene regions
KMT2A (*MLL*) rearrangements	Cytogenetics, FISH (RT-PCR)	Numerous *KMT2A* partner genes preclude efficient assessment by PCR methods
"Ph-like" genetic abnormalities	Multimodal approach including next-generation sequencing (NGS), FISH, gene expression profiling, chromosome microarray, RT-PCR	10% to 15% B-LBL and seen in childhood as well as younger adult patients Gene expression signature overlaps with true BCR-ABL1–positive B-LBL Complex group of genetic alterations including chimeric gene fusions, gene mutations, copy alterations (deletions) De-regulation of cell signaling pathways (e.g., JAK-STAT, PI3 kinase, cytokine-mediated [e.g., *CRLF2*] and Ras-MAP kinase]) and disruption of B-cell transcriptional pathways Co-occurring mutations are common (e.g., *CRLF2* rearrangement and *JAK* gene mutations, or *IKZF1* deletions) Alterations are potentially drugable (e.g., tyrosine kinase or JAK-STAT inhibition)
Minimal residual disease (MRD) positivity	Flow cytometry, leukemia-specific immunoglobulin and T-cell receptor gene rearrangements detected by clone-specific PCR or NGS	End-induction MRD of $>10^{-4}$ NGS rapidly emerging as method of choice for optimal accuracy and sensitivity to detect clone-specific gene rearrangements

very poor prognosis typified by primary chemoresistance and high relapse risk. Approximately 80% to 90% of pediatric and two thirds of adult Ph-positive B-LBL produce a BCR-ABL1 fusion mRNA transcript termed e1-a2, resulting in a 190-kD oncoprotein. Remaining cases are associated with a p210 protein and corresponding e13-a2 or e14-a2 (formerly b2-a2 or b3-a2) BCR-ABL1 transcripts. The chimeric BCR-ABL1 protein alters the normal cellular localization and regulation of the ABL1 tyrosine kinase and renders it constitutively active, leading to increased cellular proliferation. This oncoprotein is readily transforming in in vivo transduction models of leukemia, but its phenotypic outcomes as related to acute versus chronic disease are dependent on the hematopoietic cell type context (i.e., stem cell versus committed lymphoid progenitor). Molecular details of the *BCR-ABL1* fusion gene are discussed in more detail in the section on chronic myeloid leukemia (CML) and illustrated in Fig. 24.3; the *BCR-ABL1* abnormality can be readily identified by cytogenetics, FISH, or RT-PCR.

A small proportion of pediatric B-lineage B-LBL, most commonly with a "pre-B" cell (cytoplasmic IgM positive) immunophenotype, harbor an unbalanced t(1;19)(q23;p13) with the resultant formation of a chimeric *TCF3-PBX1* oncogene (the *TCF3* gene is also known as *E2A*). This abnormality joins elements of two disparate transcription factors to create a novel leukemogenic TCF3-PBX1 protein.

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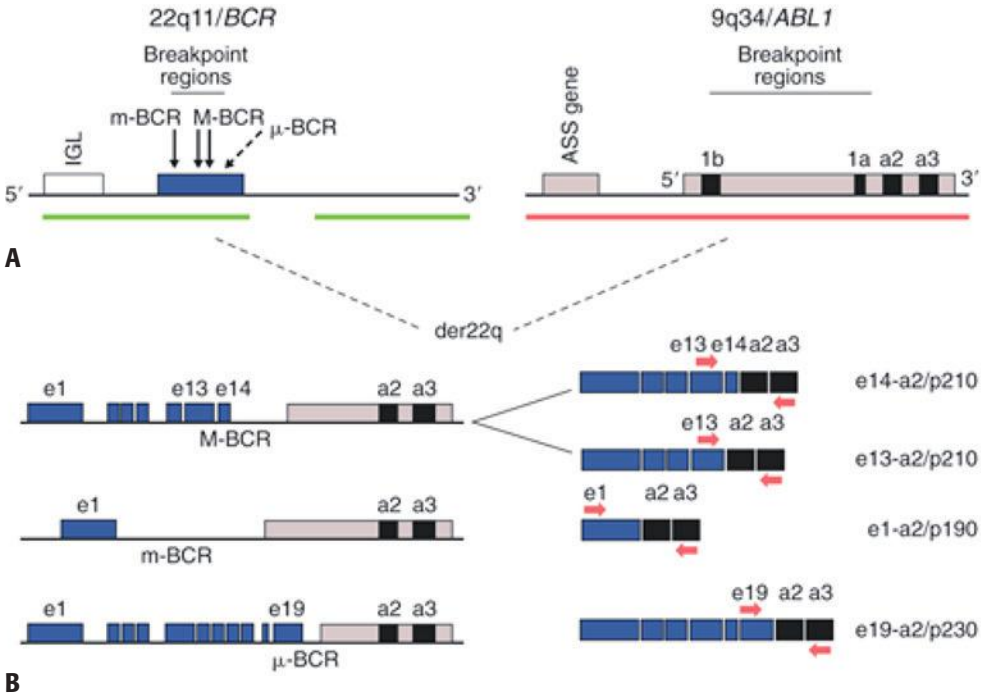


FIG. 24.3

The t(9;22)/*BCR-ABL1* abnormality. Panel A depicts the arrangement of *BCR* and *ABL1* genes on their respective chromosomes; other genes (e.g., the immunoglobulin lambda light chain gene, *IGL*) are also shown for relative orientation (gene maps are not shown to proper genomic scale, especially *BCR*, which is represented as a single locus rather than the individual exons). Genomic breakpoints in *ABL1* occur variably over a large region of DNA representing intron 1 of the gene. However, the subsequent juxtaposition with *BCR* results in the consistent presence of *ABL1* exon 2 (a2) in the *BCR-ABL1* fusion gene. *BCR* breakpoints are heterogeneous: essentially, all cases of chronic myeloid leukemia (CML) harbor breakpoints in the major breakpoint cluster region (M-BCR), whereas the majority of adult Ph+ B-lymphoblastic leukemia (Ph+ B-LBL) and most cases of pediatric Ph+ LBL are characterized by breaksites occurring in the minor breakpoint cluster region (m-BCR). However, nearly one third of adult and a small minority of pediatric Ph+ LBL may also manifest M-BCR–type breakpoints. A third breakpoint region, the μ-BCR, is situated farther 3′ in the *BCR* gene. FISH probes for the *BCR (green bars)* typically span several hundred kilobases (kb) on either side of the gene, whereas probes for *ABL1 (orange bar)* span the entire gene as well as several hundred kilobases centromeric to cover the arginosuccinate synthetase (*ASS*) gene. Deletion of the 5′ region of the reciprocal *ABL1-BCR* (which includes the latter genetic locus) occurs on the der9(34) in a subset of CML patients and has been linked in some studies to adverse prognosis. Panel B illustrates the common resulting *BCR-ABL1* fusion gene structures on the der22(q11). M-BCR locus breakpoints result in the fusion of either *BCR* exon 14 or exon 13 to *ABL1* exon a2. The resultant e14-a2 or e13-a2 chimeric mRNA species *(right side)* encode a p210 kilodalton (kD) BCR-ABL1 oncoprotein. In contrast, the m-BCR breakpoint joins the *BCR* exon e1 with *ABL1* exon a2 to form the e1-a2 transcript and a corresponding p190 kD product. The longer e19-a2 chimeric fusion produces a third type of functional leukemic protein, termed p230. Oligonucleotide primers can detect each of these common BCR-ABL1 mRNA species by RT-PCR technique *(small orange arrows)*; a single e13 primer can identify both e14-a2 and e13-a2 transcript forms, whereas separate primer reactions are required to identify the e1-a2 and e19-a2 mRNA fusions.

Continued

TCF3 is an important mediator of normal lymphoid cell and myocyte development, whereas PBX1 is a DNA binding protein that is not expressed in normal lymphocytes. Leukemia patients with this translocation fusion gene have been linked to more aggressive disease biology, although optimized therapeutic management has now improved the clinical outcome in most cases. A single chimeric TCF3-PBX1 mRNA is produced in nearly all cases, and this transcript can be readily detected by RT-PCR analysis; FISH technique can also be employed to detect the genomic abnormality. Rare occurrences of a balanced t(1;19)(q23;p13) also occur in B-LBL and do not involve the *TCF3* and *PBX1* genes but rather create novel *DAZAP1-MEF2D* and reciprocal *MEF2D-DAZAP1* gene fusions associated with poor outcome.

The *M*ixed *L*ineage *L*eukemia (*MLL*) gene, now designated *KMT2A*, is located on chromosome 11(q23) and is abnormally rearranged in approximately 5% of childhood leukemias, including the majority of cases occurring in infants less than 1 year of age. The *KMT2A* gene product encodes histone-lysine *N*-methyltransferase 2A (MLL protein), which differentially regulates a set of homeobox, or *HOX*, genes that are variably responsible for coordinated skeletal development during embryogenesis and, significantly, normal post-fetal hematopoietic progenitor cell development. In B-LBL, *KMT2A* becomes juxtaposed with a different gene locus via chromosome translocation, resulting in the formation of a chimeric gene product with concomitant disruption of normal MLL protein function. Infantile acute leukemias with *KMT2A* gene rearrangements present in a clinically aggressive fashion with a dismal prognosis, particularly when associated with a poor therapeutic steroid response. Abnormal MLL fusion proteins can promote leukemogenesis by aberrantly deregulating certain *HOX* gene subsets in hematopoietic stem cells. The central role of *KMT2A* in this neoplastic process is underscored by the numerous (>50) described reciprocal pairings at other "partner" genetic loci. Of these, the t(4;11)(q21;q23) is most commonly encountered in infantile B-LBL, resulting in the formation of the *KMT2A-AFF1* fusion. In this event, the

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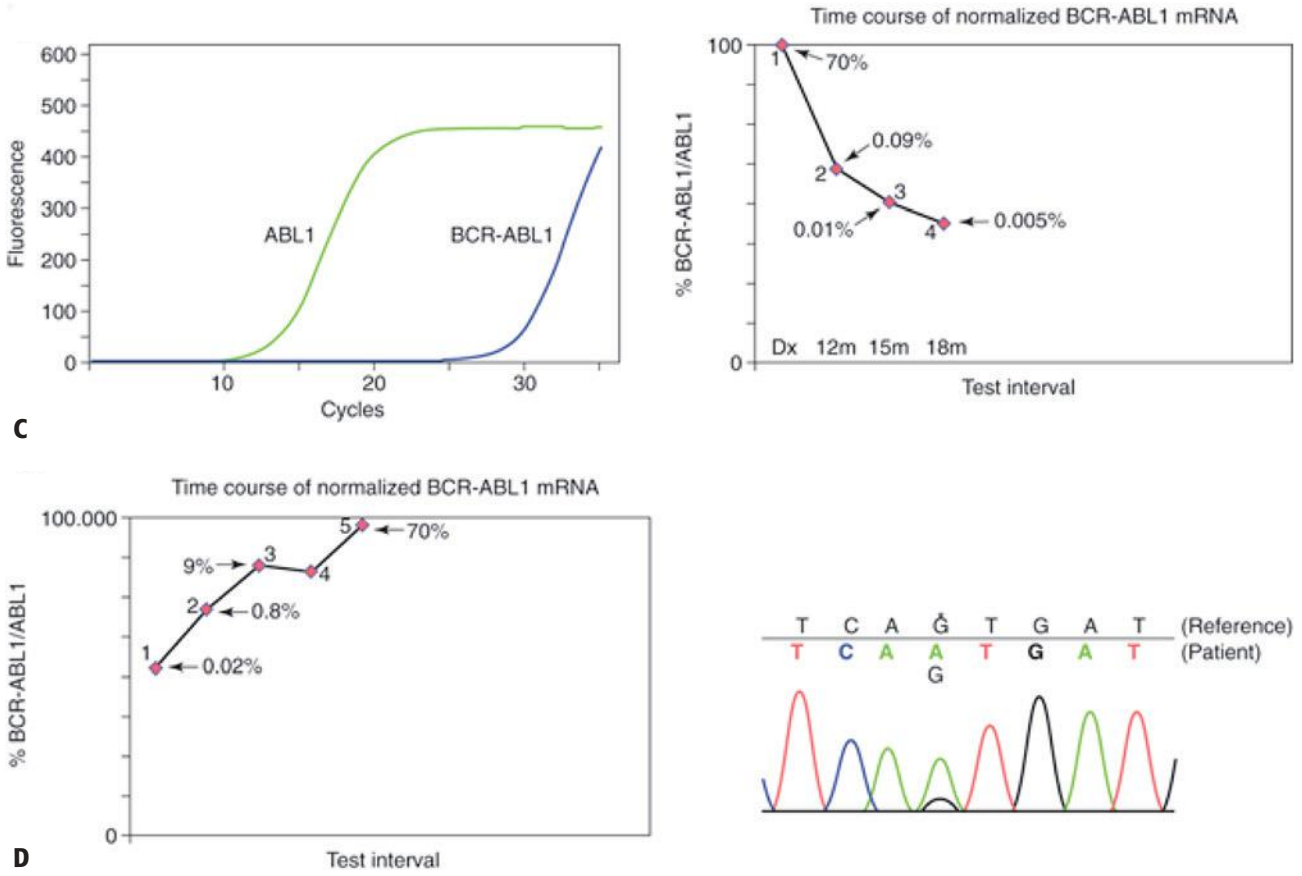


FIG. 24.3, cont'd

Panel C *(left)* illustrates results from RQ-PCR analysis for BCR-ABL1 mRNA in a patient successfully treated with the tyrosine kinase inhibitor (TKI) imatinib mesylate (*blue trace* is patient BCR-ABL1 transcript; *green trace* is an amplification plot of endogenous sample ABL1 mRNA, for normalization and calculation of a quantitative BCR-ABL1/ABL1% ratio on the International Scale, IS). The graphical data *(right)* demonstrates a desirable response over time to TKI therapy in CML. Following initiation of drug treatment at diagnosis the patient achieved rapid complete cytogenetic response (not shown), followed by a greater than 3 log reduction of normalized BCR-ABL1/ABL1 level (i.e., <0.1% IS major molecular response level or MMR) by 12 months. This situation is associated with very low risk of disease progression. Panel D conversely shows a graph of a patient with rapidly increasing normalized BCR-ABL1 transcript amounts *(left)*; within the first two consecutive time-points, an increase of over 2 logs is documented. A serial increase in BCR-ABL1 mRNA of 0.5 log (or greater) magnitude suggests TKI resistance owing to acquired *ABL1* region kinase domain mutations (KDM) in the *BCR-ABL1* oncogene. KDM analysis of the patient sample *(right)* by long transcript BCR-ABL1 RT-PCR and sequencing demonstrates the pan-resistant T315I abnormality (reverse strand sequence is shown with a G>A nucleotide change (indicated by *), corresponding to a C>T substitution at codon 315).

KMT2A gene exhibits substantial genomic breakpoint heterogeneity encompassing a large region containing exons 5 to 11. Likewise, the *AFF1* gene (also known as *AF4*) also shows variability of breakpoint location among B-LBL cases. A second translocation, the t(11;19)/*MLLT1* (or *ENL*)-*KMT2A* abnormality, is also encountered in infant B-LBL, as well as lymphoblastic and acute myeloid leukemias in older children and adults. Although one fusion transcript type is associated with any given leukemic tumor, the propensity for many possible chimeric transcripts arising from *KMT2A* gene break-fusion events coupled with the large number of potential rearranging partner genes in *MLL* translocations creates significant challenges for detection. Multiplex RT-PCR approaches have been designed to identify many different *KMT2A* gene fusions using a single platform; however, comprehensive molecular evaluation of this gene in childhood leukemias by PCR methods remains problematic owing to both biologic and technical complexity, thus limiting clinical sensitivity. FISH based on a BAP probe strategy (with probes spanning either side of the breakpoint cluster region in the *KMT2A* gene) is very powerful for the rapid identification of translocations. This strategy is diagnostically sensitive for identifying *KMT2A* rearrangements regardless of the translocation partner; D-FISH probe strategies can then be employed to specifically define the more common translocation variants. FISH analysis also discriminates between true 11(q23)/*KMT2A* locus rearrangements and other genetic recombinations not involving the gene but clustering within the 11(q22;q25) region; this distinction may be difficult to discern by routine cytogenetic analysis. On occasion, distal deletions of the *KMT2A* locus may accompany a true gene translocation event with a resulting "deletion only" signal pattern suggested by BAP FISH technique; in these situations, confirmation of an abnormal *KMT2A* gene fusion can be obtained by targeted D-FISH or possibly RT-PCR analysis.

Further underscoring the importance of MLL dysregulation in leukemogenesis is the finding of *KMT2A* translocations in the subgroup of therapy-related, or "secondary" AML

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following treatment with DNA topoisomerase II inhibiting agents (e.g., etoposide, daunorubicin). Therapy-related AML is associated with a very poor prognosis, and remarkably, the gene breakage sites situated in the 3′ region of the *KMT2A* breakpoint cluster overlap with those of de novo infant *KMT2A*-rearranged B-LBL. Several studies have examined the underlying basis for greater genetic fragility in this genomic region, and the role of in utero exposure to exogenous substances that can potentiate *KMT2A* gene breakage and translocation, leading to rapid development of B-LBL. The concept of leukemia initiating in the prenatal period is supported by intriguing investigations "backtracking" identical *KMT2A* gene rearrangements in individual patient leukemias to their corresponding neonatal blood samples. This finding has similarly been demonstrated in older children with *ETV6-RUNX1* B-LBL and now other types of ALL and AML in childhood. However, the latency period to presentation differs among subtypes of genetically defined B-LBL, suggesting that promoting mutations or environmental influences play an important role in leukemogenesis. In addition, healthy newborn or umbilical cord blood population screening studies have shown an appreciable prevalence (~0.1% to 1%) of leukemia-associated translocation events, albeit at very low quantitative levels ($<10^{-4}$); these data again point to the requirement for cooperating oncogenic mutations and a conducive background in the generation of overt leukemia. Nevertheless, the realization that at least some childhood leukemias have origins prenatally has spurred interest in identifying risk-modifying factors. In this regard, recent evidence shows that low penetrance population genetic variations in some genes (e.g., *IKZF1*) may increase the risk of developing B-LBL.

More comprehensive data from global genomic analyses have further advanced the characterization of childhood B-LBL. It is now apparent that translocation-type aberrations in B-LBL also encompass both cytokine receptor signaling deregulation and activating gene fusions involving signal transduction proteins, classically typified by *BCR-ABL1*. For example, translocations of the *IGH* gene with the erythropoietin receptor (*EPOR*) or the type 1 cytokine receptor *CRLF2* result in overexpression of these proteins, leading to autonomous receptor activity. Other gene rearrangements result in pathogenic activation tyrosine kinase genes (e.g., *ABL1*, *ABL2*, *PDGFRB*, *JAK2*). Single base mutations and small insertion/deletion (indel)-type alterations are also evident in a number of genes, including members of the Janus kinase (*JAK*) family. Importantly, many of these alterations affecting cell signaling and mitogenesis involve the JAK-STAT pathway, and these tumors share an overlapping gene expression signature similar to *BCR-ABL1*–positive (Ph^+) B-LBL (so-called "Ph-like" B-ALL). Additional genetic deletion or mutation events in critical B-cell transcription factors, notably *IKZF1* and *PAX5*, are also observed in conjunction with both *BCR-ABL1*–positive and "Ph-like" high-risk B-LBL. The proportion of B-LBL with Ph-like genetic changes increases with age, such that older children and young adult patients more frequently have these findings. Although typically considered a malignancy of "low mutation burden" (e.g., relative to many solid tumors), a greater degree of complexity is now apparent in B-LBL, with a spectrum of genetic changes spanning genomic instability (aneuploidy); transcription factor deregulation (effects on cell differentiation and proliferation); and aberrant kinase gene activation. This "mechanistic ontology" is useful for therapeutic management as well as risk stratification. The availability of JAK and tyrosine kinase inhibitor drugs has provided targets for rational treatment approaches in high-risk B-LBL patients, in addition to currently optimized chemotherapy or hematopoietic stem cell transplantation protocols. Although "genome-scale" NGS approaches (e.g., exome and RNA transcriptome sequencing) are often utilized in clinical research studies, efficient and cost-effective application of such methods in the routine molecular diagnostic setting is not optimal at present for clinical purposes. Conversely, comprehensive detection of this panoply of genetic aberrations in B-LBL is difficult using standard molecular diagnostic methods and instead requires a multimodal strategy including targeted NGS (for gene mutations, small indels, and specific translocations), FISH (for some translocations), RT-PCR (for selected expressed gene fusion mRNAs), chromosome arrays (for larger deletion/duplication analysis), and cytogenetics. A comprehensive but directed diagnostic testing approach can achieve a high rate of detection of most "favorable risk" and "high-risk" tumor genetic factors, including the more heterogeneous group of Ph-like cases. Nevertheless, a minority of these patients may still be missed in targeted analyses, and Ph-like B-LBL can possibly be more sensitively detected by a gene expression classifier method, as suggested in some pediatric hematology studies.

RISK STRATIFICATION IN CHILDHOOD ALL AND MINIMAL RESIDUAL DISEASE ASSESSMENT

The identification of prognostic predictive molecular markers in pediatric B-LBL has significantly augmented the development of refined risk-adapted therapy practices for lower risk and particularly high-risk patients. This approach is based on the premise of achieving maximum cure rate while minimizing toxic effects and late sequelae such as treatment-related neoplasms. For B-lineage ALL in particular, initial clinical risk assessment (e.g., age, white blood cell and blast count, extramedullary involvement) is further modified by the presence and type of tumor genetic abnormalities, as outlined above. The *BCR-ABL1* chimeric gene fusion, hypodiploid karyotype, *KMT2A* rearrangement, Ph-like genotype or phenotype, iAMP21, *IKZF1* deletion (often seen in Ph-like disease), or therapeutic induction failure each constitutes very high-risk patient subtypes. Conversely, B-ALL cases with hyperdiploidy and "favorable" trisomies, or the *ETV6-RUNX1* gene fusion are considered good prognostic factors. Early genetic classification (i.e., before the end-induction treatment phase) thereby influences the chemotherapy protocol and duration for a given patient. Most large cancer groups for children utilize this strategy of tumor genetic risk stratification

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in slightly different formulations. Useful as these parameters have been for more appropriately stratifying patients earlier to receive optimized therapy, a significant subset of children experiences treatment failure and leukemic relapse, indicating some enduring lack of precision in the risk assessment models. Better predictive surrogate markers of biologic response and disease clearance have therefore been sought, and minimal residual disease detection has emerged as a very powerful method of post-therapeutic prediction, contributing to a more "individualized" approach of risk evaluation. Many large clinical studies have now confirmed the utility of MRD in determining relapse potential in childhood B- and T-lineage B-LBL. Levels of MRD greater than 10^{-4} at the end of induction therapy have been shown to be highly correlated with risk of relapse compared to MRD-negative status, and this relationship has proved to be strongly independent of nearly all currently applied clinical and cytogenetic/molecular genetic factors in multivariate analyses. Targets for molecular MRD measurements include clone-specific antigen receptor gene rearrangements (e.g., *IGH*, *IGK*, *TRG*, *TRB*), tumor-specific single gene mutations, and chimeric fusion gene mRNA transcripts.

The clonal nature of immunoglobulin or T-cell receptor gene rearrangements confers a unique and ubiquitous marker for MRD assessment in lymphoid malignancies relative to leukemia-specific fusion gene transcripts, which are present in less than one third of cases. Notably in B-LBL, "illegitimate" or cross-lineage gene rearrangements also occur frequently at the T-cell receptor genes, such that most B-lineage tumors have at least one and as many as two to three unique gene rearrangements available for MRD monitoring. The highly specific nature of rearranged antigen receptor Vn(D)nJ regions in lymphoblastic leukemia cells can be amplified, then sequenced for the design and synthesis of tumor-specific PCR primers and probes for use in targeted quantitative PCR analysis following therapy induction. Poor technical reproducibility and an overall labor-intensive approach are significant limiting factors to routine implementation, despite the attractiveness of the actual tumor markers. Recent developments using NGS technology for deep sequencing of B-cell and T-cell receptor genes have indicated an alternative and very powerful means of detecting tumor-specific clones at very high analytic sensitivity (10^{-6}). Using a comprehensive consensus PCR primer strategy, a library pool of rearranged antigen receptor gene products can be prepared for high-throughput NGS using Illumina or Ion Torrent platforms. Relying on the highly specific nature of V-(D)-J segmental recombination and especially the unique CDR3 region sequence, the presence of tumor DNA can be directly and specifically queried following therapy in a single rapid assay, as long as the initial clonal rearrangement(s) of the tumor cells were determined at diagnosis. The development of commercial platforms (e.g., Lymphotrack, Invivoscribe, San Diego, CA; clonoSEQ, Adaptive Biotechnologies, Seattle, WA) has greatly facilitated clinical molecular laboratory access to this technology, as well as the related bioinformatics software to effectively analyze the large amount of sequence data generated. Other investigators have established the use of flow cytometric evaluation of MRD, based on quantitative or qualitative alterations in antigen expression by the leukemic blasts. Flow cytometric protocols can achieve comparable analytic sensitivity to molecular methods and have a potential advantage of being more easily standardized between laboratories, although at the extremes of analytic sensitivity (e.g., 10^{-5} to 10^{-6}), NGS methods appear to be more reliable. Whether a molecular- or cell marker–based technology is employed, the profound effect of early MRD detection on relapse prediction is evident, and childhood leukemia cooperative groups have begun to incorporate MRD data as a routine component of risk assessment, enabling higher risk MRD-positive patients to be rapidly stratified into more intensive therapy arms. Timing of MRD assessment is critical for the derivation of meaningful prognostic data in childhood ALL. In general, the end of induction therapy is a significant and highly informative time-point, although ongoing serial measurements can further increase the predictive value. End-induction MRD status, in conjunction with "rapid" versus "slow" blast clearance from the bone marrow has been described as further improving relapse risk prediction for individual patients.

MOLECULAR GENETIC EVALUATION OF MYELOID NEOPLASMS

ACUTE MYELOID LEUKEMIA

Cytogenetic analysis detects large structural abnormalities at the chromosomal level and is the cornerstone of AML classification and risk stratification (Table 24.4). Although many of the cytogenetic abnormalities have been well characterized in AML, the integrated molecular genetic landscape of AML is, however, heterogeneous, complex, and dynamic as revealed by numerous NGS studies in recent years. Multiple genetic aberrations can occur in individual patients with AML, from large chromosome structural alterations detectable by chromosome G-banding to gene mutations at the nucleotide level that can only be detected by molecular approaches. These aberrations commonly involve multiple pathways with intricate interplay and influence various aspects of cellular biology and functions, such as epigenetic modification (i.e., DNA methylation, chromatin histone acetylation and modification), cell signaling (e.g., kinase pathways), transcription, ribosome biogenesis, tumor suppression, and mitosis. Furthermore, individual patients often harbor a mixture of competing subclones, each with discrete variably overlapping mutation profiles that ultimately render each case genetically and evolutionarily unique. A major challenge is to integrate these multiple genetic abnormalities into a rational framework for accurate and clinically relevant prognostication and therapy. Progress to this end has been made with the aid of numerous correlative clinical studies as reflected in the 2016 National Comprehensive Cancer Network (NCCN) AML guideline and the 2016 WHO classification revision, both of which expanded the discussion of clinically relevant gene mutations

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TABLE 24.4

Common Genetic Abnormalities in AML Detected by Molecular Diagnostic Methods

Genetic Abnormality	Disease Associations	Molecular Pathogenesis[a]	Molecular Diagnostic Detection[b]	Notes
t(15;17)/*PML-RARA*	Acute promyelocytic leukemia with *PML-RARA*	Chimeric fusion protein; interference with normal myeloid maturation and differentiation	RT-PCR; FISH; RQ-PCR for MRD detection	Favorable prognosis genetic marker (good responses to atRA therapy); MRD evaluation by quantitative RT-PCR has prognostic value after therapy; rare APL-like tumors have variant *RARA* translocations and may be unresponsive to ATRA
t(8;21)/*RUNX1-RUNXT1;* inv(16) or t(16;16)/ *CBFB-MYH11*	AML with t(8;21)/*RUNX1-RUNXT1*; AML with inv(16) or t(16;16)/ *CBFB-MYH11*	Chimeric fusion protein affecting the CBF transcriptional regulatory pathway; aberrant effects on myeloid cell proliferation and differentiation	RT-PCR for *RUNX1-RUNXT1* and *CBFB-MYH11;* FISH; RQ-PCR for MRD detection	Favorable prognosis genetic markers; associated with responsiveness to Ara-C–based chemotherapy; accompanying *KIT* gene mutations may be associated with poor outcome
FLT3 mutations	AML (30%–40%), typically with normal cytogenetics	Constitutive activity of FLT3 tyrosine kinase with increased cell proliferation	DNA PCR and fragment sizing of exon 14/15 region to detect ITDs; PCR and restriction enzyme digestion to detect TKD D835 point mutations; sequencing: capture-based NGS for ITDs and TKD mutations, Sanger sequencing for TKD mutations	*FLT3*-ITD is an unfavorable prognostic marker; effects of TKD mutation in the absence of ITD not entirely clear, however, have been reported to confer acquired resistance to some FLT3 inhibitors in initial responders harboring ITDs.
NPM1 mutations	AML (30%–35%, 50%–60% de novo AML), typically with normal cytogenetics	Cytoplasmic form of mutant NPM1 with altered C-terminal sequence and loss of normal cell cycle regulation	DNA PCR of exon 12 region and fragment sizing; high-resolution melting analysis; sequencing: Sanger or NGS; RQ-PCR for *NPM1* MRD monitoring	*NPM1* mutation is a favorable prognostic marker in the absence of *FLT3* ITD mutation.
CEBPA mutations	AML (10%), typically with normal cytogenetics	Mutations alter normal function of *CEBPA* in granulocytic maturation	Sanger sequencing; NGS	Biallelic *CEBPA* mutation (commonly frameshift or nonsense in N-terminus and in-frame in C-terminus) is a favorable prognostic marker in the absence of *FLT3* mutation. Germline mutations usually occur in N-terminus.
KIT mutations	AML with t(8;21)/ *RUNX1-RUNXT1;* AML with inv(16) or t(16;16)/ *CBFB-MYH11* (30%)	Constitutive activation of KIT tyrosine kinase with increased cell proliferation	Sequencing: Sanger or NGS; AS-PCR for D816V mutations	*KIT* mutations in AML commonly involve exon 17 (tyrosine kinase domain) and exon 8 (extracellular domain). Notably, the *KIT* exon 17 D816V mutation is insensitive to imatinib but may show response to the drug midostaurin. Exon 8 mutations are sensitive to imatinib.
Other recurrent gene mutations (see Table 24.7)	AML and related myeloid neoplasms	Epigenetic modifiers; signaling and kinase pathways; NPM1; transcription factor; tumor suppressors; cohesin complex	A targeted myeloid neoplasm NGS panel is the most cost-effective approach for simultaneous evaluation of these mutations	Some mutations can be seen in healthy individuals with aging as clonal hematopoiesis of indeterminate potential (CHIP), usually as a single mutation.

[a]Major pathophysiologic effect resulting from genetic lesion.
[b]Most frequent molecular techniques used for detection.
AML, Acute myeloid leukemia; *AS-PCR,* allele-specific PCR; *ATRA,* all-*trans* retinoic acid; *CBF,* core binding factor; *FISH,* fluorescence in situ hybridization; *ITD,* internal tandem duplication; *TKD,* tyrosine kinase domain; *MRD,* minimal residual disease; *mRNA,* messenger RNA; *PCR,* polymerase chain reaction; *RQ-PCR,* real-time quantitative PCR; *RT-PCR,* reverse transcriptase polymerase chain reaction.

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in several AML subcategories. Therapeutically, encouraging efficacy has been reported using small molecular inhibitors targeting the products of mutated *FLT3*, *IDH1*, and *IDH2* genes. Indeed, the FLT3 inhibitor midostaurin has been approved for use in *FLT3*-mutated AML. Thus, *FLT3* mutational testing has become standard of care for therapeutic purposes. Overall, this field is rapidly evolving and continues to mature with adequately powered clinical studies, robust molecular methods and, critically, high-quality bioinformatics analyses.

AML WITH RECURRENT CHROMOSOMAL TRANSLOCATION ABNORMALITIES

Chromosome translocations in AML most often result in juxtaposition of one genetic locus with another unrelated gene, leading to the formation of a fusion proto-oncogene and transcription of a chimeric mRNA species that retains partially functional but aberrantly regulated features of both individual genes. Leukemogenic gene fusions frequently involve at least one partner gene with a critical transcriptional role in normal hematopoiesis; the resulting altered chimeric protein therefore manifests widespread pathophysiologic effects on cell differentiation, maturation, and apoptosis in the AML stem or progenitor cells. Although chromosomal translocations can be detected by conventional cytogenetic and FISH techniques, RT-PCR methods with primers spanning breakpoint-fusion regions are also commonly used to sensitively and specifically identify the resulting novel chimeric mRNA products at diagnosis in some subtypes of AML (e.g., acute promyelocytic leukemia). Leukemia-specific transcripts also serve as invaluable markers for detecting minimal residual disease using RQ-PCR methods in patients who have received curative-intent therapy.

ACUTE PROMYELOCYTIC LEUKEMIA WITH PML-RARA

Acute promyelocytic leukemia (APL), accounting for 5% to 10% of de novo AML, is characterized by the presence of the t(15;17)(q22;q21) abnormality, resulting in the formation of a novel fusion gene *PML-RARA* on the derivative chromosome 15q (Fig. 24.4). To emphasize the significance of *PML-RARA* fusion, which also may occasionally result from a cryptic translocation or complex cytogenetic event, the 2016 WHO classification formally recognized this type of AML as "APL with *PML-RARA*." The *RARA* gene encodes the alpha receptor (RARα) for the retinoic acid or vitamin A, and the PML gene product is a DNA-binding zinc finger protein, which in association with a macromolecular nuclear body complex, regulates cellular proliferation and apoptosis. In normal bone marrow hematopoietic cells, unbound retinoic acid receptor associates with histone deacetylase (HDAC) and other members of a nuclear protein co-repressor complex. This co-repressor unit reduces the accessibility of chromatin to transcription factors (and thus locus-specific transcriptional activity), yet is reversible in the presence of physiologic concentrations of retinoic acid. In APL, the PML-RARα oncoprotein greatly stabilizes and enhances the state of transcriptional repression by this multiprotein complex, resulting in a maturation arrest at the promyelocyte stage of myeloid differentiation, with the accumulation of neoplastic blasts and promyelocytes. Most important, the leukemogenic effects of the PML-RARα oncoprotein can be overcome by administering pharmacologic doses of all-*trans* retinoic acid (atRA), which relieve the cellular differentiation block. The discovery that arsenic trioxide (As_2O_3) can also induce remission by an independent therapeutic mechanism has further advanced the non-chemotherapy approach to the management of APL with *PML-RARA*, and induction regimens principally employing these two agents have been successfully used in patients.

The molecular genetic anatomy of the *PML-RARA* abnormality is illustrated in Fig. 24.4. One of three *PML-RARA* fusion mRNA transcripts can result from the t(15;17) event in a given patient with APL, based on breakpoint heterogeneity occurring within the *PML* gene. The two most frequent transcripts are designated BCR1 (also long [L]-form) and BCR3 (also short [S]-form), arising from breakpoints in *PML* intron 6 and intron 3, respectively; together, these transcript types are encountered in nearly 95% of APL cases. The rare BCR2 (or variable [V]-form) fusion mRNA results from unusual breakpoint events that occur within exon 6 of the *PML* gene. The BCR2/V-form transcript differs in size slightly from the BCR1/L-form, owing to a variably truncated portion of PML exon 6. *RARA* break-sites invariably involve intron 2, joining exon 3 and the remainder of the gene to *PML* in each case. Amplification of *PML-RARA* transcripts is readily achieved using RT-PCR methods, as schematically indicated in Fig. 24.4. The *PML-RARA* fusion abnormality can also be detected by FISH technique using dual-color/dual-fusion probes. FISH methods are also very rapid and of high utility for initial diagnosis of APL; however, FISH and RT-PCR should be considered complementary in this regard. FISH is also useful in identifying rare cases of "APL-like" leukemias, which share morphologic and immunophenotypic similarities with classic APL but have alternative translocations of *RARA* to genes other than *PML*. These variant AML types are characterized by t(11;17)/*ZBTB16(PLZF)-RARA*, t(11;17)/*NUMA1-RARA*, t(5;17)/*NPM1-RARA*, or t(17;17)/*STAT5B-RARA* abnormalities. Notably, myeloid leukemias with *ZBTB16(PLZF)-RARA* and *STAT5B-RARA* are not responsive to the differentiating effects of atRA and have a poor clinical outcome. FISH results for *PML-RARA* would be negative in these uncommon cases of AML; however, the presence of *RARA* region breakage is helpful for distinction from true APL and for further subclassification.

Although APL is a potentially curable type of AML, relapses are not infrequent. Typically, molecular reappearance of the *PML-RARA* mRNA precedes hematologic relapse by several weeks to months, thus enabling a window for early detection and secondary intervention. Quantitative RT-PCR–based molecular monitoring is a very powerful method for predicting

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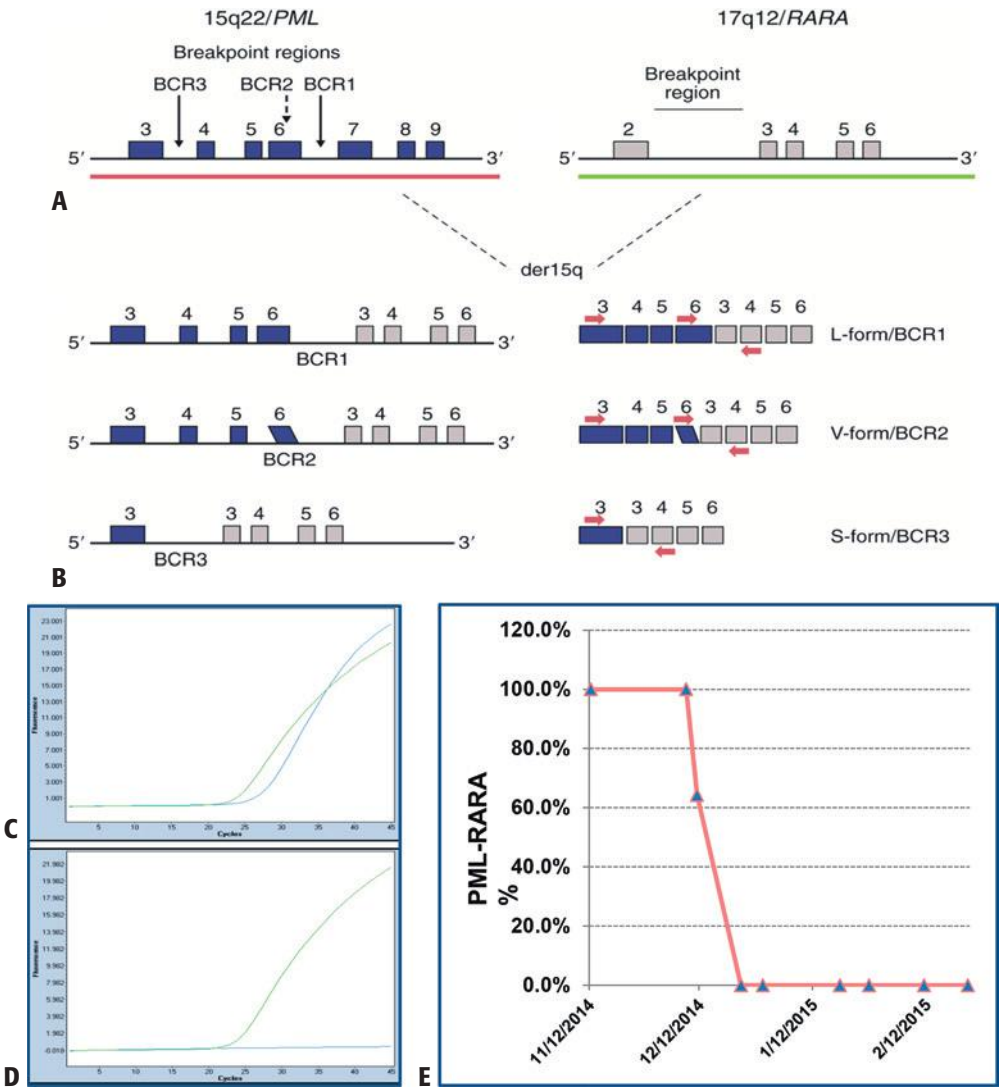


FIG. 24.4

The t(15;17)/*PML-RARA* abnormality in acute promyelocytic leukemia. Panel A depicts the arrangement of *PML* and *RARA* genes on their respective chromosomes. Exons are numbered in each gene (gene maps are not shown to proper genomic scale). Genomic breakpoints in the RARA locus occur invariantly in intron 2. This results in consistent fusion of RARA exon 3 to the PML gene. Break-sites in *PML* are heterogeneous and can involve one of three breakpoint cluster regions (BCR) in any given patient with acute promyelocytic leukemia (APL): in intron 6 (BCR1), within exon 6 (BCR2) or in intron 3 (BCR3). Of these, the BCR1 and BCR3 breakpoints are by far the most common, accounting for more than 90% of APL cases. Panel B represents the three possible PML-RARA gene fusions *(left side)* and corresponding chimeric mRNA species *(right side)*. Because the BCR1 type *PML-RARA* fusion results in the longest transcript, it is also known as the Long (L) form, in contrast to the BCR3 product, which conversely is designated the Short (S) form. The BCR2 event, owing to the inconstant intra-exonic break-fusion location in each case, is also known as the Variable (V) form transcript. RT-PCR approaches to specifically amplify these various *PML-RARA* fusion mRNA species are schematically depicted by *small red arrows* representing PCR primer locations. It is evident that the PML exon 3 primer can also detect both L-form and V-form transcript types, generating correspondingly larger amplicon fragment sizes. Panel C demonstrates a representative positive result of RQ-PCR analysis for the *PML-RARA* fusion mRNA in an APL patient at the time of diagnosis. *Blue curve*, *PML-RARA* fusion transcript; *green curve*, housekeeping gene *GUSB* transcript for normalization. Panel D demonstrates a representative negative result of RQ-PCR analysis for the *PML-RARA* fusion mRNA in an APL patient in molecular remission. *Blue curve*, *PML-RARA* transcript; *green curve*, housekeeping gene *GUSB* transcript for normalization. Panel E is a representative minimal residual disease (MRD) monitoring graph of *PML-RARA* fusion transcripts by RQ-PCR in an APL patient, who achieved molecular remission after consolidation therapy.

relapse in individual patients with APL. Clinical laboratories should achieve a minimum analytic sensitivity of 10^{-4}, and the test should be performed in the same laboratory to ensure the same level of sensitivity, given that a consensus test standard is not currently available. The timing of molecular MRD evaluation (i.e., phase of disease therapy) is very important in this regard. Monitoring of MRD at the end-induction phase is not informative for relapse potential because of the delayed clearance of the differentiating *PML-RARA*–positive leukemic cells. At the end of consolidation therapy, however, conversion to, or persistence of, PCR positivity is strongly predictive of relapse in patients who have apparently achieved a clinical complete response. The positive predictive value for overt relapse is also highest in those patients repeatedly testing positive for *PML-RARA* by RQ-PCR on consecutive occasions. Thus, a major therapeutic goal in APL is the achievement and

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maintenance of "molecular remission" (i.e., PCR-negative status). The results from several clinical studies have not only shown the benefit of using RQ-PCR to detect MRD and predict relapse risk but have also underscored the ability to successfully salvage patients with molecular disease and prevent recurrence of hematologic disease. The 2016 NCCN guideline recommends RQ-PCR on a bone marrow specimen at the completion of consolidation to document molecular remission. In patients receiving an atRA/As_2O_3 regimen, repeat testing 3 to 4 months after consolidation is recommended. With confirmed molecular remission, subsequent monitoring can be performed on peripheral blood specimens, although the sensitivity of transcript detection after treatment onset can be 1 to 2 logs lower in peripheral blood than in bone marrow. Bone marrow monitoring every 3 months for 2 years is recommended only for high-risk patients (WBC >10 × 10^9/L at diagnosis), those older than 60 years, and patients with long interruptions during consolidation or therapeutic intolerance during maintenance. The risk of relapse has been shown to be minimal for low-risk patients who achieve molecular remission at the completion of consolidation therapy, and MRD monitoring may not be necessary except for clinical trial purposes. If RQ-PCR is positive at any level, the result needs to be confirmed by a repeat testing on a bone marrow sample in 2 to 4 weeks in a reliable laboratory. Beyond diagnosis, the role of FISH for MRD monitoring in APL is virtually non-existent, since the detection sensitivity required for prognostic and clinical decision purposes cannot be reliably attained at sample time-points beyond induction therapy.

AML WITH CORE BINDING FACTOR GENE TRANSLOCATIONS

Genetic abnormalities involving the heterodimeric core binding factor transcriptional complex are relatively common in the acute leukemias. CBF is a critical transcription factor composed of α and β peptide subunits and is involved in the coordinate regulation of numerous cellular processes involved in normal hematopoietic cell differentiation. CBF binds to its cognate core enhancer DNA motif and facilitates access of other transcriptional regulators to genes associated with lymphoid and myeloid maturation. Two translocation events, the t(8;21)(q22;q22) and inv(16)(q13;q22), or related t(16;16)(q13;q22), are found in approximately 10% to 15% of de novo AML and target the components of CBF. The t(8;21) is often associated with AML with maturation, whereas the inv(16) and t(16;16) are strongly correlated with the morphology of AML with abnormal eosinophils. The t(8;21) abnormality results in the formation of a fusion gene *RUNX1-RUNX1T1*. The *RUNX1* gene encodes the DNA binding α-subunit of CBF or CBFα, whereas the normal *RUNX1T1* gene produces an unrelated transcriptional corepressor protein. The inv(16) and t(16;16) both create a chimeric fusion gene involving *CBFB* (which produces the β-subunit of CBF, or CBFβ) and a smooth muscle myosin heavy chain gene *MYH11*. Despite differences in morphologic and cytogenetic features, both *RUNX1-RUNX1T1* and *CBFB-MYH11* AML subtypes share in common the disruption of normal CBF transcriptional activity, leading to defective cellular differentiation and leukemogenesis. Clinically, these two genetically defined AML subtypes have been associated with relatively favorable chemotherapeutic responses to high-dose cytarabine-based (Ara-C) treatment, compared to de novo AML in general.

The *RUNX1-RUNX1T1* gene fusion produces a single chimeric mRNA product that is readily detectable by standard RT-PCR methods. In contrast, *CBFB-MYH11* is more complex because of substantial breakpoint heterogeneity in the *MYH11* gene, resulting in at least ten described fusion mRNA species. Nevertheless, the majority (90% to 95%) of inv(16) and t(16;16) AML cases demonstrate one common CBFβ-MYH11 transcript (type "A"), with less frequent chimeric mRNA types accounting for the remainder. RT-PCR assays have been employed to detect the common type "A" transcript, as well as many of the rare variant CBFβ-MYH11 forms. The genetic locus rearrangements in both t(8;21) and inv(16) are also readily amenable to detection using standard FISH techniques. FISH is especially helpful for diagnosing inv(16) and the related t(16;16) abnormalities, which can be more subtle and difficult to identify by standard karyotype banding. The value of post-therapeutic MRD monitoring by RQ-PCR in patients with CBF-associated AML has been examined in several studies and has shown strong utility for relapse prediction. In *RUNX1-RUNX1T1* AML, measuring log reduction of leukemic mRNA transcripts following induction therapy or transcript copy number quantity after consolidation has been correlated with relapse risk. Nonetheless, some patients with the *RUNX1-RUNX1T1* abnormality can have very low persistent levels of leukemic mRNA detected in the bone marrow, even after achieving stable long-term remission. The CBFβ-*MYH11* chimeric transcript also serves as a tumor-specific marker of post-therapy disease burden. In either case, serial time-point measurements using the RQ-PCR technique are required for most accurate prediction of relapse risk, and several clinical studies have highlighted the significance of sensitive MRD testing to identify patients at high risk of disease recurrence.

Mutations of the *KIT* gene occur in approximately 30% of CBF AML. The *KIT* gene is located on chromosome 4q12 and encodes a transmembrane receptor tyrosine kinase (KIT), which binds the cognate ligand stem cell factor (SCF). In normal hematopoiesis, KIT/SCF interaction leads to activation of PI3K/AKT, RAS/MAPK/MEK, and/or JAK/STAT pathways. Mutations in the *KIT* gene cluster within the second tyrosine kinase domain activation loop encoded by exon 17 and, less commonly, in part of the extracellular dimerization domain encoded by exon 8. The most common mutations seen in exon 17 involve substitution (missense) alterations of the Asp816 (D816) amino acid (also present in the majority of patients with systemic mastocytosis), whereas the mutations seen in exon 8 are small insertion/deletions commonly involving codons 417 to 419. The presence of *KIT* mutations has been associated with poor outcome in CBF-AML, particularly in

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patients with the t(8;21)/*RUNX1-RUNXT1*; however, this has not been confirmed universally among various clinical studies. Furthermore, some evidence has been presented that *KIT* mutations are mainly observed in the type A but not in other fusion variants in the group of inv(16)/t(16;16)/*CBFB-MYH11* AML cases. The NCCN guidelines recommend *KIT* testing in CBF-AML, and this is commonly performed by a sequencing approach (Sanger or NGS panel), given the multiple types of mutations and different exons that need to be covered.

AML WITH OTHER RARE RECURRENT TRANSLOCATION ABNORMALITIES

Several uncommon AML variants are defined by an association with specific translocation events that can be detected by routine karyotyping and each account for less than 5% of de novo cases of AML. These include the t(9;11)/*MLLT3-KMT2A* (formerly AF9-MLL) often associated with AML showing monocytic differentiation, the t(6;9)/*DEK-NUP214*, the inv(3) or t(3;3)/*GATA2, MECOM*, and the t(1;22)/*RBM15-MKL1* abnormalities, each with associated clinicopathologic correlations. Notably, the inv(3) abnormalities identified in myelodysplastic syndrome (MDS) and AML is now recognized as a juxtaposition of the distal *GATA2* enhancer with the *MECOM* (*EVI1*) gene, rather than a gene fusion. Both *GATA2* and *MECOM* encode critical and highly regulated transcription factors important in hematopoietic production and differentiation. The inv(3)/t(3;3) event leads to an overexpression of *MECOM* and a concurrent haploinsufficiency for *GATA2*. *KMT2A* gene rearrangements in AML are slightly more frequently encountered in pediatric patients. The *KMT2A* gene locus is commonly evaluated for rearrangements in AML using the BAP probe FISH technique, although this approach does not define the *KMT2A* partner gene. More comprehensive *KMT2A* D-FISH assays can be used to identify specific translocations in this setting. The role of molecular diagnostic evaluation in other rare translocation AML subtypes is limited beyond conventional karyotyping and FISH studies in routine clinical practice.

RECURRENT SINGLE-GENE SOMATIC MUTATIONS IN AML

The increasing use of NGS and its associated reduced cost relative to sequencing capacity have allowed for an unprecedented amount of information concerning the evolution and molecular landscape of AML. Whole genome sequencing (WGS), whole exome sequencing (WES), and targeted NGS panels showed that more than 95% of AML harbor at least one driver mutation with an average of three to four per case (Fig. 24.5). These alterations involve different cellular pathways and show distinct patterns of cooperation (or dependency) and mutual exclusivity. The prognostic significance of an individual mutation is, unsurprisingly, modified by the presence or absence of other driver mutations. Multiple lines of data also suggest an ordered and specific trajectory of AML development in most cases. Recurrent gene mutations have therefore become important molecular markers aiding in prognostic assessment of AML, treatment response prediction, and disease monitoring, even though the functional consequences of many gene mutations are at present incompletely understood. Currently, chromosomal karyotyping still provides the framework of AML risk stratification; however, the risk stratification in the cytogenetic intermediate-risk group is now further refined by genetic mutation data. The

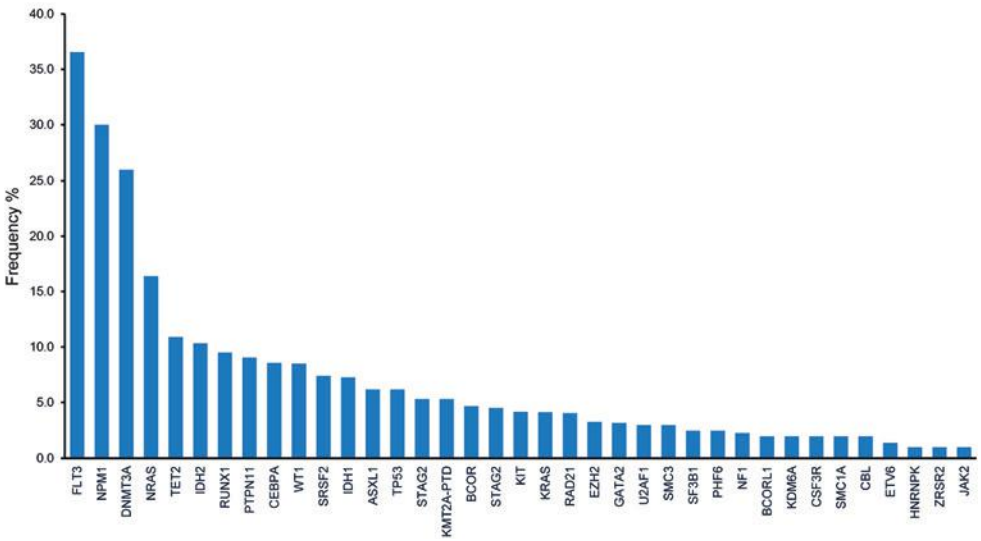


FIG. 24.5

Molecular landscape of AML. Frequency of common driver mutations identified in AML. *FLT3*, *NPM1*, and *DNMT3A* are the three most common mutations, each seen in more than 20% of patients. The median number of driver mutations is three to four per case in AML. Mutations impacting different pathways commonly coexist, and the effect of one mutation may be modified by other concomitant genetic abnormalities.

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TABLE 24.5

Acute Myeloid Leukemia (AML) Risk Stratification Based on Validated Cytogenetic and Molecular Abnormalities

Risk Status	Cytogenetics	Molecular Abnormalities
Favorable risk	inv(16) t(8;21) t(15;17)	Normal karyotype: *NPM1*$^{-}$*FLT3*-ITD^{-} or isolated biallelic *CEPBA* mutation
Intermediate risk	Normal karyotype +8 alone t(9;11) Other non-defined	
Poor risk	Complex[a] Monosomal -5, 5q-, -7, 7q- 11q23 – non t(9;11) inv(3), t(3;3) t(6;9) t(9;22)[4]	Normal karyotype: *FLT3*$^{-}$*ITD*$^{+}$ *TP53*$^{+}$

[a]Complex karyotype defined as ≥3 clonal chromosomal abnormalities.
Adapted from NCCN Clinical Practice Guidelines in Oncology, Version 2.2016.

TABLE 24.6

Functional Classification of Genes Commonly Mutated in AML and Related Myeloid Neoplasms

Functional Class	Genes
Epigenetic modifiers DNA methylation and hydroxymethylation	*DNMT3A* *TET2* *IDH1* *IDH2* *WT1*
Epigenetic modifiers Chromatin histone acetylation and methylation	*EZH2* *ASXL1* *MLL/KMT2A* *BCOR*
Spliceosome complex	*SRSF2* *U2AF1* *SF3B1* *ZRSR2*
Signaling and kinase pathway	*FLT3* *KRAS* *NRAS* *KIT* *PTPN11* *NF1* *CBL*
Transcription factors	*CEBPA* *RUNX1* *GATA2* *ETV6*
Tumor suppressor	*TP53* *PHF6*
Cohesin complex	*RAD21* *STAG1* *STAG2* *SMA1A* *SMC3*
Nucleophosmin	*NPM1*
Poorly defined	*SETBP1*

2016 NCCN AML guideline reclassifies a cytogenetically normal AML (CN-AML) with a molecular signature of mutated *NPM1* in the absence of *FLT3* internal tandem duplication (*FLT3*-ITD) or isolated biallelic *CEBPA* as favorable risk, whereas the presence of *FLT3*-ITD or *TP53* mutation upgrades a CN-AML into the poor-risk category (Table 24.5). The intricacies of AML pathogenesis signify the necessity for comprehensive genomic analysis for accurate and more personalized risk stratification, which is most effectively achieved through combined chromosomal analysis and targeted NGS testing at the time of diagnosis and relapse. Opportunities for sensitive and more comprehensive gene-specific MRD monitoring utilizing relatively stable targets, such as *NPM1*, are also possible following attainment of remission in patients with AML. The frequency of commonly mutated genes in AML is shown in Fig. 24.5, and Table 24.6 summarizes genes by functional categorization of affected pathways. Key individual genes are considered briefly below according to those specifically included in the most recent WHO classification of AML and then by other recurrently mutated genes representing functional subgroups.

Abnormalities of WHO-Defined Genes

FLT3: *FLT3* mutation is the most common mutation seen in AML (approximately one third of de novo cases) and frequently occurs as internal tandem duplications (ITD) of the juxtamembrane coding region. The open reading frame of the gene is maintained as this abnormality is always in-frame and the net result is ligand-independent, constitutive activation of the receptor and downstream RAS/RAF/MEK and the PI3K/AKT pathways. Less often (~20% of *FLT3* mutations), a heterozygous point mutation of codon D835 can be found in the absence of an ITD, affecting the "activation loop" region of the protein in the tyrosine kinase domain (*FLT3*-TKD). *FLT3*-TKD and -ITD alterations can infrequently co-occur in the same AML. Together, these two *FLT3* mutation events are found in approximately 30% to 40% of de novo adult AML, with most patients having normal cytogenetics. The prognostic significance of *FLT3* mutation is most apparent in CN-AML. *FLT3*-ITD positive AML cases with normal karyotype are associated with poor treatment response, increased relapse risk, and decreased survival, especially if the ratio of *FLT3* mutated–to–wild-type allele is high. The impact of *FLT3* D835 alteration on AML outcome in the absence of an ITD is less well understood and remains somewhat controversial. However, *FLT3*-TKD mutations involving D835 and F691 have recently been described as a mechanism of acquired resistance to some FLT3 inhibitors such as quizartinib. Some newer generation FLT3 inhibitors

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have shown promise in inhibiting both *FLT3*-ITD and *FLT3*-TKD mutations.

FLT3-ITD is not restricted to CN-AML and can be seen in other genetically defined subtypes of AML and notably in 35% of APL. Thus, the presence of *FLT3* alteration is not considered to be a disease-specific genetic finding but more so a key pathogenic feature of leukemogenesis. Interestingly, in APL the presence of a concurrent *FLT3*-ITD mutation correlates with higher initial white blood cell count but does not appear to predict an adverse outcome; this finding indicates that beyond its role as a cooperating mutation in some APL cases, *FLT3*-ITD does not otherwise substantially affect the response of neoplastic blasts and promyelocytes to current therapies, and thus, *FLT3* testing in APL is not recommended. Additionally, *FLT3* mutations are described in a smaller proportion of childhood AML and ALL.

FLT3- ITD mutations occur in exon 14 and 15 regions of the gene and the sizes range from 3 bp to 400 bp, with the vast majority between 20 and 100 bp. They are commonly detected by targeted PCR of sample DNA, followed by accurate fragment size identification (e.g., by fluorescent capillary electrophoresis) in which the *FLT3*-ITD is seen as a larger amplicon shifted away from the expected wild-type product. Although detection of the ITD abnormality is relatively straightforward, a greater degree of assay refinement is required to reproducibly evaluate mutated–to–wild-type ratios at diagnosis. Point mutations involving the D835 site can be identified by several methods, such as allele-specific PCR, digital PCR, Sanger sequencing, pyrosequencing or by restriction enzyme digestion of the PCR product. Both *FLT3*-ITD and *FLT3*-TKD can also be detected by NGS. Given the size spectrum of possible ITDs, a capture-based NGS strategy aided by adequate probe tiling is superior to an amplicon-based NGS approach in successfully detecting the larger size ITDs (see Table 24.4).

NPM1: The gene *NPM1* located on chromosome 5q35 encodes nucleophosmin, a nuclear-cytoplasmic shuttle protein previously described in the section on *ALK*-positive anaplastic large-cell lymphoma. *NPM1* is mutated in 50% to 60% of de novo CN-AML and approximately 30% of adult AML, often characterized by CD34 antigen negativity and monocytic differentiation. The presence of an *NPM1* mutation is associated with relatively favorable clinical outcome in CN-AML; however, this relationship is mitigated by a concurrent internal tandem duplication mutation in the *FLT3* gene. Alterations of *NPM1* in CN-AML are heterozygous and occur in exon 12, consisting of small insertional events. The mutations lead to frameshifts in the C-terminal region of the translated protein, which encompasses the nuclear localization signal. Consequently, abnormal NPM1 protein accumulates in the cytoplasm of leukemic cells, abrogating its normal regulatory functions affecting the cell cycle and mitosis. More than forty distinct *NPM1* mutant types have been described in CN-AML, and the majority of these (types A, B, and D, ~90%) involve a tetranucleotide insertion at hotspot codon W288 in exon 12. Because *NPM1* mutations alter the length of the exon 12 nucleotide sequence, PCR with fragment length product analysis (e.g., by fluorescent capillary electrophoresis) provides a powerful way to identify this abnormality at diagnosis. PCR methods using DNA as a template require the use of at least one intron-spanning primer in order to prevent amplification from known *NPM1* pseudogenes. Given the established gene–gene interaction with FLT3-*ITD*, clinical molecular diagnostic testing for *NPM1* must be performed in conjunction with *FLT3* mutation evaluation. Fortunately, similar molecular techniques can be employed to identify both gene mutations using leukemic cell DNA. The analytic sensitivity for typical fluorescent PCR product fragment length analysis is about 5% to 10%; this level may be marginally adequate with some patient samples (e.g., a low blast count peripheral blood) or in some cases of AML with clonal heterogeneity (e.g., a small subclone with the mutation). Additional methods for detecting *NPM1* mutations include Sanger sequencing, pyrosequencing, high-resolution melting curve analysis of PCR products, and next-generation sequencing. Some reports have described RQ-PCR methods to very sensitively detect the major *NPM1* mutations (types A, B, and D) for MRD monitoring to a sensitivity level of 10^{-5}; detection of MRD was associated with significant predictive value for AML relapse. The less sensitive standard diagnostic assays are not appropriate for MRD purposes.

CEBPA: The *CEBPA* gene located on chromosome 19q13.1 encodes *C*CAAT *E*nhancer *B*inding *P*rotein-α (*CEBPA*), an important transcription factor responsible for promoting granulocytic maturation, as well as other regulatory cellular functions. Mutations in *CEBPA* are observed in approximately 10% of de novo AML patients, mostly with normal cytogenetics. Data indicate that the favorable treatment responses and survival of *CEBPA* alteration is limited to cases with biallelic mutations, and therefore the 2008 WHO provisional entity of "AML with mutated *CEBPA*" has been redefined as a full entity of "AML with biallelic mutations of *CEBPA*" in the 2016 WHO classification revision. The biallelic *CEBPA* mutations typically present as an N-terminal (amino acids 1-120) frameshift/nonsense mutation and a C-terminal (amino acids 278-358) in-frame/missense mutation. The N-terminal frameshift/nonsense mutations usually cause premature truncation of the wild-type p42 CEBPA protein, leading to re-initiation of translation from a second downstream ATG codon, resulting in a dominant-negative p30 isoform that inhibits the function of the wild-type protein. The C-terminal in-frame/missense mutation occurs in the bZIP (p.278-358) domain and disrupts CEBPA DNA binding and/or dimerization. The concurrence of *FLT3*-ITD, although uncommon, is a dominant adverse prognostic finding in *CEBPA*-mutated CN-AML. For reasons not well appreciated, *NPM1* gene mutations are relatively rarely encountered in AML patients with *CEBPA* mutations. Because *CEBPA* mutations can be distributed throughout the gene (but mostly in 5′ and 3′ coding regions), sequencing of the entire coding single exon region by either Sanger or NGS is the most comprehensive approach to detect a *CEBPA* mutation.

Approximately 10% of *CEBPA*-mutated AML patients, usually of younger age (<50 years), may harbor a germline

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mutation. Germline alterations commonly cluster within the N-terminal region (p.1-120) and frequently coexist with an acquired, somatic C-terminal mutation (p.278-358) identified at the time of AML diagnosis. *CEBPA* germline mutations demonstrate an autosomal-dominant inheritance pattern with high penetrance and confer familial predisposition to AML without a preexisting syndromic condition, chronic hematologic disorder, or extramedullary organ dysfunction. The favorable outcome of biallelic *CEBPA* mutations is maintained in the familial cases. AML with germline *CEBPA* mutation is outlined in the 2016 WHO revision under the new classification of myeloid neoplasms with germline predisposition.

Epigenetic Modifiers

DNMT3A: Encodes DNA methyltransferase and is recurrently mutated in 20% to 30% of AML and is more commonly observed in patients with advanced age and coexisting mutations involving *NPM1*, *FLT3*-ITD, and/or *IDH1*. Approximately two thirds of *DNMT3A* mutations occur at the hotspot codon Arg882 (R882). The prognostic impact of *DNMT3A* alteration remains controversial, although the majority of the reported literature supports an association with poorer outcome.

TET2: Encodes a methylcytosine dioxygenase, which converts methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), a crucial step in the DNA demethylation process. *TET2* mutations are mutually exclusive of *IDH1/IDH2* mutations. The prognostic significance of *TET2* mutations in AML is controversial at present, but this is a relatively common event and may have role in early leukemogenesis, by establishing a clonal hematopoietic cell population subject to additional gene mutations and the subsequent development of AML.

IDH1 and IDH2: Encode the mitochondrial and cytosolic/peroxisomal enzymes IDH1 and IDH2, respectively. Both enzymes catalyze oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) and generation of NADPH from $NADP^+$. Studies have shown that mutations at *IDH1* hotspot R132 and *IDH2* hotspots R140/R172 lead to an altered enzyme ability in catalyzing the conversion of isocitrate to α-KG, coupled with a neomorphic activity of catalyzing reduction of α-KG to 2-hydroxyglutarate (2-HG), which inhibits normal TET2 function. *IDH* gene mutations in AML are predominantly associated with intermediate-risk karyotype, and their impact on AML prognosis is not completely clear. IDH1 and IDH2 inhibitors are being actively pursued in the clinical setting and have demonstrated promising activity in inducing terminal differentiation of AML blasts in individuals harboring *IDH* mutations.

ASXL1: Encodes ASXL1, a chromatin-binding protein required for normal embryonic segmentation and regulating epigenetic marks and transcription. Normal ASXL1 interacts with the polycomb repressive complex 2 (PRC2) and regulates tri-methylation of histone H3 lysine 27 (H3K27me3), a chromatin modification process associated with gene silencing. Loss of function commonly results from truncating and frameshift mutations clustered in the C-terminal plant homology domain (PHD) and have been associated with secondary AML, *RUNX1* mutations, and poor prognosis.

EZH2: EZH2 is the catalytic subunit of the polycomb repressive complex 2 (PRC2) and is involved in repressing gene expression through methylation of histone H3 on lysine 27 (H3K27). This mediates gene silencing of target genes through chromatin reorganization. In AML, loss-of-function EZH2 mutations are more commonly associated with secondary AML and appear to confer a worse clinical outcome.

Kinases and Cell Signaling Pathways

NRAS* and *KRAS: Recurrent mutations are identified in *NRAS*, *KRAS*, *PTPN11, CBL,* and *NF1* genes, with functionally similar effects culminating in the activation of downstream RAS/RAF/MEK and PI3K/AKT/mTOR pathways. The *RAS* genes encode related family members of the cytoplasmic *RAS* family of small GTPases that mediate transduction of growth signals. Mutations often occur at codon hotspots G12, G13, or Q61 in both genes. Although the prognostic significance of *RAS* oncogene mutations is unclear in de novo AML, acquisition of *RAS* mutation is associated with MDS transformation to AML and portends a poor prognosis in this setting.

PTPN11: Encodes cytoplasmic protein tyrosine phosphatase non-receptor type 11 (also known as SHP2), a signaling molecule with two tandem N-terminal SH2 domains and a phosphatase domain. PTPN11 interacts with and regulates protein tyrosine kinases and is required for effective RAS-MAPK/ERK pathway signaling. *PTPN11* mutations do not show a clear impact on AML clinical outcome. However, germline mutations of *PTPN11* are linked to Noonan syndrome and are associated with increased risk of developing myeloid neoplasms, most commonly juvenile myelomonocytic leukemia (JMML). JMML associated with neurofibromatosis, Noonan syndrome, or Noonan syndrome–like disorders is recently recognized as a distinct entity in the 2016 WHO classification.

CBL: Encodes a ring finger (RF) family member of ubiquitin ligases (E3s), which targets receptor and non-receptor tyrosine kinases (including PDGFR and FLT3) and functions as an important negative modulator of tyrosine kinase signaling. Point mutations in the linker and RF regions as well as a loss of exons 8 or both 8 and 9 are associated with a loss of *CBL* ubiquitination of receptor tyrosine kinases, such as FLT3, and induction of cellular transformation. The prognostic significance of *CBL* mutation is not well established in myeloid neoplasms but is functionally similar to Ras deregulation. Germline mutations of *CBL* are associated with Noonan-like syndrome and show an increased risk of developing myeloid neoplasms, most commonly JMML.

NF1: Encodes neurofibromin-1, a GTPase activating protein (GAP), which enhances the hydrolysis of active GTP-Ras into inactive GDP-Ras, thus negatively regulating Ras signaling. Loss of neurofibromin-1 has been shown to elevate the level of GTP-Ras and result in cellular proliferation. Direct

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inactivation of neurofibromin-1 is infrequent in de novo AML, and *NF1* deletion is associated with complex and monosomal karyotypes. Germline *NF1* abnormalities are associated with neurofibromatosis type 1, which shows an increased risk of developing myeloid neoplasms, most commonly JMML.

Transcription Factors

RUNX1: Encodes the DNA binding alpha subunit of core binding factor (CBFα), a heterodimeric transcription factor that is involved in the normal differentiation of hematopoietic cells. *RUNX1* necessarily interacts with its partner core binding factor beta (CBFβ) and targets promoter regions through the conserved Runt homology domain (RHD), thereby regulating transcription at multiple genes involved in normal hematopoietic development. *RUNX1* also has tumor-suppressor properties, and loss of function of this gene through translocation or mutation contributes to tumorigenesis in myeloid cancers. The mutations in *RUNX1* are distributed across the gene (often within the RHD) and are usually mutually exclusive of *NPM1* and *CEBPA* mutations or balanced translocations. De novo AML with mutated *RUNX1* in the absence of MDS-related cytogenetic abnormalities appears to represent a distinct biologic entity with adverse clinical outcome; thus, a provisional entity of *AML* with mutated *RUNX1* has been added in the 2016 WHO classification. Germline mutations of *RUNX1* are also associated with familial platelet disorder (FPD), and these individuals have a predisposition to develop AML/MDS later in life. Myeloid neoplasm with germline *RUNX1* mutation is also recognized as a distinct entity in the 2016 WHO classification.

WT1: The wild-type Wilms' tumor suppressor gene product *WT1* has a critical role in normal renal and genitourinary tract development, and gene mutations are commonly observed in Wilms' tumor. *WT1* is also mutated in 5% to 15% of de novo AML patients, and most alterations are truncation or frameshift events that result in loss of the DNA-binding zinc finger regions. *WT1* mutations appear to be more prevalent in AML patients with the *FLT3*-ITD abnormality. The presence of *WT1* mutations has been associated with mostly poor clinical outcome, independent of *FLT3*-ITD, although this finding was not consistently observed in all studies. Overexpression of *WT1* has also been shown to correlate with poor prognostic features. The WT1 protein has recently been shown to interact with *TET2* in DNA binding, and, intriguingly, *WT1* mutations demonstrate similar DNA hypermethylation signatures as *TET2* and *IDH1/2* mutations, although they are mutually exclusive. These findings suggest that the mechanism of action of *WT1* mutation derives from an epigenetic effect (similar to *TET2* and *IDH* mutations) rather than loss of a traditional tumor-suppressor function.

Splicing Machinery

SF3B1, U2AF1, SRSF2, and ZRSR2: Encode protein components of the splicing machinery, and these genes are often mutated in MDS. Mutations are mutually exclusive of each other and demonstrate distinct disease phenotype associations: *SF3B1* is seen in 70% to 90% of MDS with ring sideroblasts and myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), and *SRSF2* is associated with more aggressive disease and chronic myelomonocytic leukemia (CMML). Spliceosome mutations contribute to tumorigenesis by inducing abnormal splicing during gene transcription, with impacts on epigenetic modification, transcription, and genome integrity. These alterations have a frequency of approximately 10% of AML, and studies have reported that mutations in *SRSF2*, *SF3B1*, *U2AF1*, or *ZRSR2* along with *ASXL1*, *EZH2*, *BCOR*, and *STAG2* are reported as relatively specific markers for secondary AML with poor clinical outcome.

Tumor Suppressor Genes

TP53: The p53 protein is a well-studied tumor suppressor with key functions in maintaining genome stability through induction of apoptosis or growth arrest. Normal p53 is upregulated in response to a variety of cellular proliferative stress and DNA damage signals. Somatic *TP53* mutations are seen in approximately 6% to 8% of AML and 5% to 10% of MDS, more commonly associated with secondary or therapy-related tumors rather than de novo AML (17% vs. 4%). *TP53* mutations often coexist with complex karyotypes (genomic instability) and confer chemoresistance with poor prognosis. Germline mutations in *TP53* are associated with Li-Fraumeni syndrome with predisposition to developing multiple solid and hematologic cancers.

Cohesin Complex

SMC1A, SMC3, RAD21, and STAG1 or STAG2: Recurrent cohesin complex gene mutations are present in approximately 5% of non-APL AML and are proportionately higher in cases of secondary AML. The cohesin complex is a multi-subunit ring-like structure composed of SMC1A, SMC3, RAD21, and STAG1 or STAG2 proteins and functions in the processes of anaphase sister chromatid exchange (mitotic checkpoint), DNA repair regulation, and transcription control. Cohesin complex gene mutations are prognostically neutral in de novo AML and commonly associated with *NPM1*, *RUNX1*, *BCOR*, and *ASXL1* mutations.

CLONAL HETEROGENEITY AND EVOLUTION IN AML AND THE CONCEPT OF AGE-RELATED CLONAL HEMATOPOIESIS OF INDETERMINATE POTENTIAL

CLONAL HETEROGENEITY IN AML

As is evident, the genetic profile of AML is relatively more complex than previously appreciated, and tumors at the time of diagnosis can harbor several distinct somatic driver

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mutations. Because of the emerging knowledge regarding recurrent gene mutation patterns and fractional allele burdens (made possible by NGS approaches), the origins and subsequent progress of many leukemias can be partially deduced. Any individual case of AML may carry several clonal subpopulations, of which the major clones are responsible for the initial presentation of clinical disease. However, accumulating data from longitudinal clinical research studies have shown that minor populations play a significant role in re-constituting disease relapse, following eradication of the major leukemic population. More sensitive or more comprehensive NGS studies also show these subclonal populations may reside at very low quantity, sometimes below the usual analytic sensitivity of typical clinical assays (e.g., 1% to 5%). As such, these investigations have resulted in a better understanding and mapping of the detailed pathways of AML development and progression (Fig. 24.6). As early events, for example, hematopoietic stem or progenitor cells acquire founder-type mutations in epigenetic modifiers (e.g., *DNMT3A, TET2, IDH1/2*, and *ASXL1*), establishing the presence of functionally immortalized preleukemic clones that still maintain the normal capacity for pluripotent differentiation. In AML, mutations of these genes commonly coexist, suggesting that although they facilitate clonal expansion, they are by themselves insufficient to induce overt leukemogenesis. *NPM1* mutations and activating alterations in signaling genes (e.g., *RAS*, *FLT3*, *JAK2*) usually emerge later during AML progression and confer a competitive growth advantage to the subclones harboring the initiating mutations, leading to the outgrowth of the dominant leukemic clone at the time of AML diagnosis. Mutations involving spliceosome complex genes (e.g., *SF3B1*, *SRSF2*, *U2AF1*) may also represent early events that destabilize hematopoietic cells through aberrant RNA splicing, and these abnormalities are often seen in the setting of secondary AML, implying a specific role in disease initiation. A sobering observation from "reverse engineering" AML biology by comprehensive mutation profiling of clonal architecture is that each tumor is an evolutionary phenomenon and prediction of clinical behavior or recurrence may be very difficult to achieve. Preleukemic stem cells can be persistent in remission samples of patients with AML, indicating that these cells can survive chemotherapy and serve as a potential reservoir for future relapse and resistance by following a stochastic rather than deterministic trajectory.

CLONAL HEMATOPOIESIS OF INDETERMINATE POTENTIAL

Following the widespread availability of sensitive PCR methods in the 1990s, low levels of *BCR-ABL1* and *IgH-BCL2* fusions were reported in the peripheral blood of healthy individuals without chronic myeloid leukemia or lymphoma, respectively. Within the past several years, chromosomal abnormalities commonly observed in hematologic neoplasms such as 20q, 11q, 5q, and 17p were observed in approximately 2% of apparently healthy people older than 70 years in genome-wide association studies (GWAS). Later, *TET2* mutations were identified in elderly women with normal blood counts with features of age-related hematopoietic clonal skewing. With the recent development of NGS technology, several large population-based studies have detected mutations

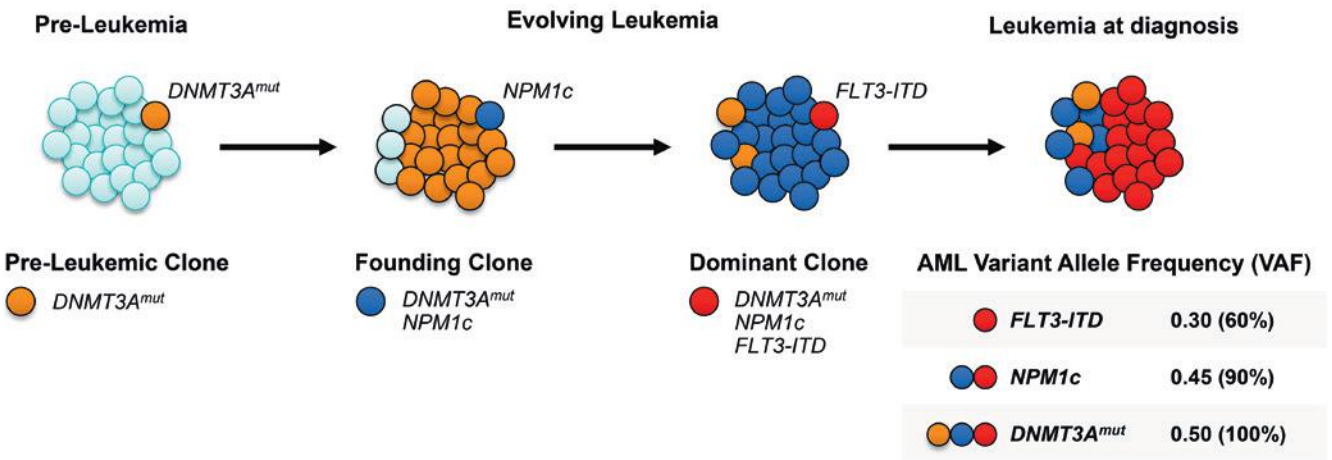


FIG. 24.6

AML clonal heterogeneity and evolution. Representative schema of clonal evolution of AML in a hypothetical patient harboring cooperative driver mutations in *DNMT3A*, *NPM1*, and *FLT3*-ITD at the time of AML diagnosis. The earliest event is the *DNMT3A* mutation occurring in the pre-leukemia stage. It facilitates clonal expansion (pre-leukemic clone, *orange*) but is not sufficient to induce overt leukemogenesis. *NPM1* mutation then emerges within this pre-leukemic clone and defines the founding AML clone *(blue)*, which becomes leukemogenic and is evolving into AML. This leukemia expansion phase is further accelerated by acquisition of an *FLT3*-ITD mutation leading to the dominant clone *(red)* present at the time of diagnosis. For simplicity, only a linear evolution path is shown, although branching evolution is frequently encountered. The evolutionary path of any given AML is also variable, although some common patterns are recognized. The relative variant allele fractions (VAF) of the mutated genes indirectly reflect the temporal order of mutation acquisition and clonal hierarchy of the bulk disease analyzed. Mutations with higher VAF are suggestive of earlier events in the disease process and those with lower VAF likely emerge later. Higher VAF may also be encountered when there is concurrent acquired uniparental disomy (copy neutral loss of heterozygosity) at the genetic locus where the specific mutation occurs.

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Box 24.1 Characteristic Features of Clonal Hematopoiesis of Indeterminate Potential (CHIP)

Age: Older (>60 years), increasing incidence by decade after 50 years
Number of mutations: Commonly single somatic mutation of myeloid-associated genes, rarely two genes
Mutation burden: Variant allele fraction (VAF) ≥2%, most not exceeding 20% to 30%
Common genes: *DNMT3A, TET2, ASXL1, SF3B1, JAK2, TP53, SRSF2, U2AF1, CBL, BCOR, GNB1, CREBBP, CUX1, MLL2, SETD2, SETDB1, PPM1D, BCORL1*
Risk association: 0.5% to 1% per year progression rate to subsequent hematologic neoplasms
Does not meet diagnostic morphologic features of hematologic neoplasms
Does not meet diagnostic criteria for PNH, MGUS, or MBL

MBL, Monoclonal B-cell lymphocytosis; *MGUS,* monoclonal gammopathy of undetermined significance; *PNH,* paroxysmal nocturnal hemoglobinuria.
Adapted from Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood* 126:9–16, 2015.

in genes commonly associated with myeloid neoplasms in individuals without apparent hematologic disease, most frequently involving the epigenetic modifiers *DNMT3A*, *TET2,* and *ASXL1*. The incidence of this finding increases with age, being relatively rare in people younger than 40 years, and approaching 10% to 20% between those aged 65 to 90 years. These individuals have an increased risk of developing hematologic neoplasms at a rate of approximately 0.5% to 1% per year (hazard ratio, ~13 compared to unaffected individuals lacking gene mutations); in this regard, this phenomenon is reminiscent of monoclonal gammopathy of uncertain significance and monoclonal B-cell lymphocytosis (Box 24.1). The presence of hematopoietic cells carrying clonal mutations is also associated with increased all-cause mortality and a higher risk of coronary artery disease. The age-related acquisition of somatic mutations in hematopoietic stem/progenitor cells producing a clonal expansion *in the absence of significant cytopenias, morphologic dysplasia, or neoplasia* has been termed clonal hematopoiesis of indeterminate potential (CHIP) or age-related clonal hematopoiesis (ARCH), reflecting its associated pre-malignant nature and biologic uncertainty. The presence of CHIP reflects an inability to eliminate or suppress the growth of mutated hematopoietic subclones that are occurring at appreciable rates in many individuals with advancing age.

Typical CHIP mutations are characterized by the presence of a single somatic mutation similar to those seen in myeloid neoplasms, with a variant allele frequency usually not exceeding 20% to 30%. A minimum threshold of 2% VAF has been proposed to define the presence of CHIP based on the assay detection limits and clinical data from published studies. However, even lower levels of clonal hematopoiesis have been demonstrated using more sensitive techniques, although the clinical significance of very low-level somatic mutations is uncertain. For example, in one study using an NGS assay with an analytic sensitivity of 0.01% and designed to correct for NGS sequencing errors, clonal hematopoiesis involving *DNMT3A* and *TET2* was identified in 95% of healthy individuals at ages 50 to 60 years with a median VAF of 0.2%. Importantly, these clonal mutations were stable with longitudinal follow-up and present in multiple hematopoietic compartments, highlighting the almost ubiquitous nature of very low-level, clinically silent clonal hematopoiesis. The emergence of more readily detectable levels of clonal hematopoiesis in the absence of overt disease is associated with both cell-intrinsic and cell-extrinsic factors and is modified by additional cooperative or mitigating mutations and environmental stressors such as chemotherapy, infection, or inflammation. Unsurprisingly, the presence of CHIP mutations has been associated with an increased risk of developing therapy-related myeloid neoplasms in patients with solid tumor receiving chemotherapy and in those with non-Hodgkin lymphoma following autologous stem-cell transplantation. Furthermore, in patients with unexplained cytopenias but insufficient pathologic features of a myeloid malignancy, a spectrum of mutated genes similar to CHIP has been observed that overlaps with alterations seen in patients meeting formal diagnostic criteria for MDS. This finding has been termed *clonal cytopenia(s) of uncertain significance* (CCUS), and these patients are generally considered to be at a higher risk of subsequently developing a hematopoietic neoplasm than individuals with CHIP. CCUS differs from the scenario of ICUS (idiopathic cytopenia of uncertain significance), in which a clonal genetic abnormality cannot be demonstrated by standard cytogenetics or molecular diagnostic (e.g., NGS) approaches in a patient with unexplained cytopenia(s). However, the association between CHIP, CCUS, ICUS, and established myeloid neoplasia requires further investigation to more precisely define their inter-relationships and delineate the relative risks for overt neoplastic progression. The existence of ICUS and CCUS very commonly presents a substantial diagnostic dilemma for clinical hematologists and hematopathologists in daily practice. Recognition of CHIP and CCUS is also critical to avoid the misdiagnosis of a myeloid neoplasm when not truly present or when assessing posttreatment AML specimens for persistent gene mutations suggestive of residual disease.

MYELODYSPLASTIC SYNDROMES

CYTOGENETIC ABNORMALITIES IN MDS

Recurring chromosomal abnormalities, in conjunction with the level of cytopenias and peripheral blood and bone marrow blast percentage, are an integral component of risk stratification in MDS in both the International Prognostic Scoring System (IPSS) and Revised International Prognostic Scoring System (IPSS-R). Chromosomal abnormalities are seen in approximately 50% of MDS cases and are characterized predominantly by loss of genetic material rather than translocation events. The presence of MDS-defining chromosomal abnormalities

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demonstrated by conventional karyotyping in patients with persistent cytopenias of unexplained etiology remains essentially unchanged in the 2016 WHO classification revision. Therefore, cytogenetic analysis of newly diagnosed MDS is a required component of evaluation. In this regard, multiple studies have consistently demonstrated a limited diagnostic and prognostic value of targeted FISH testing in MDS when karyotyping studies are deemed adequate. A value and evidence-based cytogenetic testing approach in MDS is recommended by several national/international guidelines, which reserves MDS FISH analysis for cases with inadequate karyotyping (<20 metaphase cells available) or cases with karyotype ambiguity that require FISH clarification per the cytogeneticist's discretion.

RECURRENT SOMATIC MUTATIONS IN MDS

Similar to AML, MDS is also a complex and heterogeneous neoplastic process with various types of mutations involving multiple genes and pathways. Recurrent somatic mutations have been reported in more than 40 genes, and 80% to 90% of MDS cases harbor at least one somatic mutation, with a median of two to three mutations per case. Common affected pathways involve spliceosome machinery, epigenetic modifiers, transcription factors, cell signaling and kinase pathways, tumor suppressors, and the cohesin complex (Fig. 24.7). Spliceosome genes and epigenetic modifier mutations are most common and observed in greater than half of MDS cases, highlighting their importance in the early pathogenesis of MDS. During disease progression, clonal expansion occurs from the emergence of additional mutations in signaling pathway (*NRAS/KRAS*, *FLT3*, *PTPN11*), transcription factor (*RUNX1*, *ETV6*), and tumor suppressor (*TP53*, *PHF6*) genes, illustrating a complex cooperativity that confers a competitive growth advantage to the dominant clone in a cellular background characterized by the initial deregulated pre-mRNA splicing and epigenetic abnormalities.

In the diagnostic evaluation of MDS, the presence of recurrent MDS-associated mutations provides evidence of a clonal process, particularly in cases with normal karyotype, and further supports the diagnosis of MDS in cases fulfilling clinical and morphologic criteria. At the same time, as nearly all cases of MDS harbor at least one gene mutation, the negative predictive value to exclude the possibility of MDS is relatively high in the absence of mutations when using a well-designed targeted NGS panel with adequate genetic coverage. In these cases with negative NGS findings, non-neoplastic causes of cytopenias should be thoroughly evaluated and excluded before a diagnosis of MDS can be established. For cases suspected of MDS, detection of one or more mutations helps to establish clonal hematopoiesis and may help to rule out benign causes of cytopenias in the appropriate clinical setting. As MDS-associated mutations overlap with CHIP (seen in 10% of healthy individuals >70 years), the detection of similar alterations does not presently constitute sufficient laboratory evidence of MDS if definitive morphologic features of dysplasia are lacking. As already indicated, the term *clonal cytopenia(s) of undetermined significance* has been proposed for patients with clonal hematopoiesis, unexplained cytopenia(s), and not fully meeting the morphologic features diagnostic of MDS. Further clinical studies and follow-up are required to elucidate the natural history and optimal management of CCUS, which may also prove to be of value in the future as related to risk prediction for the development of myeloid neoplasia.

The inclusion of the new WHO 2016 subgroup of MDS-RS with multilineage dysplasia is largely based on the frequent association of *SF3B1* mutations with ring sideroblasts (80%), the presence of which confers a distinct genetic expression

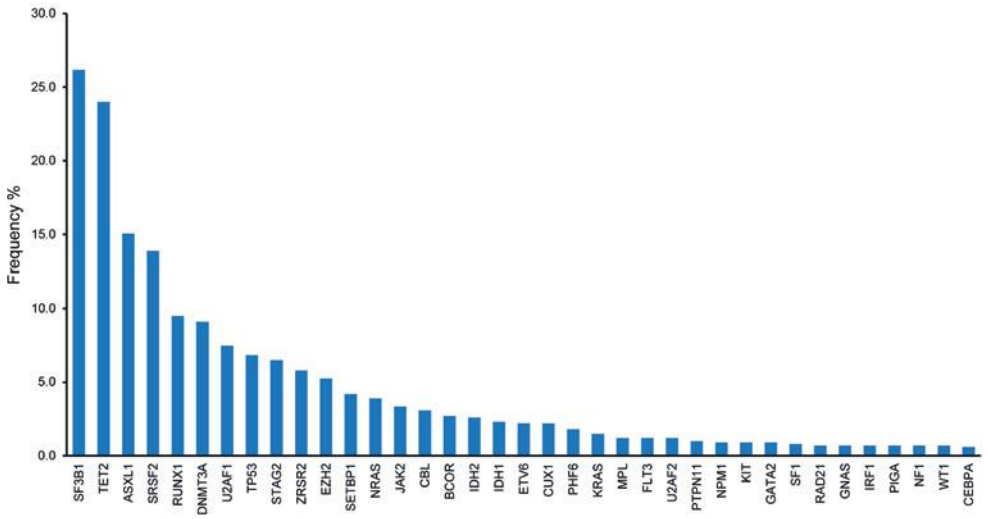


FIG. 24.7

Molecular landscape of myelodysplastic syndrome (MDS). Frequency of common driver mutations identified in MDS. Spliceosome and epigenetic modifier mutations in genes *SF3B1*, *TET2*, *AXSL1*, and *SRSF2* are the most common mutations seen in MDS, each present in more than 10% of patients. MDS disease progression is associated with acquisition of additional mutations in the kinase signaling pathway, transcription factors, and tumor suppressor genes. *Note: The presented frequency graph includes subsets of CMML and MDS/MPN-RS-T cases.

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profile and favorable outcome of low-risk MDS. The diagnostic criteria of MDS-RS requires 15% or more ring sideroblasts in the absence of a *SF3B1* mutation but 5% or more ring sideroblasts in the presence of an *SF3B1* mutation. *SF3B1* mutations are usually missense alterations and can occur at codons E622, Y623, R625, N626, H662, T663, K666, K700, I704, G740, G742, and D781, spanning exons 14 to 16. The hotspot mutation Lys700Glu (K700E) accounts for more than half of somatic *SF3B1* mutations. Multiple studies have also demonstrated additional prognostic value provided by the numeric quantity and pattern of mutations in MDS, as shown by the finding that a higher number of gene mutations is correlated with inferior leukemia-free survival and enhanced risk of leukemic transformation. Mutations in *TP53*, *EZH2*, *ETV6*, *RUNX1*, and *ASXL1* were found to be independent risk factors for poor overall survival in multivariate studies. A clinical study including 14 genes in combination with conventional risk factors of gender, age, and IPSS-R exhibited higher prognostic power than IPSS-R or the gene model alone. Patients with MDS harboring *TET2* or *DNMT3A* mutations are also more likely to initially respond to DNA methyltransferase inhibitor therapy, such as azacitidine and decitabine, although some longitudinal investigations have demonstrated transient efficacy and ongoing dynamic clonal shifts when using epigenetic modifier therapies. Given the poor lenalidomide response observed in *TP53*-mutated del5q-positive MDS patients, the 2016 WHO revision has recommended evaluation of *TP53* mutation status in these cases. Various types of *TP53* mutations exist, including nonsense, frameshift, splice site, and missense mutations, commonly involving the DNA-binding domain spanning exons 4 to 9. *TP53* gene mutation is also notable in general as a poor prognostic factor in MDS and AML. Although gene mutations are not currently included in the IPSS/IPSS-R or other risk scoring systems for MDS, it seems inevitable that key alterations will become integrated as part of a more comprehensive framework for MDS prognosis. Several national and international guidelines have recommended or suggested molecular profiling of recurrent gene mutations to augment diagnosis and prognostic risk assessment in MDS.

MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS

CHRONIC MYELOMONOCYTIC LEUKEMIA

Clonal cytogenetic abnormalities are seen in 20% to 40% of CMML cases, and they overlap with other myeloid neoplasms and thus are not considered disease defining. Recurrent somatic gene mutations have been identified in up to 90% of CMML cases, and the commonly mutated genes in the order of descending frequency are *TET2* (40% to 60%), *SRSF2* (40% to 50%), *ASXL1* (40% to 50%), *RUNX1* (15% to 20%), *NRAS* (10% to 20%), and *SETBP1*, *CBL*, *EZH2*, *KRAS*, *SF3B1*, *ZRSF2*, *U2AF1*, *IDH2*, *JAK2*, *FLT3*, *NPM1*, *DNMT3A*, *IDH1*, and *TP53* (each 1% to 10%). Comutation of *TET2* and *SRSF2* is highly associated with a CMML disease phenotype, characterized by monocytosis and morphologic dysplasia. *TET2*, *SRSF2*, and *ASXL1* mutations are seen in more than 80% of CMML cases, therefore providing a set of clonal genetic markers in cases diagnostic or highly suspicious for CMML; however, as mutations in each of these genes are also encountered in healthy aging people as CHIP, detection of a solitary alteration, especially at low (<20%) VAF, should not be used as definitive evidence of CMML. Among the recurrent somatic mutations, a frameshift and truncating *ASXL1* mutation has been confirmed to be an independent poor prognostic marker in multiple studies, and this abnormality has been incorporated into some risk stratification schemes for CMML. Mutations in the *RAS*, *CBL*, and *JAK2* genes likely represent secondary mutations in CMML and are typically associated with myeloproliferative disease progression. Of note, *NPM1* mutations can rarely be seen in CMML and appear to portend an aggressive clinical course with rapid progression toward AML. As for other myeloid neoplasms, the range of genetic mutations in CMML is also optimally detected using a targeted NGS strategy.

JUVENILE MYELOMONOCYTIC LEUKEMIA

Clonal cytogenetic abnormalities are seen in 35% of JMML cases, with nearly 70% showing monosomy 7. Unlike CMML, which is more commonly seen in older adults, JMML is a childhood clonal hematopoietic disorder, and, consequently, the spectrum of genetic mutations is somewhat different from CMML, with more than 90% of the recurrent abnormalities in JMML converging on the *RAS* oncogene pathway. The commonly mutated genes in JMML thus include *KRAS/NRAS* (each 20%), *NF1* (30%), *PTPN11* (35%), and *CBL* (10% to 15%). Both somatic and germline mutations are present, and germline mutations in *PTPN11* and less commonly *KRAS/NRAS, CBL*, and *NF1* mutations are associated with Noonan syndrome, Noonan-like syndrome, and neurofibromatosis, respectively. JMML in these clinical contexts is recognized under the 2016 WHO classification of myeloid neoplasms with germline predisposition; spontaneous regressions of germline *CBL* mutation-associated JMML have been described.

MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASM WITH RING SIDEROBLASTS AND THROMBOCYTOSIS

In MDS/MPN-RS-T, *SF3B1* mutations are present in 70% to 90% of cases and frequently coexist with *JAK2* V617F (50% to 65%) or less commonly (<10%) mutations involving *CALR* or *MPL*. The combination of *SF3B1* with driver mutations in *JAK2* V617F, *CALR*, or *MPL* provides the biologic basis to explain the mixed MDS/MPN phenotype exhibited in MDS/MPN-RS-T. *JAK2*, *CALR*, and *MPL* gene mutation screening is also further detailed in the section on myeloproliferative neoplasms. *SF3B1* mutations are best evaluated by Sanger sequencing or NGS, given the different possible

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mutation sites documented; the hotspot mutation K700E accounts for more than half of somatic *SF3B1* mutations.

ATYPICAL CHRONIC MYELOID LEUKEMIA, BCR-ABL1 NEGATIVE

Clonal cytogenetic abnormalities are seen up to 80% of atypical chronic myeloid leukemia (aCML), most commonly +8 and del(20q). Recurrent somatic mutations have been identified in *NRAS/KRAS*, *SETBP1*, and *ETNK1* genes with a frequency of approximately 30%, 20% to 30%, and 9%, respectively. *CSF3R* is a gene commonly mutated in CNL, and mutations of this gene were initially reported in aCML; however, this finding was not confirmed in two subsequent studies employing a strict pathologic definition of aCML. Although the *JAK2* V617F mutation has been reported in 4% to 8% of aCML, *CALR* or *MPL* mutations are rarely encountered in aCML. Therefore, the pattern of gene mutations (e.g., detected by targeted NGS) can be useful to help establish a diagnosis of aCML in cases that are morphologically overlapping with CNL, MPN-unclassifiable, or CMML, although some genes that are common to the pathogenesis of these myeloid neoplasms may be less informative in this situation.

Box 24.2 Myeloid Neoplasms With Germline Predisposition

Myeloid Neoplasms With Germline Predisposition Without a Preexisting Disorder or Organ Dysfunction

AML germline *CEBPA* mutation
Myeloid neoplasms with germline *DDX41* mutation

Myeloid Neoplasms With Germline Predisposition and Preexisting Platelet Disorders

Myeloid neoplasms with germline *RUNX1* mutation
Myeloid neoplasms with germline *ANKRD26* mutation
Myeloid neoplasms with germline *ETV6* mutation

Myeloid Neoplasms With Germline Predisposition and Other Organ Dysfunction

Myeloid neoplasms with germline *GATA2* mutation
Myeloid neoplasms associated with BM failure syndromes
Myeloid neoplasms associated with telomere biology disorders
JMML associated with neurofibromatosis, Noonan syndrome, or Noonan syndrome–like disorders
Myeloid neoplasms associated with Down syndrome

AML, Acute myeloid leukemia; *BM,* bone marrow; *JMML,* juvenile myelomonocytic leukemia.
Adapted from Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood* 127:2391–2405, 2016.

MYELOID NEOPLASMS WITH GERMLINE PREDISPOSITION

Although most myeloid neoplasms are considered to arise in a sporadic or de novo manner, recent evidence has demonstrated that a subset of affected patients can instead harbor germline mutations associated with underlying familial syndromes showing an intrinsic predisposition to develop myeloid cancers. This information was included in the 2016 WHO classification as a distinct entity of myeloid neoplasms with germline predisposition (Box 24.2). Multiple genes have been implicated in this category, each denoting a specific type of familial syndrome with variable disease manifestations including hematologic abnormalities (i.e., owing to ineffective bone marrow function), protean extramedullary tissue or organ disorders, and the emergence of myeloid tumors across a wide age range. Some genes are common to both germline conditions and somatic myeloid neoplasms (e.g., *RUNX1*, *CEBPA, ETV6*) and are thus likely to be included in tumor-specific NGS panels; however, other important genes would not be identified by this approach. Although many of these genes could be included in a more comprehensive targeted NGS panel, some practical and operational aspects require consideration. Most important is the recognition that "marrow failure syndromes" represent a very large group of diverse conditions spanning hematology, other internal medicine, immunology, and clinical genetics practices, necessitating a coordinated effort to identify these patients effectively. Second, a large number of genes are associated with these conditions, including those encoding the Fanconi anemia protein complex, telomeropathies (e.g., dyskeratosis congenita), familial platelet disorders (*ANKRD26*, *RUNX1,* etc.), Noonan syndrome, and several "orphan" conditions such as mutations involving *SRP72* or *DDX41*. This indicates that the advantages of smaller targeted NGS panels begin to lose ground to more comprehensive approaches (WES, WGS) when the evaluation of familial predisposition syndromes is a consideration in test development. Related to this aspect is the increased complexity of the alterations in a number of germline predisposition genes, including changes in copy number or intrinsic genomic instability, requiring additional methodologies to achieve sufficiently high clinical sensitivity. Third, the requirement to include full exon sequencing of an increasingly larger number of genes begins to blur the boundary between cancer and germline testing indications. Practically, this entails a sufficiently large panel design (or WES) with the attendant additional analytic complexity; delineating true pathogenic mutations from numerous variant of uncertain significance (VUS) alterations or rare "private" polymorphisms; and providing requisite support for further evaluation when a germline change is identified (i.e., screening of family members, genetic counseling, and risk assessment, etc.). Targeted panel NGS testing performed on peripheral blood and bone marrow samples for myeloid neoplasms may therefore return findings in some genes that do not allow direct differentiation between a heterozygous germline variant and somatic mutation, particularly when the detected VAF is near 50%. To confirm the germline nature of a specific mutation, follow-up testing can be performed on a buccal swab sample, cultured skin fibroblasts, or possibly flow-sorted,

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highly purified CD3-positive T cells. Germline genetic testing has a profound psychosocial impact, and the importance of thorough genetic counseling in this scenario is paramount.

MOLECULAR DIAGNOSTIC EVALUATION OF OTHER CHRONIC MYELOID NEOPLASMS

The chronic myeloid tumors represented in this section include several neoplasms grouped by common clinicopathologic characteristics. Specific genetic abnormalities are continuing to subdivide and better categorize seemingly related members of these diseases between and within existing classifications (Tables 24.9 and 24.10). These entities include the "classic" myeloproliferative neoplasms (*BCR-ABL1*+ chronic myeloid leukemia, polycythemia vera, primary myelofibrosis, essential thrombocythemia, chronic neutrophilic leukemia), myeloid and lymphoid neoplasms associated with eosinophilia, and systemic mastocytosis.

CHRONIC MYELOID LEUKEMIA: t(9;22)/ BCR-ABL1

The t(9;22)(q34;q11)/*BCR-ABL1* abnormality was initially recognized as the "Philadelphia chromosome" (Ph) associated with CML nearly 50 years ago; detailed study of this genetic lesion over four decades has resulted in a detailed understanding of the pathobiology of *BCR-ABL1* leukemogenesis, as well as a paradigm for use of targeted pharmacologic therapies in cancer. The *BCR-ABL1* chimeric gene is required for the diagnosis of CML and is also central to the pathogenesis of 20% to 25% of adult and 3% of childhood B-lineage acute lymphoblastic leukemia (ALL). Two major forms of *BCR-ABL1* exist based on different breakpoint-fusion sites within the *BCR* gene (see Fig. 24.3). The major breakpoint cluster region (M-BCR), encompassing a 5.8-kD DNA span, is involved in nearly all cases of CML. Breakpoints in *ABL1* occur in a large intronic region upstream of exon 2. The *BCR-ABL1* gene fusion on the derivative 22(q11) results in the production of an e14-a2 or e13-a2 fusion transcript (formerly, b3-a2 or b2-a2, respectively), with a corresponding 210-kD chimeric oncoprotein (p210). Not infrequently, alternative splicing of the *BCR* exon 14 fusion generates both e13-a2 and e14-a2 transcripts in the same leukemia. In contrast, Ph-positive B-ALL cases are associated with *BCR* breakpoints occurring in the minor breakpoint cluster region (m-bcr) located farther 5′ in the *BCR* gene. The resultant single e1-a2 chimeric mRNA is associated with the production of a 190-kD protein (p190). However, *BCR* gene break-sites and disease phenotypes are not exactly correlated. Although the e14 (or e13)-a2 product (p210) is essentially pathognomonic of CML in the appropriate clinical and pathologic setting, unusual cases have been rarely described with a myelomonocytic type of hematologic presentation and the e1-a2–derived p190 *BCR-ABL1*. By extension, approximately one third of adult and a smaller number of pediatric Ph-positive patients with B-ALL are associated with *BCR* breakpoints in the M-BCR and the presence of a p210 chimeric transcript. These exceptions illustrate the importance of cellular context (e.g., pluripotent stem cell vs. committed lymphoid progenitor) in the generation of *BCR-ABL1*–associated diseases. A third less common type of *BCR-ABL1* event involves a 3′ breakpoint cluster region termed the *μ-BCR* (see Fig. 24.3), resulting in the formation of an e19-a2 fusion transcript, encoding a p230 protein. This latter anomaly is identified in very rare cases of CML, exhibiting a variant of the disease often with more prominent neutrophilic differentiation. Additional very rare alternative forms of *BCR-ABL1* have also been described in CML or Ph-positive B-ALL, including the e6-a2 type transcript, chimeric mRNA fusions involving BCR exon 8, and also translocation events involving *ABL1* exon a3 (i.e., e1-a3, e13-a3). RT-PCR assays can be appropriately designed to detect most of these uncommon variants, but in extremely unusual cases, targeted PCR methods may miss the presence of a complex or cryptic *BCR-ABL1* event that would otherwise be evident from cytogenetics or FISH testing.

Timely detection of the *BCR-ABL1* abnormality is critical to correctly establish the diagnosis of CML and exclude potential overlapping entities (e.g., the Ph-negative classic myeloproliferative neoplasms, or myeloproliferative/myelodysplastic processes like chronic myelomonocytic leukemia [CMML] and atypical CML). The identification of the *BCR-ABL1* is important to distinguish CML from cases of true chronic neutrophilic leukemia (CNL), which are by definition *BCR-ABL1* negative and instead harbor mutations in the *CSF3R* receptor gene. As indicated, rapid detection of *BCR-ABL1* is also of importance in B-ALL, to identify those patients with high-risk disease. The schematic of Fig. 24.3 illustrates the strategy for RT-PCR amplification of specific *BCR-ABL1* fusion mRNA types. Both qualitative and quantitative PCR methods can be employed in this regard, depending on the clinical scenario (e.g., diagnosis versus post-therapy monitoring). Standard cytogenetics and D-FISH analyses can also be valuable at diagnosis; however, the role of cytogenetics and FISH in follow-up management has become more circumscribed in the era of tyrosine kinase inhibitor (TKI) monotherapy as discussed further below. The remarkable success of TKI therapy has led to the development of sophisticated strategies for evaluating response and risk of treatment failure (Table 24.7). Major therapeutic "milestones" with the use of TKI include achievement of complete hematologic response and subsequently a complete cytogenetic response (CCR). Ideally, CCR, defined by the absence of the Ph chromosome in a good quality bone marrow karyotype study, should be attained within the first 6 to 12 months of therapy. The application of real-time quantitative RT-PCR technique for sequential monitoring of *BCR-ABL1* transcript levels has greatly individualized the management of patients with CML receiving TKI therapy (see Fig. 24.3). The earliest landmark clinical trial studies of imatinib in CML established a log reduction scale that correlated with response and outcomes. In this manner the achievement of a major molecular response (MMR), defined as a 3-log reduction

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TABLE 24.7

Optimal Response Criteria for Chronic Myeloid Leukemia Treated With Tyrosine Kinase Inhibitors

Time Point	Summary of Tests and Optimal Response Criteria: NCCN (2015) and ELN (2013)[a]
Diagnosis	CG, or FISH, or qualitative RT-PCR RQ-PCR for baseline BCR-ABL1 p210 mRNA level
3 months	RQ-PCR ≤10% BCR-ABL1 (IS) or CG <35% Ph^+ (PCyR)
6 months	RQ-PCR ≤10% BCR-ABL1 (IS) or CG <35% Ph^+ (PCyR)
12 months	CG CCyR RQ-PCR ≤0.1% (IS) MMR desirable
18 months	CG CCyR and/or RQ-PCR ≤0.1% (IS) MMR

[a]Note: FISH is not preferred for minimal residual disease detection.
CG, Cytogenetics performed on a bone marrow specimen; *CCyR,* complete cytogenetic response (0% Ph^+ metaphases); *ELN,* European LeukemiaNet; *MMR,* major molecular response (0.1% on International Scale, IS); *NCCN*, National Comprehensive Cancer Network; *PyCR,* partial cytogenetic response (1% to 35% Ph^+ metaphases); *RQ-PCR,* real-time quantitative RT-PCR.

in normalized *BCR-ABL1* mRNA level (relative to a pool of diagnostic-level patient samples), was associated with a very low risk of disease relapse or progression. Adoption and standardization of reproducible RQ-PCR methods to reflect the initial study benchmarks had proved challenging for molecular diagnostic laboratories until the development of *BCR-ABL1* e13/14-a2 (p210) WHO secondary reference materials, which enabled the commercial manufacturing of high-quality reagents and standards to improve the adoption of more universal and accurate testing. Accordingly, the International Scale (IS) has been introduced to interpret *BCR-ABL1* RQ-PCR levels from 100% to 0.01% (and even lower), with 0.1% defined as the MMR; these values can alternatively be expressed on a "molecular response" (MR) log reduction scale, such that 0.1% = MR3, for example. *BCR-ABL1* p210 mRNA levels are reported as a normalized ratio relative to that of a well-chosen housekeeping gene transcript. Most commonly, native *ABL1* is used for this purpose and, although there is some inaccuracy at the very high end of the quantitative range, *ABL1* is otherwise a very acceptable and stable choice over the required critical dynamic range (i.e., analytic measurement range [AMR]) of the RQ-PCR assay; other gene transcripts for this purpose have been described, including GUSB and BCR.

The routine use of RQ-PCR from the time of diagnosis and onward during TKI therapy provides ongoing predictive data for optimal clinical management of CML cases; a normalized *BCR-ABL1* mRNA level less than 10% (IS) at 3 months and/or less than 1% (IS) at 6 months is correlated with a high probability of achieving CCR and a favorable long-term outcome. Patients who obtain CCR with TKI therapy have a greater than 85% chance of staying in long-term remission and those who meet or exceed MMR have a sustained response with very low rates of hematologic relapse or blastic transformation. Failure to reach these early treatment response goals may indicate the need for other TKI agents, chemotherapy and interferon therapy, or allogeneic hematopoietic stem cell transplantation. Cytogenetic bone marrow evaluation can be repeated at 6 months but is required at 12 months post therapy to assess for CCR, as well as to determine if any non-Ph clonal genetic findings are present that may have an impact on TKI response or indicate emerging new bone marrow pathology. Although D-FISH technique may be useful at diagnosis, it is not recommended for residual disease monitoring because of insufficient analytic sensitivity and the potential risk of technical false positivity when scoring very small numbers of apparent fusion signals. Rapid and accurate detection of *BCR-ABL1* p210 mRNA levels at 2- to 3-month intervals using RQ-PCR methods is the standard of care for CML, and laboratories routinely performing these assays should be able to achieve replicate precision within 0.5 log for clinical use. It should be noted that *BCR-ABL1* levels are detected at similar concentrations in peripheral blood and bone marrow specimens of CML patients. Published consortium guidelines for the management of CML cases have been advocated by both the European LeukemiaNet (ELN) and National Comprehensive Cancer Network (https://www.nccn.org/), which comprehensively detail expected response parameters and timelines, as well as warning signs for patients at risk of TKI treatment failure and disease relapse or progression (see Table 24.7).

A rising amount of *BCR-ABL1* (>0.5 log) precedes loss of MMR or CCR and can thus be used as a surrogate marker for inadequate or refractory treatment response. Therapeutic resistance is relatively uncommon in CML patients treated with TKI agents and not fully understood, but approximately 50% of these individuals develop acquired point mutations in the *ABL1* kinase domain region of the *BCR-ABL1* oncogene. Kinase domain mutations (KDM) involve exons 4 through 9 of *ABL1* and affect codons in the phosphorylation (P) loop, activation (A) loop, catalytic domain (CD), or at residues known to be critical for drug contact and binding. Over 100 KDM have been described, including the most prevalent amino acid changes with known imatinib resistance: M244V, G250E, Q252H, Y253F/H, E255K/V (P-loop), H396P/R (A-loop), M351T, F359V (CD), and T315I, F317L (contact sites). Because KDM are spread throughout a relatively large region of *ABL1*, they are usually detected using qualitative RT-PCR and Sanger sequencing. One practical consideration with KDM analysis is related to the quantitative *BCR-ABL1* mRNA level: with lower transcript abundance (e.g., near the MMR or 0.1% IS), successful RT-PCR amplification of a longer *BCR-ABL1* mRNA template encompassing the entire KDM region is more difficult to achieve. Thus, an optimized RT-PCR, typically employing a nested round of internal *ABL1* amplification, is required to provide sufficient high-quality product for Sanger sequencing of the KDM. Even with a well-designed assay, however, the sensitivity of Sanger sequencing is limited to approximately 20% for detecting

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acquired KDM in the *BCR-ABL1*–positive cell population. Other diagnostic methods include targeted point mutation screening using high-resolution melting curve analysis of PCR products, pyrosequencing, or allele-specific PCR for common mutations; these approaches are capable of lower level mutation detection (e.g., 5%) but have lower clinical sensitivity. Kinase domain mutations differ in their resistance profiles, and for some changes simply escalating imatinib dosage can reverse the adverse effects. More resistant mutation types can often be successfully overcome by switching to newer generation TKI drugs (e.g., dasatinib, nilotinib, bosutinib). Most significantly, patients with CML developing the Thr315Ile (T315I) abnormality show widespread insensitivity to nearly all currently employed TKI agents, with the exception of ponatinib. KDM are also more frequently identified in CML patients with accelerated phase or blast crisis, as well as in patients with *BCR-ABL1*–positive B-LBL treated with TKI agents. Knowledge of drug resistance patterns and relative therapeutic indexes is important to guide optimal management. Some studies have also reported very low-level mutations in patients in the early chronic phase CML using ultrasensitive methods, which indicates that KDM are present in some individuals and can be selected under treatment pressure. Current recommendations do not require highly sensitive KDM screening at diagnosis. Indications for *ABL1* KDM testing can include (1) serial increases in normalized *BCR-ABL1* levels pointing to loss of MMR or CCR; (2) failure to achieve CCR after sufficient initial TKI treatment (e.g., by 12 months); (3) rare instances of primary therapeutic resistance; (4) development of blast crisis; or (5) when considering a switch to a different TKI agent (e.g., due to drug intolerance). Finally, it is worth noting that two or occasionally more KDM can be present in a given patient and these events have been found to occur as both compound clones (i.e.. cumulative events in a single cell population) or, less frequently, as multiple separate clones (polyclonal mutations). The presence of multiple KDM appears correlated with poorer therapeutic response to TKI agents.

BCR-ABL1-NEGATIVE CLASSIC MYELOPROLIFERATIVE NEOPLASMS: PRIMARY MYELOFIBROSIS, ESSENTIAL THROMBOCYTHEMIA, AND POLYCYTHEMIA VERA

The classic *BCR-ABL1*–negative myeloproliferative neoplasms include polycythemia vera (PV), primary myelofibrosis (PMF), and essential thrombocythemia (ET). Clonal cytogenetic abnormalities are seen in 20% to 30% of PV and PMF, most commonly +8, +9, del(20q), and del(13q). Cytogenetic abnormalities are rare in ET (5% to 10%) and include +8, 9q abnormalities, and del(20q). Cumulative research over the past decade has greatly enhanced our understanding of the genetic basis of MPN pathogenesis and corresponding diagnostic capability. The molecular landscape of these three disorders has been largely defined by recurrent driver mutations in the *JAK2*, *MPL*, or *CALR* genes. Mutations in one of these genes are identified in essentially all cases of PV and more than 90% of PMF and ET (Fig. 24.8). In PV, at least 95% of patients harbor the *JAK2* Val617Phe (V617F) point mutation in exon 14, whereas the remainder have small indel-type mutations within *JAK2* exon 12. In PMF and ET, the most common mutation is also the *JAK2* V617F, comprising approximately 50% to 60% of cases, followed by mutations in *CALR* (20% to 30%), and *MPL* (3% to 5% in ET and 5% to 10% in PMF). *JAK2*, *CALR*, or *MPL* mutations activate cell signaling via the JAK-STAT pathway and are capable of inducing a MPN phenotype in murine models. These mutations are essentially mutually exclusive of each other, and the common convergence on the JAK-STAT pathway also explains the observed responses to therapeutic

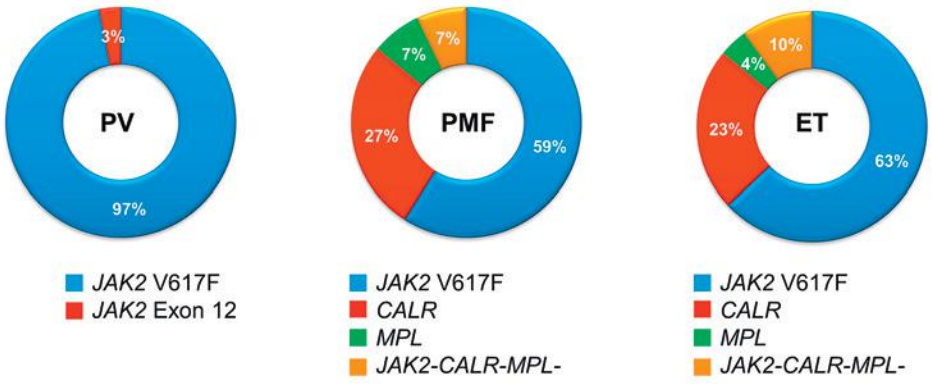


FIG. 24.8

Molecular landscape of major driver mutations in BCR-ABL1–negative classic myeloproliferative neoplasms (MPN). Distribution of the major driver mutations in *BCR-ABL1*–negative classic MPNs. In polycythemia vera (PV), more than 95% of cases harbor the exon 14 *JAK2* V617F mutation. The rest are essentially all positive for *JAK2* exon 12 mutations. In primary myelofibrosis (PMF) and essential thrombocythemia (ET), *JAK2* V617F account for approximately 50% to 60% of cases, and the second most common mutation is *CALR* (20% to 30%), followed by *MPL* (3% to 5% in ET and 5% to 10% in PMF). The rest are triple-negative (*JAK2-CALR-MPL-*), which is a high-risk molecular signature in PMF. The mutations converge on the JAK-STAT pathway and are essentially mutually exclusive.

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JAK inhibitors regardless of the specific driver mutation. In morphologically equivocal cases or patients with unexplained cytosis or myelofibrosis suspected of an MPN, identification of one of these gene mutations is supportive of the diagnosis in the appropriate clinicopathologic setting. Further to its diagnostic value, the type of gene mutation also serves as a prognostic marker for clinical outcome in patients with PMF or ET who have a risk of thrombotic complications: in PMF, several large studies have demonstrated a more favorable outcome in *CALR*-mutated cases and inferior survival for "triple negative" cases (i.e., lacking *JAK2*, *CALR*, or *MPL* alterations) and in ET, *CALR* mutations are associated with a lower risk of thrombosis.

JAK2 MUTATIONS

Although precise subclassification still requires consideration of key clinical, laboratory and histopathologic findings, the presence of an activating *JAK2* mutation provides strong evidence for the diagnosis of an MPN in suspected cases and has been an integral component of the WHO diagnostic criteria for all three entities. The mutation results in a constitutively activated cytokine (EPO, TPO, GCSF) receptor/JAK2 complex that can recruit and phosphorylate one of several STAT proteins (signal transducer and activator of transcription), leading to downstream effects eventuating in increased cell division. A single point mutation (G1849T) in exon 14 replacing valine with phenylalanine at codon 617 (*JAK2* V617F) is found in the majority of MPN. This mutation involves the pseudokinase (JH2) region of JAK2, which normally negatively regulates and inhibits autophosphorylation of the adjacent kinase domain in the absence of associated receptor-ligand activity. The amino acid substitution alters the inactive conformation of *JAK2* and abrogates its intrinsic regulatory control, resulting in autonomous cell signaling independent of receptor-cytokine engagement, with constitutive activation of the central JAK-STAT pathway as well as the ERK/MAPK and PI3K/AKT pathways.

The *JAK2* V617F abnormality is most prevalent in PV (>95%) and present in the majority of PMF and ET (50% to 60%). The relative amount of mutated JAK2 V617F alleles to wild type alleles varies considerably between different individuals with the same disease type. Furthermore, patients with advanced PV and a subset of patients with PMF have biallelic homozygous mutations arising from a copy-neutral loss of heterozygosity event (acquired uniparental disomy) of chromosome 9(p24). The heterogeneous distribution of the V617F *JAK2* allele in tumor cells from different patients also indicates that relatively sensitive assays are required for detection. It has been recommended that molecular analytic assays should be able to minimally detect a level of 0.1% mutated DNA in a patient specimen. A variety of methods have been advocated for detecting the *JAK2* V617F, including high-resolution PCR product melting curve analysis, Sanger sequencing or pyrosequencing, and variations of allele-specific PCR (e.g., amplification refractory mutation system [ARMS] and RQ-PCR). Of these, allele-specific PCR approaches using DNA as a template reproducibly achieve the highest analytic sensitivity (see Table 24.8). A quantitative reporting of mutation burden is not presently required for diagnostic or therapeutic purposes, but the development of newer generation *JAK2* inhibitors may change this aspect as more data emerge.

It should be noted that the *JAK2* V617F is also found in patients with MDS, CMML, atypical CML (*BCR-ABL1* negative), and de novo AML (5% or less each), as well as in most cases of MDS/MPN-RS-T. Thus, the presence of a *JAK2* V617F mutation does not necessarily specify the diagnosis of a classic MPN and should not be used in the context of conclusively distinguishing MPN from other rare, atypical myeloid tumors with proliferative morphologic features. Conversely, a negative *JAK2* V617F analysis does not exclude the diagnosis of a classic MPN, if definitive clinical and morphologic findings are otherwise present. The use of the high sensitivity of PCR methods may also identify apparent V617F mutations at very low levels (<0.1%), and the presence of rare mutated cells in healthy subjects has been described. By extension, the finding of a very low-level *JAK2* V617F abnormality in a patient with unexplained minimal thrombocytosis or leukocytosis could be misleading. Therefore, molecular diagnostic laboratories must be aware of the wide dynamic range of detection for *JAK2* V617F and understand the relative clinical validity of positive values, especially at low levels (<1%), when reporting such results. Recently the *JAK2* V617F has been reported in several large population studies as part of the CHIP gene spectrum with an incidence of approximately 0.2%. Although the mutation was detected at significantly higher VAF than most instances of CHIP involving other genes, it is important to note that not all patients harboring *JAK2* V617F had a well-documented hematologic evaluation in these reports. In one study involving over 12,000 subjects with 2 to 7 years of longitudinal follow-up, a sizeable proportion of individuals (7/24 cases) with *JAK2* V617F mutation either were found to have an existing MPN or developed MPN during the study. Further studies are thus warranted to establish whether the presence of *JAK2* V617F at relatively high VAF is truly a CHIP-associated, age-related finding in these rare individuals; current data are inconclusive in this regard.

A small group of patients with PV lack the *JAK2* V617F but have been found instead to have small in-frame insertion/deletion (indel) events, or base substitutions involving codons 536-547 in exon 12 of the gene, such as N542-E543del, H538-K539del, F537-K539delinsL, R541-E543delinsK, and K539L. These alterations occur in the region adjacent to the start of the *JAK2* pseudo-kinase domain and disrupt the interaction between the pseudo-kinase domain and kinase domain of *JAK2*. Morphologically these cases may show subtler histologic changes in the bone marrow compared to typical findings in PV and demonstrate endogenous erythroid colony formation in vitro, like classic PV. Techniques, such as Sanger sequencing, NGS, or PCR with high-resolution melting curve analysis, can detect *JAK2* exon 12 mutations. Additional mutations of *JAK2* in MPN have also been

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TABLE 24.8

Common Genetic Abnormalities in the *BCR-ABL1*–Negative Classic Myeloproliferative Neoplasms Detected by Molecular Diagnostic Methods

Genetic Abnormality	Disease Associations	Molecular Pathogenesis[a]	Molecular Diagnostic Detection[b]	Notes
JAK2 V617F mutation (exon 14)	PV PMF ET	*JAK2* V617F is a point mutation in autoinhibitory domain of *JAK2,* causing constitutive activation of JAK-STAT pathway, as well as ERK/MAPK and PI3K/AKT pathways.	AS-PCR High-resolution melting curve analysis Sanger sequencing Pyrosequencing NGS	Subset of PV and advanced PMF cases show biallelic V617F mutations; also frequent in MDS/MPN-RS-T, rare cases of AML, MDS, CMML
JAK2 exon 12 mutations	PV	Small in-frame insertion and deletions in *JAK2* exon 12, result in gain of function.	Sanger sequencing; High-resolution melting curve analysis; NGS	Essentially exclusively seen in PV
CALR exon 9 mutations	PMF ET	Frameshift insertion/deletion mutations in the last exon, resulting in a C-terminal neoepitope, and active JAK-STAT pathway through interaction with TPO-receptor c-MPL.	PCR and fragment analysis; High-resolution melting curve analysis Sanger sequencing NGS	Not seen in PV In-frame *CALR* mutations are of uncertain clinical significance and most are polymorphisms. They do not create the pathogenic C-terminal neoepitope.
MPL exon 10 mutations	PMF ET	Point mutation causes constitutive activation of thrombopoietin receptor (c-MPL) and JAK-STAT signaling; the most common mutation site is W515.	AS-PCR Sanger sequencing NGS	Not seen in PV S505N germline mutation associated with familial ET

[a]Major pathophysiologic effect resulting from genetic lesion.
[b]Most common molecular techniques used.
AML, Acute myeloid leukemia; *AS-PCR,* allele-specific polymerase chain reaction; *CMML,* chronic myelomonocytic leukemia; *ET,* essential thrombocythemia; *MDS,* myelodysplastic syndrome; *MDS/MPN-RS-T,* myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis; *NGS,* next-generation sequencing; *PCR,* polymerase chain reaction; *PMF,* primary myelofibrosis; *PV,* polycythemia vera.

identified by more comprehensive gene sequencing studies; however, these anomalies constitute very rare events of yet uncertain pathogenic significance and do not currently justify this broader screening approach in routine molecular diagnostic evaluation.

CALR MUTATIONS

The *CALR* gene, located on chromosome 19p13.2, encodes calreticulin, a multi-functional protein involved in endoplasmic reticulum (ER) protein folding, calcium homeostasis, as well as cellular proliferation and apoptosis. Recently, two groups simultaneously reported highly prevalent somatic *CALR* mutations in *JAK2* and *MPL* mutation-negative ET and PMF patients but not in PV patients. *CALR* mutations occur in the terminal exon 9 of *CALR* and are always frameshift indel-type mutations resulting in 1-bp frameshift and production of a specific alternative reading frame. This alteration generates a novel C-terminus in the mutant CALR protein with loss of most of the acidic amino domain and the "KDEL" motif ER-retention signal. This novel peptide can be detected in paraffin sections by a mutant-specific antibody using routine immunohistochemistry. At least 40 variant somatic indels have been documented thus far, and all follow the stereotypical pattern of (3n+1) bp for deletions or (3n+2) bp for insertions. The two most common mutations are designated type 1 (52-bp deletion, c.1092-1143del, p.Leu367fs*46) and type 2 (5-bp insertion, c.1154_1155insTTGTC, p.Lys385fs*47), and these account for approximately 53% and 32% of the *CALR*-mutated cases, respectively. *CALR* mutations have been identified in the neoplastic hematopoietic stem and progenitor cells of MPN; these cause constitutive signaling through the JAK-STAT pathway by direct activation of the TPO receptor c-MPL (encoded by *MPL*) independent of TPO ligand activation. *CALR* mutants were also sufficient to induce an MPN phenotype mimicking ET with later progression to myelofibrosis in a retroviral mouse model.

Given the relative sizes of indel mutations in *CALR*, these alterations can be effectively identified by PCR of exon 9 and fragment sizing analysis or sequencing by Sanger sequencing or NGS methods (Fig. 24.9). One caveat of fragment sizing is that it only identifies the overall base pair differences indicative of insertion or deletion length changes but cannot differentiate the nature of events that are of

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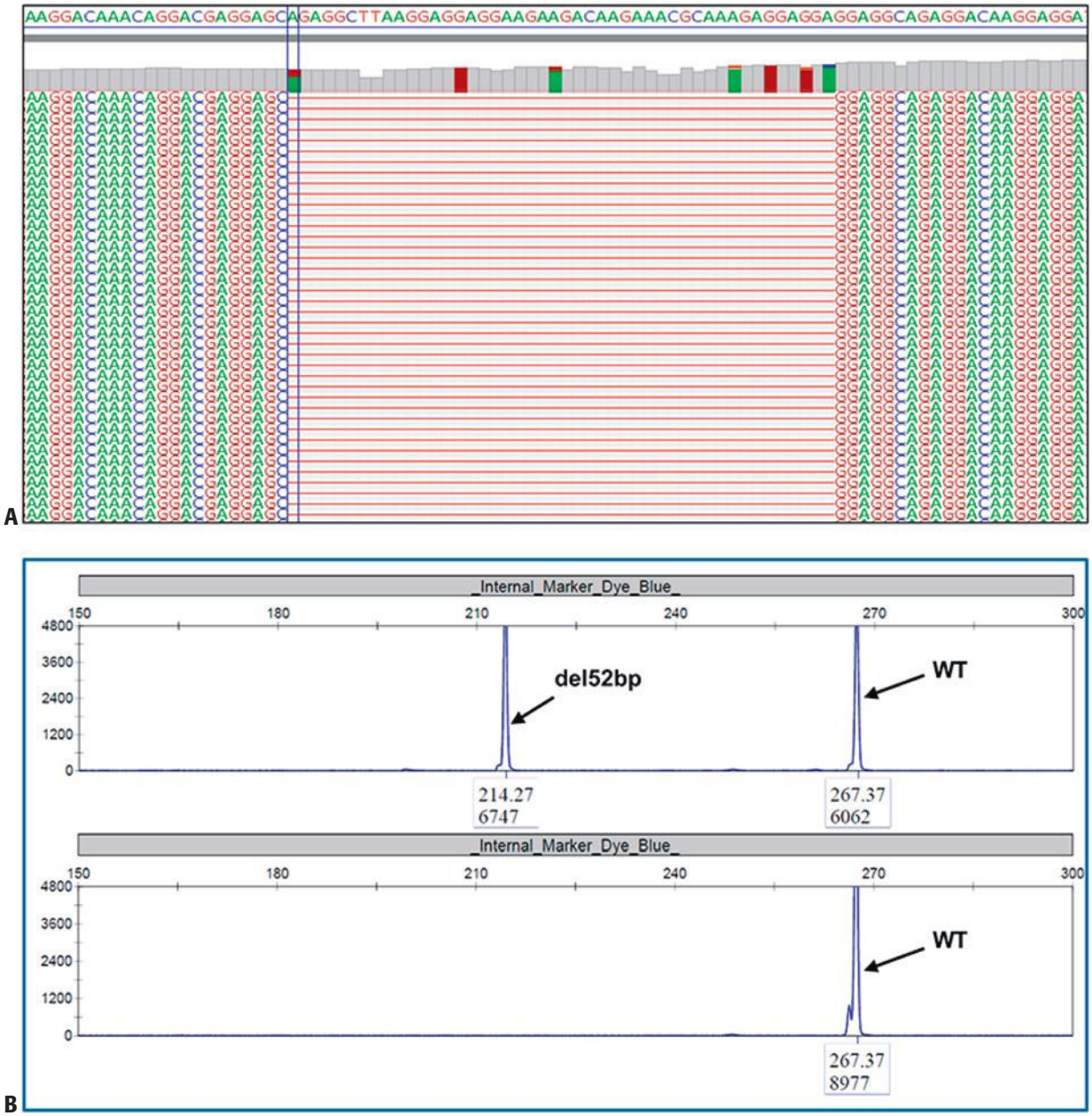


FIG. 24.9

NGS and fragment sizing analysis of *CALR* mutation. Representative sequence reads and tracings of *CALR* exon 9 mutational analysis from NGS and fragment sizing platforms, respectively. Panel A, A capture-based targeted NGS panel detected a c.1094_1139del, a 46-bp deletion resulting in a pathogenic frameshift mutant of p.Gln365Argfs*50 (reference transcript NM_004343.3; GRCh 37 genome build). Panel B, Exon 9 of *CALR* was amplified by PCR, and the size of the PCR products was analyzed by fluorescent capillary electrophoresis. The *lower panel* shows the wild-type amplicon from a normal control, and the *upper panel* exhibits a mutant amplicon with 52-bp deletion to the left of the wild-type amplicon in a positive sample.

identical size change (e.g., different deletion mutants can generate a 52-bp deletion size, but these can arise from a variety of different locations within *CALR* exon 9). Knowing the precise mutant sequences may be of clinical significance in light of some recent reports suggesting that the favorable clinical outcome of *CALR* mutations in PMF patients is restricted to type 1 and so-called type 1–like cases but not type 2 and type 2–like cases, although this finding was not confirmed in other studies. The reported classification of the type 1–like and type 2–like mutations also required amino acid sequence-based analysis, yet currently no consensus is established for the precise definition of type 1–like or type 2–like mutations. Therefore, more detailed peptide-based analysis of individual *CALR* mutations is not routinely required at the current time. It is also important to note that a low frequency of in-frame indels of *CALR* have been observed, and these alterations are distinctly different from pathogenic frameshift mutations; the collective available data support that in-frame indels are of doubtful clinical significance and are generally considered to be consistent with

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germline polymorphisms. Since in-frame *CALR* variants can occasionally be identified during routine screening analysis, as well as in PMF and ET patients with a pathogenic *JAK2* V617F or classic frameshift *CALR* mutation, recognition is important to avoid errant interpretation. One additional caveat is that the typical resolution of many fragment size analysis platforms is ±1 to 3 bp depending on the size of the PCR product and platform used; for rare pathogenic *CALR* mutations not matching the typical type 1 and type 2 indel size patterns, supplemental sequencing may be required for confirmation (see Table 24.8).

MPL MUTATIONS

A minority of PMF (5% to 10%) and ET (~3% to 5%) patients lack *JAK2* or *CALR* gene mutations but instead harbor coding alterations in the *MPL* gene. The *MPL* gene located on chromosome 1p34 encodes the transmembrane tyrosine kinase receptor for thrombopoietin (TPO, also called c-MPL). It is the major regulator of proliferation and differentiation of megakaryocytes and platelets. The receptor-ligand interaction initiates cell signaling through the *JAK-STAT* pathway. In this case, *MPL* mutations render the TPO receptor constitutively active. *MPL* mutations mostly occur in exon 10 involving codon W515 and result in amino acid substitutions of W515L (76%), W515K (11%), and W515R/S/A/G (<10% each). Somatic and germline S505N mutations have been reported, and the latter situation is associated with rare cases of familial ET. Although the W515L mutation can be interrogated using allele-specific PCR methods, sequencing of *MPL* exon 10 can identify multiple possible changes in this region, as well as any other additional rare sequence variants (see Table 24.8).

MPN DIAGNOSTIC MOLECULAR TESTING ALGORITHM

The current health care environment emphasizes value-based, cost-effective clinical molecular diagnostic testing for optimal and efficient patient care. The mutually exclusive nature of *JAK2, CALR,* and *MPL* mutations in ET and PMF patients and corresponding differences in disease-associated frequencies make these genes ideal candidates for reflexive testing based on a sequential analysis of *JAK2* V617F, *CALR*, and finally *MPL* mutations, with the process halted once a positive result is encountered (Fig. 24.10). This algorithmic approach was adopted in practice in some clinical laboratories and was recently incorporated into the 2017 NCCN guideline for the diagnostic workup of MPN. Similarly, if the clinicopathologic indication for evaluation is PV, a reflexive testing approach of *JAK2* V617F assessment followed by *JAK2* exon 12 sequencing is recommended; this approach reduces the need

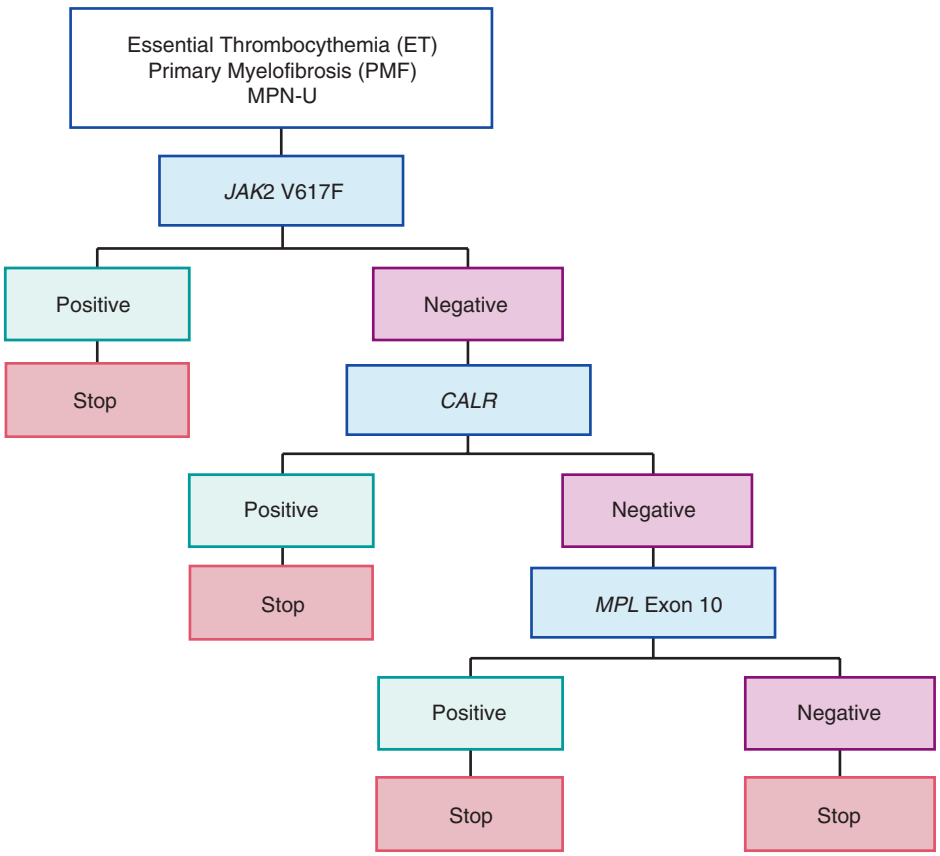


FIG. 24.10

Reflexive molecular diagnostic testing algorithm in primary myelofibrosis (PMF) and essential thrombocythemia (ET). Reflexive testing algorithm of *JAK2* V6127F, *CALR*, and *MPL* during an MPN diagnostic workup. When the clinicopathologic suspicion is PMF, ET, or MPN-U, *JAK2* V617F, *CALR*, and *MPL* mutations are sequentially tested until a positive result is encountered. The testing algorithm is based on the nearly mutually exclusive nature of *JAK2*, *CALR*, and *MPL* mutations in ET and PMF patients and their significant differences in disease-associated frequencies.

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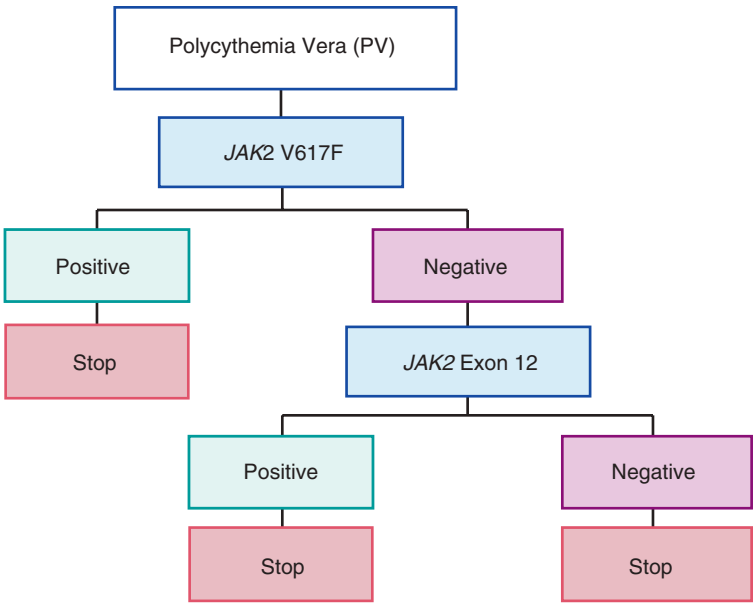


FIG. 24.11

Reflexive molecular diagnostic testing algorithm in polycythemia vera (PV). Reflexive testing algorithm of *JAK2* V617F and *JAK2* exon 12 mutation during an MPN diagnostic workup. When the clinicopathologic suspicion is PV, *JAK2* V617F and *JAK2* exon 12 mutations are sequentially tested until a positive result is encountered. A negative result virtually excludes PV. The testing algorithm is based on the marked predominance of *JAK2* V617F mutations seen in PV (>95%) with rare V617F-negative cases showing other mutually exclusive mutations in exon 12.

for *JAK2* exon 12 analysis given the very high prevalence of V617F mutation in the majority of cases of PV (Fig. 24.11). *JAK2* exon 12 analysis is not recommended for non-PV MPN evaluation.

OTHER SOMATIC MUTATIONS IN MPN

Beyond the trio of MPN-associated gene mutations in the current WHO diagnostic criteria for *BCR-ABL1*–negative classic MPNs, other somatic mutations have been identified by WES and targeted NGS, most frequently in PMF patients. These mutations commonly involve the spliceosome machinery (*SF3B1*, *U2AF1*, *SRSF2*) and epigenetic modifiers (*TET2*, *DNMT3A*, *IDH1/2*, *EZH2*, and *ASXL1*). In patients with PMF, the presence of *ASXL1*, *SRSF2*, *IDH1/2*, or *EZH2* mutations has been associated with shortened survival, but only *ASXL1* mutations remain independently significant in the context of the current IPSS/DIPSS+ (Dynamic International Prognostic Scoring System+) risk stratification scoring systems. Correlative studies have also identified several high-risk molecular signatures including two or more mutations in *ASXL1*, *SRSF2*, *EZH2,* or *IDH1/2* and *CALR*-/*ASXL1*+ or "triple negative" (*JAK2*/*CALR*/*MPL*–) mutation status. In addition, *TP53* somatic mutations coupled with loss of heterozygosity are associated with leukemic transformation. *CALR* mutations, in contrast, have been shown to favorably impact survival in PMF, independent of the number of total mutations, *ASXL1* mutational status, and the IPSS/DIPSS+ score. These observations have led to proposals for a new prognostic strategy incorporating updated molecular information, in addition to standard clinical and pathologic measures. In ET and PV, additional somatic mutations have also been identified (e.g., *TET2*, *DNMT3A*, *ASXL1*) less frequently than in PMF; however, little current evidence strongly supports expanded clinical molecular testing for these abnormalities in ET or PV patients.

CHRONIC NEUTROPHILIC LEUKEMIA

Approximately 90% of CNL cases show normal cytogenetic findings. Somatic mutations in *CSF3R* have been identified in 50% to 80% of these patients. There are two types of *CSF3R* mutations: extracellular domain/membrane proximal point mutations and cytoplasmic tail truncation mutations. The membrane proximal mutations constitutively activate the JAK-STAT pathway, resulting in ligand-independent cell growth and demonstrating sensitivity to JAK kinase inhibitors in vitro. Cytoplasmic truncating nonsense or frameshift mutations are less frequent and result in loss of the negative regulatory domain in the C-terminus of the CSF3R (G-CSF) protein. In CNL, the most prevalent mutation is the membrane proximal p.Thr618Ile (T618I) point mutation, although cytoplasmic truncation mutations can also occur, often coexisting with the T618I. The strong association of *CSF3R* mutation with CNL has been included in the diagnostic criteria for this disorder in the 2016 WHO classification revision. *CSF3R* mutational analysis can be performed by Sanger sequencing of exons 14 and 17, or NGS. The most frequent point mutation T618I can also be evaluated by high-resolution melting curve analysis and AS-PCR. Notably, somatic cytoplasmic truncation mutations have also been reported in approximately 30% of patients with severe congenial neutropenia (SCN) as well as in 80% of SCN patients with leukemic transformation, often following administration of G-CSF therapy. However, the precise role of *CSF3R* mutation in leukemic transformation in this clinical setting is uncertain.

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MYELOID/LYMPHOID NEOPLASMS WITH EOSINOPHILIA AND REARRANGEMENTS OF *PDGFRA, PDGFRB, FGFR1,* OR *PCM1-JAK2*

Progress in tumor genetic classification has improved the definition of several hematopoietic tumors that are loosely linked by the presence of accompanying neoplastic eosinophilia. These entities share in common gene rearrangements resulting in the deregulation of several tyrosine kinases. Chromosomal rearrangement at 4(q12) involving the platelet-derived growth factor receptor α (*PDGFRA*) gene underlies the central pathogenesis in a significant subset of chronic eosinophilic leukemias (CEL). These patients present with a profound blood and bone marrow eosinophilia in which the tumor cells harbor a gene fusion between *PDGFRA* and an adjacent genetic locus termed *FIP1L1* on the same chromosome. Less commonly, the *FIP1L1-PDGFRA* can be seen in cases of T-lymphoblastic leukemia/lymphoma (T-LBL) or AML with eosinophilia. Most significantly, CEL patients with the *FIP1L1-PDGFRA* fusion gene exhibit a high response rate to single-agent imatinib therapy, often at relatively low dosage. Identification of this genetic marker is thus critical to guide treatment. The intrachromosomal del4(q12q12) alteration is relatively small and thus not easily recognized by conventional karyotyping studies; however, an intervening gene, *CHIC2*, is deleted during formation of the *FIP1L1-PDGFRA* fusion, and the loss of *CHIC2* can be readily identified by locus-specific BAP FISH technique. RT-PCR analysis has also been used to detect the chimeric *FIP1L1-PDGFRA* mRNA transcript but can produce false-negative results because of breakpoint heterogeneity in *FIP1L1*. The related *PDGFRB* gene, encoding the beta receptor of platelet-derived growth factor, is also disrupted in rare myeloid neoplasms, typically associated with hematologic features of chronic myelomonocytic leukemia with eosinophilia. In these cases, cytogenetic studies may reveal a t(5;12)(q33;p13), resulting in formation of the fusion gene *ETV6-PDGFRB*, although several variant translocations involving *PDGFRB* have also been described. These tumors also appear relatively sensitive to imatinib treatment. *PDGFRB* locus abnormalities can be detected by conventional karyotyping or interphase FISH analysis. A third group of aggressive hematopoietic neoplasms with prominent eosinophilia is characterized by translocation-induced rearrangements of the fibroblast growth factor receptor 1 gene (*FGFR1*) on 8(p11). *FGFR1*-associated malignancies encompass a variety of myeloid and lymphoid tumors including T-LBL, AML, CEL, mixed phenotype acute leukemias, and B-LBL; these tumors are unfortunately resistant to imatinib therapy. *FGFR1* rearrangements are detectable by conventional karyotyping and confirmed by interphase BAP FISH analysis. Myeloid neoplasms with the t(8;9)(p22;p24.1)/*PCM1-JAK2* abnormality represent a new provisional entity added to this category in the 2016 WHO classification. The common clinicopathologic features include eosinophilia, left-shifted erythroid hyperplasia, lymphoid aggregates, and frequently myelofibrosis resembling PMF. These tumors may also rarely present as T- or B-LBL. *PCM1-JAK2*–positive neoplasms may be sensitive to JAK inhibitors, leading to the recent inclusion as a distinct WHO entity. The t(8;9)(p22;p24.1) is detected by conventional karyotyping, and interphase FISH analysis or RT-PCR analysis can identify the *PCM1-JAK2* gene fusion. Hematolymphoid malignancies with a clonal eosinophilic component are in summary morphologically diverse, and the characterization of specific disease entities based on abnormalities of *PDGFRA*, *PDGFRB, FGFR1*, and *PCM1-JAK2* genetic abnormalities is valuable for more precise classification and, in a substantial number of these patients, for guiding effective targeted therapy with tyrosine kinase or JAK pathway inhibitors (Table 24.9).

MASTOCYTOSIS

Mast cell diseases have a broad clinical and pathobiologic spectrum, from localized, relatively indolent cutaneous lesions to debilitating systemic presentations involving skin, visceral organs, and bone marrow. Point mutations of the *KIT* gene are highly characteristic of mastocytosis, and the large majority results in a change from aspartic acid to valine at codon 816 in exon 17 (D816V). The *KIT* gene encodes the tyrosine kinase receptor for stem-cell factor, which is critically required for normal hematopoietic stem/progenitor-cell proliferation and expansion. Because mastocytosis is an uncommon disease, the frequency of the D816V abnormality varies among studies but also differs when considering clinical subtypes of mast cell disease (e.g., cutaneous versus systemic), or the age of onset (i.e., adult versus childhood presentations). In adults with systemic mast cell disease the D816V is found in approximately 80% of cases, although with more sensitive molecular detection methods and cell enrichment techniques, nearly 95% of cases are mutation positive. In general, systemic mastocytosis (SM) is associated with a very high prevalence of the *KIT* D816V; however, this appears to differ according to adult versus childhood (pre-pubertal) age of disease development (~90% vs. 50%). When assessing patients with cutaneous mastocytosis (CM), the presence of the D816V appears to be much less frequent, but this too is controversial among reported series. Notably, younger patients with SM or CM seem to have a higher incidence of somatic *KIT* mutations occurring at other codon sites in exon 17 or exons 8, 9, or 11. Nevertheless, because the largest proportion of individuals (80% to 90%) with SM harbors the D816V mutation, targeted molecular analysis is an important aspect of diagnosis.

Molecular assays specifically detecting the D816V will be associated with a risk for clinical false negativity, since a small percentage of SM cases will have alternative nucleotide changes at this site, or may involve mutations elsewhere in exon 17 or other exons. The focal and fibrotic nature of bone marrow mast cell lesions also may result in a negative D816V result because of sampling bias. Therefore, the absence of the D816V does not rule out a diagnosis of SM, if the clinical and pathologic findings are otherwise diagnostic. Accordingly,

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TABLE 24.9

Myeloid/Lymphoid Neoplasm With Eosinophilia and Rearrangement of *PDGFRA, PDGFRB,* or *FGFR1* or With *PCM1-JAK2* and Mastocytosis

Genetic Abnormality	Disease Associations	Molecular Pathogenesis[a]	Molecular Diagnostic Detection[b]	Notes
PDGFRA, *PDGFRB*, or *FGFR1* rearrangement *PCM1-JAK2* fusion	Myeloid and lymphoid neoplasms with eosinophilia	Deregulation of receptor and non-receptor tyrosine kinases	Karyotyping (except *PDGFRA* which is commonly cryptic and need FISH) FISH for specific gene locus rearrangements RT-PCR	Improves classification of a diverse group of malignancies with prominent neoplastic eosinophil component Identifies subsets of hematologic neoplasms responsive to imatinib mesylate therapy (*PDGFRA, PDGFRB*) or JAK2 inhibitor therapy (*PCM1-JAK2*)
KIT mutations	Mastocytosis	Deregulation of KIT tyrosine kinase (receptor for stem cell factor)	Allele-specific PCR RQ-PCR High-resolution melting analysis Sanger sequencing NGS	*KIT D816V* in exon 17 is the most common mutation, present in 80% to 90% of systemic mastocytosis; lower frequency of detection in cutaneous mastocytosis; D816V is not responsive to imatinib therapy; *KIT* mutations also seen in CBF-AML, commonly involving exon 17 or exon 8

[a]Major pathophysiologic effect resulting from genetic lesion.
[b]Most common molecular techniques used for detection.
FISH, Fluorescence in situ hybridization; *PCR,* polymerase chain reaction; *RQ-PCR,* real-time quantitative PCR; *RT-PCR,* reverse transcriptase polymerase chain reaction; *NGS,* next-generation sequencing; *CBF-AML*, core binding factor AML.

the presence of the *KIT* D816V is included as one of the "minor" WHO criteria in establishing the diagnosis of SM. The broader application of imatinib therapy for deregulated tyrosine kinase activity in several hematopoietic cancers has unfortunately not been successful in SM with *KIT* D816V mutation, although responses are observed for other variants, such as exon 8 and 9 mutations. The observation that imatinib is also variably effective in other *KIT* mutation–positive tumors (e.g., some gastrointestinal stromal tumors and melanomas) underscores the importance of additional factors such as the mutation location and cellular context in conferring sensitivity or resistance to particular TKI agents. The *KIT* D816V can be sensitively identified using allele-specific PCR. Because the mutation is heterozygous in most cases, separate primers recognizing mutant and wild-type alleles at the D816 codon site can be used to determine the presence of both mutated and wild-type *KIT* to ensure adequate amplification of the DNA template. RQ-PCR analysis using allele-discriminating primers or probes can similarly be utilized for this purpose. Alternative techniques for mutation detection include high-resolution melting analysis or Sanger sequencing of PCR products. Simultaneous multiple-exon evaluation of *KIT* may be achieved using Sanger sequencing or NGS technology. However, given the high prevalence of the *KIT* D816V mutation in SM and the higher cost of NGS, it is more cost-effective in the routine clinical practice to screen for the D816V alteration using a dedicated and sensitive assay (see Table 24.9).

MOLECULAR DIAGNOSIS OF RED BLOOD CELL DISORDERS

Abnormalities of red blood cells (RBCs) involve many different components of the cell. These include abnormalities of the hemoglobin molecule, disorders of hemoglobin production, abnormalities of the RBC enzymes, defects in the cellular oxygen-sensing pathway, and RBC membrane or cytoskeletal anomalies. Clinical manifestations of these disorders range from harmless or compensated conditions to hemolytic anemias, erythrocytosis, impaired hemoglobin function with poor RBC survival, and thalassemic syndromes. For many of these conditions, well-established assays are available to provide adequate diagnostic information; however, an increasing number of molecular assays can be applied appropriately

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in specific situations, particularly if an RBC disorder is associated with clinical symptoms.

HEMOGLOBINOPATHIES

Common methods, such as alkaline–acid hemoglobin (Hb) electrophoresis, isoelectric focusing, high-performance liquid chromatography, and capillary electrophoresis are able to identify the common (and clinically significant) Hb variants S, C, and E. Other less common variants are difficult to identify precisely because many of these have similar electrophoretic mobility or retention times depending on the given analytic platform. The combination of two or more routine techniques helps to enhance the identification of these less common Hb types; however, because many Hb variants are clinically harmless, it is not always necessary to definitively subclassify them by standard approaches. In contrast, variants associated with clinical symptoms (e.g., altered oxygen affinity, unstable Hb) are important to identify precisely by the addition of more complex protein methods and genotyping, if needed. Owing to the small size of the human globin genes (three exons and two introns), these genomic loci are amenable to PCR amplification and direct Sanger sequencing, although allele-specific probes or primers have also been used to detect relatively common mutations.

THALASSEMIAS

α THALASSEMIAS

Most α thalassemia mutations involve large deletions of one or both α globin genes (*HBA1*/*HBA2*) at the genetic locus on chromosome 16p13. Therefore, routine direct DNA (Sanger) sequencing will not detect the most common α thalassemia mutations. Detection of such mutations can be accomplished with Southern blot or gap-PCR testing—the former technique is laborious and not commonly used. Gap-PCR involves primer sets straddling known genomic regions that are brought close together in the presence of a deletion, enabling amplification of the abnormality. This technique assesses for specific mutations selected by the assay and cannot detect novel deletions. Multiplex ligation-dependent probe amplification (MLPA) assay uses a series of DNA probes to hybridize at various points along an extended area of the chromosome that includes the α gene complex. Absence of hybridization and subsequent site-specific ligation of a particular probe region indicates deletion of that area of the gene. MPLA can detect novel deletions that affect the probed regions but cannot confirm the exact nucleotide position of the deletion and is therefore not as specific as PCR. These methods will, however, not detect the less common, but clinically significant, point mutations responsible for nondeletional α thalassemia phenotypes. The latter abnormalities, however, can be identified by standard DNA-sequencing methods.

β THALASSEMIAS

In contrast to α thalassemias, the vast majority of β thalassemias are associated with single base substitutions, small insertions, and small deletions within or flanking the β globin gene (HBB) located on chromosome 11p15. These mutations cause different modes of decreased protein expression and can affect transcription, RNA processing, or mRNA translation. Most β thalassemia mutations can be detected by PCR and direct DNA sequencing of the β globin gene. This method will identify both known and novel mutations but cannot detect large deletions. In areas of the world where a small number of β thalassemia mutation subtypes are particularly common, allele-specific oligonucleotide probes or allele-specific PCR amplification may identify the majority of these cases, although a negative result will not exclude the presence of other mutations not in the assay. There is a less common subset of β thalassemia mutations that arises from large deletions (105 bp–67 kb) (ref Thein Cold spring harbor 2013) of the β globin gene or the locus control region. These all completely abolish all protein expression from the affected allele (β zero thalassemia) and usually have higher Hb A2 levels and slightly elevated Hb F levels.

HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN AND Δβ THALASSEMIA

These disorders have in common an elevation in Hb F beyond the first year of life. Similar to the case for α thalassemia mutations, these abnormalities involve large deletions in the β globin gene complex and can be detected with similar methods (Southern blot, gap-PCR, and multiplex ligation-dependent probe amplification). There is also nondeletional hereditary persistence of fetal Hb mutations that involve the promoter region of the γ genes; however, it is rarely clinically necessary to detect the exact molecular mutation in these cases.

PRACTICAL WORKUP OF HEMOGLOBINOPATHIES AND THALASSEMIAS

For many of these situations, routine protein analytic methods are adequate to provide an appropriate answer. These methods include simple measurement of Hb A2 for β thalassemia trait or evaluation of Hb variants by electrophoretic methods. Although exact identification of some Hb variants may not be possible by routine electrophoretic methods, such accuracy may not be necessary if the variant is clinically harmless. Molecular identification is most helpful in situations that are clinically significant, including variants that produce hemolytic anemias or altered Hb oxygen affinity (i.e., erythrocytosis or physiologic anemia). For thalassemia syndromes, identification of the molecular abnormalities in cases of β thalassemia major or intermedia is warranted, particularly in parents undergoing

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prenatal counseling. Similarly, identification of α thalassemia mutations in two parents who are both microcytic is warranted to assess for the possible severity of disease in an affected child. The molecular diagnostic approach to hemoglobinopathies and thalassemic disorders thus requires an appropriate screening strategy, knowledge of clinical parameters, and experience with variant globin identification. Molecular methods therefore highly complement standard protein electrophoretic techniques in specific circumstances. Although tempting, assigning hemoglobin classification using molecular data alone is prone to misdiagnosis and should not be recommended. Molecular data are important to correlate with electrophoresis results to allow for correct assignment of phasing (whether the mutation is in *cis* or *trans* conformation) when multiple mutations are detected and assigning clinical significance to findings. For example, DNA sequencing of homozygous Hb S and the compound heterozygous Hb S/HPFH would have the exact sample results; however, the former is a clinically significant sickling disorder, and the latter is a disorder that is essentially similar to Hb S trait owing to the protective effect of the homocellular gamma chains present in the red blood cell. Electrophoresis data showing markedly increased Hb F levels are required to ascertain the need to perform MLPA testing on the case to detect the large beta complex deletion causing the HPFH phenotype.

ERYTHROCYTOSIS (Fig. 24.12)

Erythrocytosis (or polycythemia) is present when there is increased red cell mass (RCM). RCM is not a routinely measured parameter, and hemoglobin and hematocrit levels are more conveniently used as a surrogate. Recent criteria for polycythemia vera updated in the prepublication primer for the upcoming 2016 WHO classification set hemoglobin and hematocrit benchmarks for erythrocytosis in men (>16.5 g/dL; >49%) and women (>16.0 g/dL; >48%). It is important to note that the RBC count is not a criterion, as this can be elevated in thalassemic syndromes and in isolation does not indicate erythrocytosis (polycythemia). Most erythrocytoses are compensatory because of conditions such as chronic cardiac or pulmonary dysfunction, high-altitude dwelling, chronic smoking, sleep apnea, morbid obesity, and hypoventilation syndromes. However, when reactive and paraneoplastic conditions have been excluded and a primary neoplastic (i.e., PV) or a heritable cause is suspected, the molecular evaluation of erythrocytosis is warranted. The development of molecular tests for mutations associated with erythrocytosis has shed light on classification and has defined more clear etiologies, thereby decreasing the numbers of true idiopathic cases. With the aid of molecular techniques, many cases are confirmed as neoplastic in etiology, and some erroneously assumed to be neoplastic are reclassified as heritable.

The most common cause of primary erythrocytosis is PV, an acquired somatic mutation in hematopoietic stem cells that carries a risk of evolution to myelofibrosis and acute leukemia. PV is most often diagnosed in middle-aged to older individuals but can occasionally be seen in children and young adults. It is important to differentiate PV from heritable causes of erythrocytosis, the latter of which can be passed to progeny but does not carry the risks of clonal evolution associated with PV. Treatment strategies are much better defined for PV than for the heritable causes because of a more complete understanding of the risks and natural history of the disorder. The clinicopathologic and molecular evaluation of PV is discussed previously and should be undertaken in the initial stages of an evaluation of erythrocytosis, especially when the serum erythropoietin (EPO) level is low, because PV is much more common than hereditary (congenital) erythrocytosis.

TABLE 24.10

Molecular Testing Available for the Evaluation of Erythrocytosis

Involved Gene	Inheritance	Serum Erythropoietin	p50
JAK2 V617F	Acquired	Decreased	NL
JAK2 exon 12	Acquired	Decreased	NL
EPOR	Dominant	Decreased	NL
PHD2	Dominant	NL to elevated	NL to slightly decreased
BPGM	Dominant	NL	Decreased
β globin	Dominant	NL to elevated	Decreased
α globin	Dominant or recessive	NL to elevated	Decreased
HIF2α	Dominant	NL to elevated	NL
VHL	Recessive	Nl to elevated	NL

NL, Normal range level.

Mutations causing abnormalities in hemoglobin (high oxygen-affinity hemoglobin variants), hemoglobin effector proteins (2,3 bisphosphoglycerate [2,3 BPG] deficiency), the erythropoietin receptor and in oxygen-sensing pathway proteins (hypoxia-inducible factor, prolyl hydroxylase domain, and von Hippel-Lindau) can result in erythrocytosis (Table 24.10). Of the heritable causes, high oxygen-affinity hemoglobin variants are the most common. Most of the remaining conditions are much less common; however, the true prevalence remains to be elucidated with wider investigation of patients previously classified as idiopathic. Serum EPO levels and p50 (hemoglobin oxygen affinity test) results help to guide efficient testing choices according to the most likely etiologies (Table 24.11). The most studied genetic causes currently associated with erythrocytosis follow and are discussed separately.

2,3-BISPHOSPHOGLYCERATE

The RBC 2,3-BPG (also known as 2,3-DPG) molecule stabilizes the deoxygenated form of hemoglobin by allosteric

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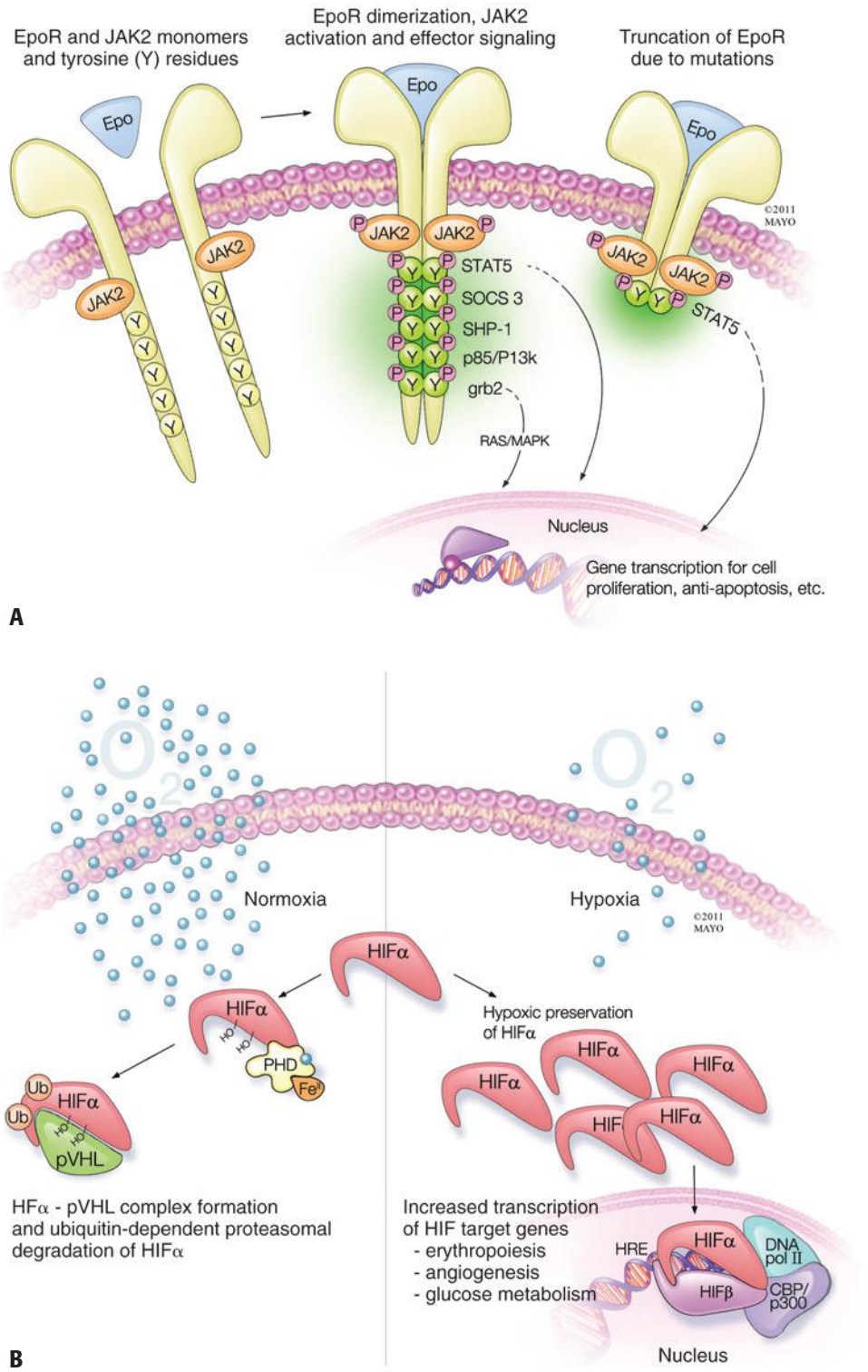


FIG. 24.12

Erythropoietin receptor (EPOR) signaling and the oxygen sensing pathway in the regulation of erythroid cell production. A, Erythropoietin receptor signaling pathway. Extracellular (EPO)-erythropoietin receptor (EpoR) binding causes EpoR dimerization; this in turn activates Janus kinase 2 (JAK2) to autophosphorylate multiple sites within the cytoplasmic domain of EpoR. These phosphorylated sites allow binding of transcription factors, including STAT5, which results in the production of more red cells. The process is regulated by the binding of SHP1 to a cytoplasmic binding domain on EpoR. Polycythemia vera results from a constitutively activated JAK-STAT pathway because of a somatic gain of function mutation in JAK2. Heritable erythrocytosis has been associated with heterozygous germline EPOR mutations that have interrupted the binding of SHP1 to EpoR. Most result in a truncated EpoR protein resulting in the loss of the cytoplasmic SHP1 domain and are located in exon 8. B, Oxygen-sensing pathway. In the presence of adequate oxygen, the transcription factor protein hypoxia-inducible factor α subunit (HIFα) is prepared for and ultimately degraded by a complex of enzymatic steps. Important components are oxygen-dependent hydroxylation by a family of prolyl hydroxylase domain (PHD) proteins, which in turn is required for the colocalization of ubiquitin and proteosomes by the von Hippel-Lindau (vHL) protein, which results in degradation of HIFα. Under hypoxic conditions, no hydroxylation occurs and HIFα is allowed to enter the nucleus, bind to its β subunit, and turn on the transcription of many genes, including EPO. Mutations along this pathway that allow HIFα to survive under normal oxygen concentrations have been associated with heritable erythrocytosis. Mutations in HIFα, PHD2, and vHL have been reported. *(Used with permission of Mayo Foundation for Medical Education and Research.)*

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TABLE 24.11

Erythrocytosis Testing Stratified to Serum Erythropoietin Level[a]

Decreased Erythropoietin Level	Normal Erythropoietin Level	Increased Erythropoietin Level
JAK2 V617F	*JAK2* V617F	p50
JAK2 exon 12	*JAK2* exon 12	Hemoglobin electrophoresis
EPOR	p50	HBB and HBA1/A2 globin genes
	Hemoglobin electrophoresis	Methemoglobin evaluation
	HBB and HBA1/A2 globin genes	*VHL*
	2,3-BPG enzyme assay	*EPAS1/HIF2α*
	2,3-BPG mutase gene	
	Methemoglobin evaluation	
	EGLN1/PHD2	
	EPAS1/HIF2α	
	EPOR	
	VHL	

[a]Recommend test ordering in a reflexive manner beginning with more common etiologies for the clinical picture. If focused testing guided by erythropoietin levels is negative, recommendation is to extend the testing panel as the etiology may be multifactorial.
BPG, Bisphosphoglycerate.

binding and facilitates oxygen release at tissue sites. Decreased 2,3-BPG concentrations create increased oxygen affinity by the normal hemoglobin tetramer; therefore, a deficiency is associated with a left-shifted oxygen dissociation curve (decreased p50 value) and subsequent erythrocytosis. A known mechanism of 2,3-BPG deficiency is caused by mutations in the gene coding the converting enzyme, bisphosphoglycero-mutase (*BPGM*). The *BPGM* gene is located on chromosome 7, spans 22 kb, and contains three exons. Point mutations and small frameshift mutations have been reported and can be identified by PCR and Sanger sequencing. The reported cases are associated with normal serum EPO.

HIGH OXYGEN-AFFINITY HEMOGLOBIN VARIANTS

High oxygen-affinity hemoglobin variants are defined by an associated left-shifted oxygen dissociation curve (decreased p50 value), and more than 300 different mutations have been reported. Usually these are the result of point mutations or small deletions involving loci important in cooperative binding of α and β subunits, the heme pocket, the binding of 2,3-BPG, or C-terminal conformation stabilization; approximately one third of known high oxygen-affinity variants are associated with clinically evident erythrocytosis. The remaining high oxygen-affinity variants are not associated with erythrocytosis for various reasons, most often owing to a decreased amount of the variant hemoglobin (unstable or α chain variants). The degree of erythrocytosis owing to a high oxygen-affinity hemoglobin variant is usually proportional to the degree of p50 decrease; however, compound heterozygotes with concurrent β thalassemia can show a more severe polycythemia than expected. The erythrocytosis is EPO driven, and serum EPO levels are usually inappropriately normal but can be elevated. If notation of erythrocytosis is forwarded as an indication for testing, a thorough hemoglobin electrophoresis evaluation by an experienced laboratory will identify the majority of abnormal hemoglobin variants. It is important to note that a significant subset of high oxygen-affinity variants do not resolve from normal hemoglobins by conventional methods; therefore, mass spectrometry and/or sequencing of the β and α globin genes (discussed earlier) is warranted in negative cases to exclude these electrophoretically silent variants. Correlation of the molecular data is crucial for correct classification as the quantity of variant can affect the clinical phenotype and quantitation requires protein evaluation.

ERYTHROPOIETIN RECEPTOR

The erythropoietin receptor (EpoR) is present on erythroid progenitor cells. When EPO binds to EpoR, apoptosis is inhibited through an intracellular signaling cascade producing increased numbers of RBCs. EpoR-modulated gene transcription is regulated by SHP1 binding. Truncating mutations in the *EPOR* gene result in the loss of the intracellular–cytoplasmic SHP1 binding domain, which translates functionally to EPO hypersensitivity and erythrocytosis (see Fig. 24.12A). *EPOR* is located on the short arm of chromosome 19, spanning 7 kb and containing eight exons. These are heterozygous truncating mutations localized to exon 8 and are detectable by direct sequencing methods. Serum EPO levels are decreased similarly to PV cases. P50 results are normal.

MUTATIONS IN OXYGEN-SENSING PATHWAY GENES

The oxygen-sensing pathway, which is found in all cell types, contains multiple factors that regulate RBC mass, and mutations favoring less control over RBC proliferation result in erythrocytosis (see Fig. 24.12B). The most studied components of this pathway are hypoxia-inducible factor (HIF), prolyl hydroxylase domain (PHD), and von Hippel-Lindau (vHL) proteins (see Fig. 24.12B). HIF is a heterodimer protein composed of α and β subunits, and it functions as a sensor of depleted oxygen concentration. When present, oxygen serves as a substrate in a cascade for HIF α subunit degradation. This cascade includes hydroxylation of HIF2α by PHD at two proline sites, P405 and P531, to which vHL can then

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bind and coordinate ubiquitination of the protein. In the absence of oxygen, degradation does not take place, and the α subunit is available to dimerize with the HIF β subunit (see Fig. 24.12B). In the heterodimerized state, HIF then binds to the hypoxia response elements of promoters and enhancers for more than 100 hypoxia response genes, including those for EPO, transferrin, vascular endothelial growth factor, and glucose transporter 1. The α subunit has three isoforms: HIF1α, HIF2α, and HIF3α. HIF1α is ubiquitous and may drive immediate response to hypoxia, whereas HIF2α is not found in as many organs and cell types and is important in subacute and chronic hypoxia. HIF3α is less understood and may have inhibitory functions. The *EPAS1* gene encoding HIF2α is located on chromosome 2, contains 15 exons, and spans 120 kb. The overwhelming majority of clinically significant mutations are localized within exon 12. The mutations are heterozygous and usually result in an amino acid substitution that interferes with the proline residue at amino acid position 531, resulting in an abnormal gain-of-function phenotype. The interference blocks degradation of the α subunit, allowing hypoxia response element binding to continue despite properly oxygenated conditions. *EPAS1* mutations are associated with normal or increased EPO levels and normal p50 results. *EPAS1* mutations located in exon 9 (near proline 405) have been recently discovered in sporadic neuroendocrine tumors such as paragangliomas and pheochromocytomas. These patients sometimes have a history of long-standing erythrocytosis that can predate the presence of any associated tumor symptoms. *EPAS1* exon 9 or 12 mutations present in the tumoral tissue are not always present in the patient's germline DNA, recently shown to be sometimes caused by mosaicism.

The vHL protein functions in oxygen regulation through colocalizing the proteins necessary to rapidly destroy the oxygen-sensing protein HIF, initiating its ubiquitination and proteasomal degradation. It does so by binding the HIF α subunit, although this interaction first requires prolyl hydroxylation by a specialized protein—PHD (see Fig. 24.12B). Erythrocytosis resulting from a germline missense mutation at nucleotide 598 C to T (R200W) in the VHL gene has been found endemically in more than 10,000 people in the Chuvash region of the former Soviet Union and is therefore named Chuvash polycythemia (CP). Further investigations have detected this mutation in unrelated patients from diverse ethnic groups including Asians, Caucasians, and African Americans, and haplotype studies imply a founder effect. In contrast to all the other mutations we have discussed regarding hereditary erythrocytosis, transmission of CP is autosomal recessive, and patients have inappropriately normal or elevated EPO levels and normal p50 results. Homozygous CP patients are hypersensitive to EPO, with a correspondingly increased risk for cerebrovascular and embolic complications; heterozygous relatives (carriers) are usually not affected. The CP mutation is not associated with the risk of neoplasias typically present in vHL syndrome (e.g., pheochromocytomas, hemangioblastomas, renal cell carcinomas). Mutations in the *VHL* gene other than the classic CP mutation have also been linked to erythrocytosis and are commonly at or near the R200 codon. It is important to note that *VHL* mutations resulting in the vHL syndrome are for the large part exclusive from the intragenic mutation causing CP. Patients with vHL syndrome are heterozygous for a germline mutation (usually 5′ from the CP mutation locus) and sporadically acquire a random somatic "second hit" in the remaining VHL allele resulting in complete loss of vHL function (i.e., localized homozygosity) in various tissue and organ sites, with focal tumor formation. In contrast, CP patients are germline homozygotes with a polycythemic presentation and do not suffer from tumors.

The regulation of HIFα by vHL protein-mediated ubiquitination and proteasomal degradation requires prolyl hydroxylation (see Fig. 24.12B). Enzymes important in the hydroxylation of HIF are the PHD proteins, which have three isoforms, PHD1, PHD2, and PHD3. The most significant isoform associated with erythrocytosis is PHD2. PHD enzymes are oxygen-dependent and have an iron-containing active site; therefore, activity theoretically could be modulated by low iron and ascorbic acid levels as well as by low oxygen, although this has not been substantiated experimentally. The *PHD2* (also *EGLN1*) gene is located on chromosome 1, spans 66 kb, and has 5 exons. Clinically significant mutations are heterozygous and have been found in all exons. They tend to cluster near regions important in nuclear importation, the β2β3 loop involved in specificity for HIFα CODD/NODD domains and 2OG-Fe(II) oxygenase domains. These mutations are associated with inappropriately normal EPO. The majority have normal p50 levels, although a subset can have slightly left-shifted oxygen dissociation curves (p50 = 21–29 mm Hg, normal range 24–30 mm Hg). At least for *PHD2*, monoallelic mutations appear to be sufficient for a clinical phenotype, by either dominant negative activity of the mutated gene product or a gene dosage effect (haploinsufficiency).

HEMOLYTIC ANEMIAS ASSOCIATED WITH RED BLOOD CELL ENZYME DEFICIENCIES

Deficiencies in enzymes important for RBC survival result in hemolysis of varying forms and severity. Many common clinically significant enzyme deficiencies involve the glycolytic pathway, such as pyruvate kinase (PK), but by far the most common is from the hexose monophosphate shunt pathway, glucose-6-phosphate dehydrogenase (G6PD). Although G6PD deficiency is commonly associated with acute hemolytic episodes triggered by medication, illness, or fava bean ingestion, most RBC enzyme deficiencies result in a chronic nonspherocytic hemolytic anemia. Some enzymopathies are also associated with neurologic deficits or developmental delay, whereas others result in glycogen storage diseases with myopathic symptoms. Symptoms of underlying chronic hemolysis are frequently present, such as pigmented gallstones, jaundice, and splenomegaly. The genetic features of the two most common are briefly discussed.

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GLUCOSE-6-PHOSPHATE DEHYDROGENASE

Glucose-6-phosphate dehydrogenase (G6PD) is a cytosolic enzyme that produces NADPH, the electron donor molecule important in neutralizing harmful oxidizing agents. Normal conditions use 2% of the enzyme, leaving an abundant reserve. In a G6PD-deficient person, situational demand overwhelms the insufficient enzyme reserve, resulting in hemolysis. Attributed to malaria pressure, hundreds of mutations have been described with wide ranging levels of enzyme activity. The mutations are usually single base substitutions or rarely small deletions. The G6PD gene is located on the X chromosome, spans 18.5 kb, and contains 13 exons. Inheritance is X-linked; however, because of the high prevalence of the disorder (>400 million people), homozygous females are not uncommon, and some heterozygous females can manifest symptoms because of mosaicism or lyonization of the X chromosome. G6PD deficiency symptoms most commonly include neonatal jaundice, and acute episodic hemolysis. Assay by enzymatic activity is the mainstay of diagnosis with genotyping reserved for ambiguous cases. A subset of cases with mutations in exon 10 are associated with chronic nonspherocytic hemolytic anemia more phenotypically similar to other RBC enzyme deficiencies.

PYRUVATE KINASE

Pyruvate kinase is an allosteric enzyme involved in glucose metabolism converting phosphoenol pyruvate to pyruvate. It exists in different isoforms coded by two genes, *PKM* on chromosome 15 and *PKLR* on chromosome 1. *PKLR* contains 12 exons, spans 10 kb, and is the gene associated with RBC PK enzyme activity. Approximately 160 mutations have been reported, usually involving alterations in substrate affinity or enzymatic stability. Occasional mutations affect the interaction of fructose 1,6-diphosphate, an allosteric activator. Inheritance is autosomal recessive, and symptoms are associated with homozygous or compound heterozygous genetic states. Simple heterozygotes for *PKLR* mutation are clinically normal, although they usually have moderately decreased measured enzyme activity levels. PK deficiency can be a particularly challenging diagnosis at risk for false-negative findings as some patients can have unexpectedly higher enzyme activity, including values within the normal range, owing to brisk reticulocytosis. This can paradoxically occur in those with the most severe symptoms. This phenomenon has led to the recommendation that PK enzyme levels should be interpreted in the context of other enzyme activities and not as a stand-alone test. It is useful to use a ratio to another enzyme, hexokinase (i.e., PK/HK ratio or G6PD/PK ratio), as an internal standard that helps indicate a masked deficiency. More often than in G6PD, molecular methods are required to fully exclude it as an etiology of long-standing or neonatal hemolysis. PK deficiency is relatively common in humans and other mammals, including beagles and Abyssinian cats, and has shown protective effects against malaria morbidity. Clinical symptoms include chronic nonspherocytic hemolytic anemia of varying degree but can result in hydrops fetalis or severe neonatal/pediatric anemia responsive to splenectomy.

NEXT-GENERATION SEQUENCING OF NON-NEOPLASTIC RBC DISORDERS

The availability of massively parallel sequencing techniques, termed *next-generation sequencing*, as a clinically available test has greatly improved the ability to accurately diagnose many incompletely understood disorders. It is particularly useful in identifying pathogenic mutations in large genes with many exons and allows the interrogation of many gene regions simultaneously. Consequently, NGS is well suited for the diagnosis of RBC membrane or enzyme abnormalities. The number and size of the exons required to establish a definitive diagnosis of hereditary spherocytosis, hereditary elliptocytosis, and hereditary pyropoikilocytosis had previously inhibited widely available genetic testing for these disorders. NGS panels also allow an extended panel for the identification of hereditary (congenital) erythrocytosis mutations, and whole exome/whole genome sequencing holds the promise of identification of the as yet undiscovered etiology for many recalcitrant idiopathic cases. Currently, limitations of NGS include adequate coverage in areas with high homology and large deletions and duplications are not routinely or sensitively identified. Until these technical aspects are satisfactorily addressed, practical use of the method for the comprehensive diagnosis of hemoglobinopathies and thalassemias will be suboptimal and better performed using classic molecular techniques. Despite these current limitations, the impact of NGS on the diagnosis of RBC membrane, and enzymatic and hereditary erythrocytic disorders will likely be profound.

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The references for this chapter and supplemental material can be found online by accessing the accompanying Expert Consult website.

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POLYMERASE CHAIN REACTION TECHNIQUES AND SAMPLE CONSIDERATIONS

The ubiquitous technique of PCR is employed as a stand-alone analytic procedure or as one component of more complex methodologic assays in the majority of molecular diagnostic laboratory operations. PCR using oligonucleotide primers and thermostable DNA polymerases can be performed using genomic DNA or messenger RNA transcripts as templates, in the latter case also requiring a reverse transcription (RT) step to generate complementary DNA (cDNA) prior to amplification (i.e., RT-PCR). A myriad of PCR variations have been developed (e.g., allele-specific, "asymmetric," use of modified oligonucleotides) to enhance analytic specificity according to particular assay requirements. Detection of PCR-amplified products can be achieved by a number of methods, including agarose or polyacrylamide gel electrophoresis and by capillary electrophoresis; in the latter approach, PCR primers are typically covalently labeled with a fluorescent dye moiety to permit highly accurate fragment length determination following laser scanning of the capillary gel matrix. Routine PCR methods are of great utility in hematopathology, including the determination of B-cell or T-cell monoclonality and the detection of chromosomal translocations characteristic of many leukemias and lymphomas. Translocation events can either produce a novel chimeric gene, which is subsequently transcribed as a unique fusion mRNA species (this is typical of the acute leukemias), or the translocation can result in aberrant overexpression of a highly regulated proto-oncogene owing to its juxtaposition with an unrelated region of DNA containing a strong transcriptional promoter or enhancer (this is most often seen in the non-Hodgkin lymphomas). Other types of genetic rearrangements (e.g., small insertion/deletion events, point mutation changes in genes) can be detected by standard PCR methods, along with additional post-PCR procedures such as fluorescent PCR product sizing, restriction enzyme digestion, oligonucleotide probe hybridization, high-resolution melting curve analysis using DNA intercalating dyes, or automated DNA sequencing. Finally, non-PCR or "isothermal" amplification techniques have been employed in the molecular diagnostic setting, including ligation-mediated amplification, and nucleic acid sequence–based amplification (NASBA), but these alternatives occupy a relatively small niche in relation to the abundance of PCR-based methods.

A variety of tissue or sample sources can be used for the molecular genetic investigation of leukemia and lymphoma, including fresh or frozen tissues and cells, as well as formalin fixed, paraffin-embedded (FFPE) tissue material. Blood and bone marrow specimens should be obtained in anticoagulant (e.g., EDTA, acid citrate dextrose [ACD], or heparin) to ensure that the leukocytic component is adequately available for DNA or RNA extraction. Paraffin-embedded tissue blocks are a common source of material for DNA PCR assays, although fixed tissues are prone to nucleic acid degradation and cross-linking, often reducing the quality of the DNA template. Although technically more difficult, RNA can also be obtained from FFPE tissue; however, the nucleic acid quality is significantly less optimal compared with RNA from fresh or viably cryopreserved cells. Analyzable DNA can also be reliably obtained from air-dried glass slide smears or touch preparations of blood, marrow, or tissue; in this case, unstained slides are preferred, as commonly employed histologic and hematologic dyes may adversely inhibit successful PCR. Regardless of tissue source, the molecular diagnostic laboratory must have adequate procedures to ensure the quality and integrity of each individual sample. Such measures include assessing the amount, purity, and adequacy of amplification of the obtained nucleic acids. RNA samples are particularly labile and subject to rapid degradation by RNases. Given the sensitive nature of PCR technique, isolation of nucleic acid preparations must be done very carefully to avoid sample cross-contamination and rigorous separation of "pre-PCR" (nucleic acid isolation) from "post-PCR" (analysis) areas of the laboratory to prevent pre-analytic sample contamination arising from "floater" or carry-over PCR-amplified products. Standard amplification controls are required in all PCR assays and should include (1) relevant positive and negative nucleic acid controls to ensure specificity; (2) an internal control (i.e., a "housekeeping" gene) of sufficient fragment size to ensure integrity of the RNA or DNA within the expected amplicon size range; and (3) a "no template" control to assess for possible contamination. Assay sensitivity controls (e.g., standard curve or related calibrator reagents for quantitative assays and low-level positive controls near the limit of detection for qualitative assays) are also included to monitor per-run accuracy over the stated analytic range.

REAL-TIME QUANTITATIVE PCR METHODOLOGY AND GENERAL ASPECTS OF MINIMAL RESIDUAL DISEASE DETECTION

Specialized "real-time quantitative" PCR (RQ-PCR) platforms have become invaluable in the molecular diagnostic evaluation of several hematolymphoid neoplasms. RQ-PCR is ideally performed in an automated, multi-sample, closed system format in which the initial amount of a specific nucleic acid target can be accurately quantified with exceptional precision. Commercially available RQ-PCR instruments are based on two major technologies: the 5′ fluorescent nuclease assay, also known as "Taqman" (Applied Biosystems Inc., Foster City, CA) and the dual fluorescent probe hybridization assay based on the concept of fluorescence resonance energy transfer, or FRET (Roche LightCycler, Roche Molecular Diagnostics, Indianapolis, IN). The ability of each platform to deliver highly reproducible quantitative PCR results is very similar, and Taqman chemistry is commonly employed in many molecular diagnostic laboratories. Although standard "end-point" PCR methods can be designed to identify the presence of very small amounts of a target nucleic acid, the plateau phase of PCR product detection (e.g., by gel electrophoresis) often does not reliably reflect the starting concentration of

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the species of interest, mainly because of late cycle amplification effects on PCR efficiency. In contrast, measurement of the initial quantity of a specific mRNA or DNA species by RQ-PCR is possible by relying on the early detection of PCR product during the exponential phase of PCR. The initial appearance of a fluorescent signal above background at the so-called threshold cycle (Ct) or crossing point (Cp) during this phase of PCR is directly and quantitatively related to the starting amount of the target DNA or RNA in the sample of interest. A standard curve prepared by plotting observed Ct numbers against serial 10-fold dilutions of a given target molecule should produce a linear relationship in which each 3.3-cycle interval represents a change of 1 log of concentration (assuming 100% PCR efficiency). In an ideal PCR assay, the concentration of amplified DNA template doubles with each cycle. In practice, however, PCR efficiency is affected by a variety of issues, such as the composition of the specific target region (GC nucleotide content, or homopolymer repeat region), placement of primers and probes (avoiding known polymorphic nucleotides or pseudogene regions), optimized reagent concentrations and type of polymerase used, among others. Achieving a reliable and precise RQ-PCR assay often requires substantial expertise and some trial-and-error in order to approach the theoretical ideal assay output over the biologically relevant range of detection.

One method of quantifying a nucleic acid of interest in an unknown sample using RQ-PCR is thus to directly read the concentration from a standard curve generated from co-amplified serial dilutions of a positive control template, in essence deriving an "absolute" measurement of the analyte. The standard curve method requires that a quantitative unit value for a particular target be normalized to that of an amplified, unrelated "housekeeping" gene or mRNA transcript, in order to compensate for sample, technical, and PCR condition variability. Alternatively, one can choose to measure the relative quantity of a specific PCR product by comparison to a reference reagent standard or "calibrator," again after normalizing both the unknown sample and calibrator amplification Ct result to a housekeeping gene or transcript value. This comparative approach also assumes that the sample gene target and calibrator amplify with a PCR efficiency equivalent to the normalizing control gene region, in which case relative changes in the normalized quantities between the unknown sample and calibrator can be reproducibly measured. The comparative method is also known as the $2^{-\Delta\Delta Ct}$ method, reflecting its mathematical derivation; if carefully validated, the requirement for a formal standard curve with each experiment can be avoided, and the quantitative results are expressed as a-fold or percentage change relative to the calibrator, rather than an absolute numeric measurement. Notably, for RNA analytes, a reverse transcription (RT) step is performed to generate cDNA templates prior to the actual RQ-PCR; the RT reaction is generally assumed to be close to 100% efficient, such that the subsequent RQ-PCR accurately reflects the mRNA target abundance in the sample. Poor RT efficiency can thus adversely affect the reliability of RQ-PCR data. Additional considerations for successful and robust RQ-PCR assays include the appropriate choice of standards (e.g., serial dilutions of positive control cell line DNA, RNA, or cloned template of a particular genetic target), adequate controls (positive, low positive or dilute control, negative, and no template samples), and the careful selection of the normalizing or "housekeeping" gene or transcript. Normalization controls for RNA-based RQ-PCR are typically selected to possess relatively stable gene expression levels in both tumor cells and background cells, to show equivalent PCR efficiency, to have degradation characteristics similar to the target mRNA over time, and to show minimal tendency for significant deviation among clinical subjects. The RQ-PCR assay must also exhibit excellent linearity throughout the testable analytical measurement range (i.e., AMR). The proper evaluation of positive control reagents is also a critical consideration; some cell lines, for example, may have amplification of the target gene or a highly active transcription of an expressed fusion gene relative to clinical samples from individual patients. The choice of positive controls should ideally reflect the biologic range of target values expected in the patient population with the disease when accurate quantitative measurements are required; one approach to assess positive controls is by using the digital PCR (DPCR) technique, as detailed briefly in the ensuing section, to calculate gene or transcript copy number. Finally, the choice of cell line control and housekeeping transcript affects the relative value of normalized target quantity in reverse transcription RQ-PCR assays that are not reported according to a standard consensus (e.g., the International Scale for BCR-ABL1 p210 monitoring); therefore, direct comparisons of measurements from the same patient analyzed in different laboratories may demonstrate some quantitative variability, even though the relative range of target detection (e.g., degree of log-level change) is similar.

If adequately constructed, RQ-PCR analyses can demonstrate remarkable reproducibility and achieve a high level of analytical sensitivity (i.e., 5–6 log dynamic range). In addition to obvious applications in post-therapy MRD detection, RQ-PCR can also be used to identify and quantify single base changes in nucleic acids for "allelotyping" assays. The sensitive measurement of MRD using RQ-PCR is now an integral component in the management of many hematologic cancers. The rationale of MRD evaluation is to provide more definitive prognostic or predictive information for individual patients in order to monitor treatment efficacy and determine the need for additional intervention. Beyond the concerns inherent in developing high-quality RQ-PCR assays, a variety of other issues are germane to MRD determination, such as the sample source (i.e., blood versus bone marrow), the frequency of monitoring required, and the biologic characteristics of the hematolymphoid tumor being evaluated. In general, serial MRD assessments at key time intervals post treatment are most likely to provide the best predictive data on tumor biologic behavior in a given patient, rather than single measurements at only one or two time points. Although elimination of molecular residual disease (i.e., PCR negativity) is a desirable major goal of treatment (e.g., acute promyelocytic leukemia with *PML-RARA*), it is also well known that some

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long-term leukemia survivors (e.g., patients with *BCR-ABL1* chronic myeloid leukemia or *RUNX1-RUNX1T1* acute myeloid leukemia) still harbor detectable molecular MRD, albeit at low levels. Since it is not possible to definitively predict which patients have low-level MRD consisting of "dormant" residual tumor cells versus those with minor tumor populations capable of re-constituting overt disease relapse, any low-abundance PCR-positive result must be considered as potentially significant; the ability of RQ-PCR technology to reproducibly assess temporal disease level fluctuations in individual patients is therefore of obvious benefit in this situation.

The detection of "rare events" by highly sensitive RQ-PCR methods can also lead to interpretive difficulties. At the analytic limits of assay detection, one may encounter stochastic effects, such that a sample may test positive in one reaction aliquot but negative in a duplicate one or in a later sample from the same patient, despite the same approximate minimal level of disease being initially present in each case. It is important that laboratories performing molecular MRD detection establish a rigorous definition of the reproducible limit of target detection (LOD) for each quantitative assay. Outlying or spurious low-level results can then be better interpreted in light of these precision standards. Of note, several leukemia-associated fusion gene transcripts (such as the *BCR-ABL1* abnormality) have been described at very low levels but with relatively high prevalence in the blood of healthy individuals. These data are consistent with the hypothesis that an abnormal gene fusion is necessary but likely insufficient on its own for the production of overt leukemia. Although these intriguing findings would appear to potentially complicate the interpretation of RQ-PCR MRD analyses in leukemia patients, the detection of such low-abundance fusion transcripts in normal subjects has required the use of modified PCR methods with extended sensitivities typically one or two orders of magnitude greater than the maximal limits obtained by current clinical RQ-PCR assays (i.e., 10^{-5} to 10^{-6}). Thus, the chance of identifying a confounding positive PCR result arising from possible rare "bystander" cells should be negligible in a well-designed RQ-PCR assay for clinical use.

DIGITAL PCR

Digital PCR (D-PCR) technique has become a recent and powerful addition to the stable of quantitative PCR-based applications and exhibits some important differences compared to RQ-PCR methods. For example, D-PCR quantification relies on end-point product detection in contrast to early exponential phase detection for RQ-PCR. Unlike qualitative end-point PCR assays, however, D-PCR employs a very large partition system of many thousands or millions of small individual "micro-reactors," each containing nanoliter (or smaller) quantities of a DNA template copy and associated PCR reagents. In theory, adequate partitioning of a sample volume containing the target nucleic acid should create many individual reactions containing either one or no DNA copies in a single compartment. A common mode of sample preparation involves generation of a micro-droplet emulsion suspension from a standard sample volume containing nucleic acid template and PCR reagents. Each tiny reaction is then co-amplified to a cycle maximum, and the individual compartments are analyzed by fluorescence signal detection to identify the presence or absence of PCR product. In this sense, D-PCR is a form of automated, quantitative limiting dilution PCR technique. Accordingly, simple digital counting and scoring of each reaction is a direct reflection of the starting quantity of molecules-of-interest, and if the sum of all compartment volumes is known (i.e., essentially the total input volume dispersed in the digital array), then the starting concentration of the target (copies/sample volume) can be directly computed. Accuracy and precision of D-PCR depend on the target concentration range and at very high levels, non-random errors are introduced to the system (e.g., multiple template copies per partition, or saturation), causing inaccuracies in digital counting and quantitation. At lower target levels, in which fewer PCR-positive events are distributed among a sufficiently large population of negative reaction partitions, the system becomes both highly accurate and sensitive. Furthermore, the probability of a compartment containing some or no copies of a target, along with the degree of measurement uncertainty, can be accurately estimated using the Poisson distribution. The accuracy and sensitivity of D-PCR is optimized when the platform partition space or total number of compartments is very large. D-PCR is gaining popularity because of its advantages in direct determination of target DNA or RNA quantity over useful biologic ranges without the complexities of traditional RQ-PCR methods (e.g., a calibrator reagent for deriving quantitative results is not strictly required). Various commercial platforms employ different strategies to generate micro-reactor compartments (e.g., Raindance Technologies, Billerica, MA, and BioRad, Hercules, CA) and to perform digital counting calculations. The current challenges for D-PCR include the relative inability to process multiple samples at a time, a lack of higher order target multiplexing, and variable lower limit of detection (analytical sensitivity) among different platforms.

NEXT-GENERATION SEQUENCING

Following completion of the first draft sequence of the Human Genome Project in 2000, an intense period of technology advances resulted in the emergence of several "next-generation" NGS platforms. An ideal tool for the genomic characterization of cancer is one that could simultaneously provide information about copy number variations, allelic information, somatic rearrangements, insertion/deletion (indel) events, and nucleotide level mutations in a single experiment. In this regard, the advent of NGS is moving analytic capabilities closer to this goal. Traditional DNA sequencing is performed by Sanger method or alternative approaches like pyrosequencing; however, these are limited in scalability and/or sensitivity.

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In contrast to Sanger sequencing (which the Human Genome Project was based upon), NGS has a number of distinct advantages (see Table 24.1). NGS methodology essentially is composed of four key process elements: (1) choice of sample type (e.g., DNA, RNA, fresh/frozen, or FFPE sources); (2) novel sample preparation techniques to produce sequence-ready libraries; (3) proprietary high-throughout, massively parallel sequencing platforms to detect DNA sequence alterations; and (4) sophisticated bioinformatics analysis pipelines to assess quality parameters, quantitate and classify numerous varied sequence alterations, and graphically display data against a reference genome for localization and visual inspection. A very critical additional element is the careful human assessment of detected variants to determine clinical and biologic significance in the context of disease diagnosis, prognosis, and treatment (i.e., "curation" of the data). NGS technology is truly revolutionary and has quickly found applications in DNA-based sequencing (targeted gene panels to whole exome or genome), RNA sequencing (transcriptomics and expressed mRNA fusions from translocations), mate pair sequencing (detection of larger chromosome or genomic rearrangements), and determination of copy number alterations. Specialized applications including epigenomics (methylation profiling) and structure-function mapping (e.g., CHiP-Seq) have also been developed. NGS has been applied to the detection of antigen receptor gene rearrangements, providing powerful tools for detecting clonal lymphocyte populations and greatly facilitating MRD monitoring in lymphoid neoplasms, as well as deepening the basic understanding of adaptive and cellular immune responses. The depth and breadth of NGS also provides an unprecedented opportunity for unraveling more complex processes, such as genetic mosaicism or tumor genetic heterogeneity and the pathways of neoplastic molecular evolution.

Despite the variety of nucleic acid sources that can be used for NGS analysis, careful considerations are needed for each. High-quality DNA can be readily extracted from fresh (or cryopreserved) tissues, but FFPE samples are prone to degradation and DNA breaks from cross-linking. Nevertheless, FFPE tissues are very commonly used for NGS as long as adequate sample quantity and quality standards are met. RNA must be rapidly extracted and either stored or converted to cDNA to preserve the relative quantities of different transcript species and avoid degradation effects due to RNases. Library preparation methods are numerous, but commonly employed approaches include highly multiplexed PCR amplification of desired target regions, or oligonucleotide probe ("bait") capture of the regions of interest in a sheared DNA specimen. Each has advantages and disadvantages, such as higher potential sensitivity with PCR-based methods versus more uniform target coverage using capture probe systems. In any library preparation, many steps are involved to incorporate flanking universal "adapter" sequences, sequencing primer sites and unique indexes (or DNA bar codes) to enable downstream sequencing, and analysis of the internal segments of DNA (inserts). The addition of molecular bar codes or indexes enables the simultaneous multiplexing of numerous patient samples, in turn achieving maximum utility of the sequencing capacity of the instrument and providing higher sample throughput with lower cost. Modern NGS platforms use different, innovative approaches to generate large amounts of high-quality data, producing gigabytes to terabytes of data from hundreds of millions of individual sequence reads. These technologies include semiconductor, solid phase support, and "long read" (e.g., nanopore and single molecule) methods. Importantly, NGS instrumentation for most clinical applications requires relatively small fragments of DNA derived from patient samples, referred to as "short read" sequencing; these numerous short sequence reads are subsequently bioinformatically mapped and assembled to produce a consensus final sequence for each target region. Because many hundreds to thousands of individual sequence reads are generated per target region (the depth of coverage), NGS assays routinely reach an analytic sensitivity of approximately 1% to 5%, and even higher sensitivity can be gained with novel chemistry adaptations.

The two most commonly employed commercial platforms include the Ion Torrent (Thermo Fisher, Waltham, MA) and HiSeq or MiSeq (Illumina, San Diego, CA) systems (Fig. e24.1). Ion Torrent sequencing relies on PCR amplification of a prepared library of target DNA fragments attached to millions of microbeads in a droplet emulsion to generate clonal single-stranded DNA sequencing library templates, which are then distributed onto an array of microwells on a semiconductor sequencing chip. Multiple rounds of unlabeled nucleotide additions sequentially elongate the immobilized DNA fragments. Each time a new base is incorporated in an elongating sequence strand, a pyrophosphate molecule and hydrogen ion (H^+) are released, and these tiny changes in pH generate a voltage fluctuation that can be measured across the many thousands of individual microwells. By knowing the order of bases introduced with each cycle, the corresponding nucleotide sequence is deduced by determining which wells "experienced" a pH change. Illumina sequencers use a solid support system in which channels ("lanes") are coated with adapter molecules that can bind complementary sequences attached to the prepared library fragments. After attachment of sheared, adapter-ligated DNA fragments to the surface of the lanes of the sequencing chip, a limited cycle PCR step is performed to accomplish "bridge amplification" of each spot on the lane, resulting in the formation of millions of individual clonal colonies of fragments. Sequencing is then initiated from consensus sequence primer sites using multiple rounds of fluorescently labeled reversible dye terminator nucleotide addition, digital imaging of the chip, and dye cleavage. If only a single adapter is added during the library step, then sequencing output is termed *single-ended*. However, Illumina methodology allows for the addition and selection of two adapters for each pair of double-stranded DNA molecules within the library. This permits a second step during sequencing (turn-around phase) in which the initial single-ended sequence product can be re-bridged to the second complementary adapter on the chip surface and another round of sequencing obtained in the opposite direction. So-called

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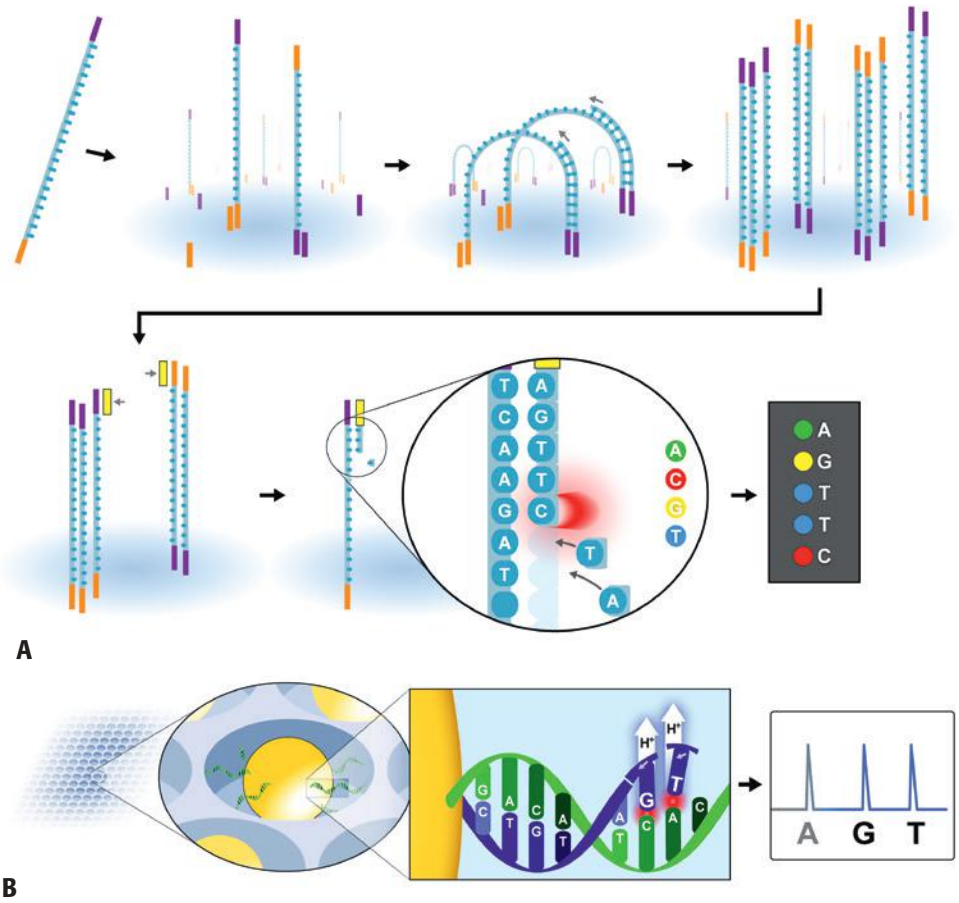


FIG. e24.1

Two common next-generation sequencing (NGS) platforms. This figure is a basic overview of the two major commercial platforms for NGS. Panel A shows a schematic of the Illumina NGS technology that is common to the MiSeq and HiSeq instrument platforms (Illumina, San Diego, CA). A library of sheared, adapter-ligated single-strand DNA fragments is attached to the sequencing chip in flow cells that are composed of two or more "lanes." The surface of the lanes is coated with covalently bound oligonucleotides complementary to each of the two adapter sequences (called P5 and P7, depicted as *purple* and *orange bars*), which were incorporated in a specific orientation into the sample DNA fragments during library preparation. Each single-strand DNA fragment is then "bridged" to a corresponding adapter oligonucleotide on the chip surface and extended to produce complementary strands. Several rounds of bridge PCR amplification are performed to expand the tethered single-strand DNA fragments and generate 1000s of clonal copies (clusters) of each unique DNA fragment. One of the adapter ends (P5) is then cleaved, and sequencing is initiated from the free end of the remaining sample fragments. Sequencing typically produces 100 or 150 base lengths and proceeds through repetitive cycles of adding fluorescent dideoxynucleotides and imaging the chip area to determine which bases were added in each cluster. The sequencing nucleotides have reversible terminator chemistry such that following each cycle the terminator (blocker) and the fluorescent dye are removed prior to the next round of nucleotide addition. One advantageous feature of Illumina technique is the ability to utilize the same DNA library to sequence target fragments from the reverse strand, known as paired-end sequencing. This occurs by re-bridging the already sequenced, chip-bound DNA strands, additional clonal amplification cycles, cleavage of the other adapter (P7), and subsequent cycles of new sequence generation. Paired-end sequencing improves the ability to map the insert DNA more accurately and unambiguously to the genomic reference. In this diagram, the paired end component is not shown for simplicity. The raw or primary sequencing data are converted to FASTQ file format that can be used to accurately align sequences to the reference and detect a range of sequence alterations using downstream software applications. Panel B illustrates the Ion Torrent sequencing platform (ThermoFisher, Waltham, MA). A prepared library of adapter-ligated DNA fragments is captured onto microbeads for emulsion PCR. This process creates millions of clonally amplified single-strand DNA targets anchored to the bead surfaces. The emulsion is applied to the surface of a specialized semiconductor chip containing numerous tiny reaction micro-wells in a manner that generates a single bead per well. The chip and an enlarged section of an individual well are depicted. Successive cycles of unlabeled nucleotide additions are performed to produce an elongating sequence product. With the incorporation of each base, release of H^+ and pyrophosphate molecules occurs and the small changes in pH are detected as voltage differentials. By knowing the order of base additions, the sequence of the target molecules can be derived. Ion Torrent sequencing obviates the need for specialized nucleotide modifications. Primary sequence data are processed by on-board software and FASTQ files are generated for downstream analytic applications. *(B, Adapted from* Nat Rev Genet *2016 May 17;17(6):333–51. doi: 10.1038/nrg.2016.49.PMID: 27184599.)*

paired-end reads are generated, which improves the ability of analytic software algorithms to achieve higher alignment accuracy to the reference genome.

Following sequencing by any NGS method, the raw sequence information is converted to FASTQ file format for initial alignment (mapping) to the genomic reference, base quality scoring, and variant calling and data filtering. The basic process of the bioinformatics "pipeline" involves a variety of software applications that perform sophisticated analyses for accurate alignment of sequenced reads to the reference genome (i.e., genomic and selected coding transcript positions, relative to the specific human genome build used), base substitution detection, and insertion/deletion calling (initial annotation). An extensive quality report is typically

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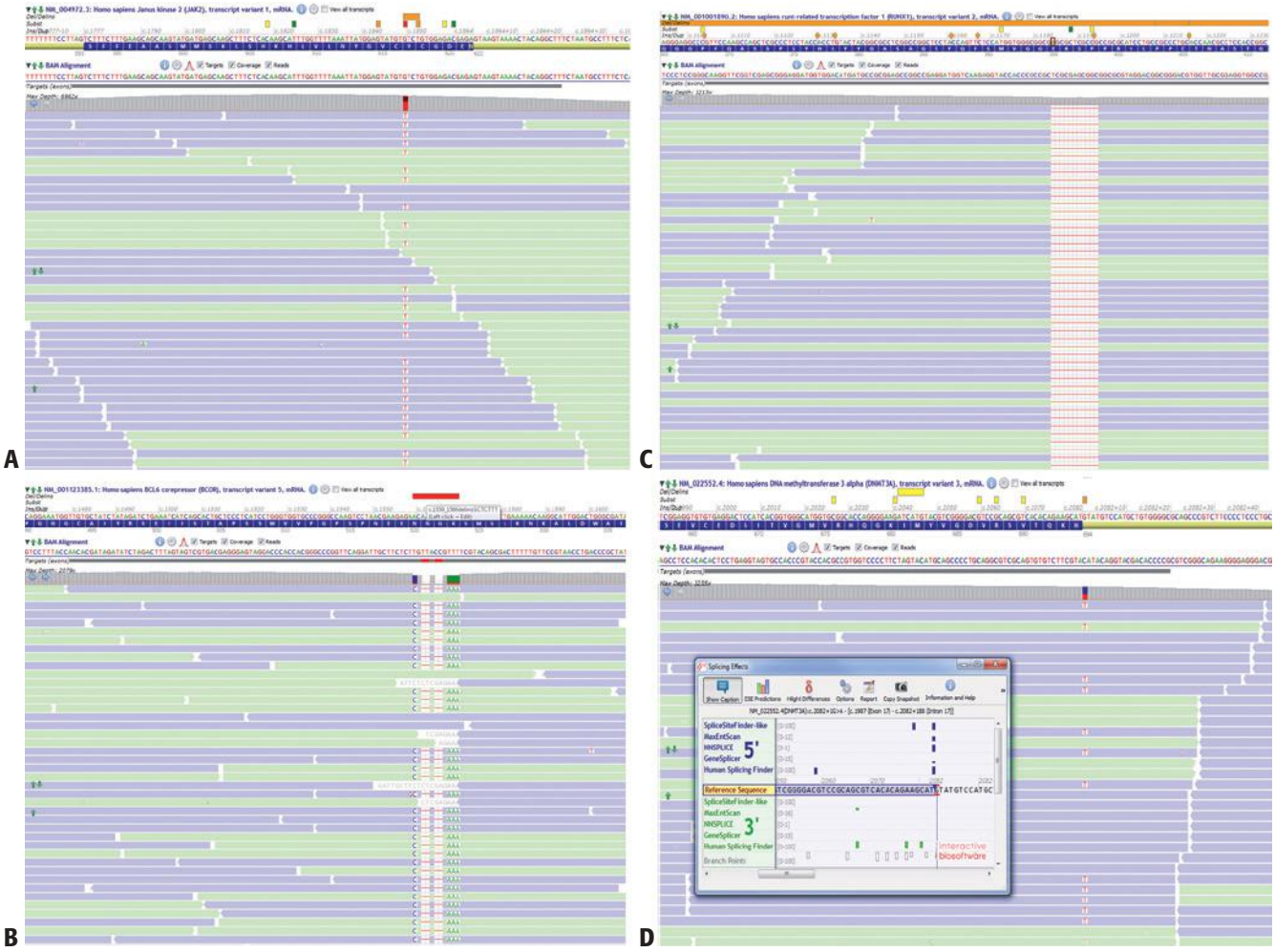


FIG. e24.2

Examples of DNA alterations detected by next-generation sequencing. Each image demonstrates binary sequence alignment map (BAM) file data loaded into the Alamut Visual genomic viewer (www.interactive-biosoftware.com). Different tracks can be displayed, including the genomic reference coordinates, preferred reference transcript sequence, databases of known human polymorphisms, hereditary disease associations, cancer mutations, species conservation of sequence data, and others. The selected transcript nucleotide sequence (relative to standard genomic reference coordinates) and underlying coding region amino acid sequence (indicated by *blue lettered boxes*) are shown along with intervening intron regions (*yellow bars, images A and D*). The consensus sequence is displayed below in the BAM file alignment. The *gray vertical bars* below the alignment display the depth of base sequence reads at each nucleotide position. Below are shown tiled bars representing sequences of blue (forward) and green (reverse) reads for each DNA fragment *(insert)* generated by 2 × 150 base paired-end reads on the Illumina MiSeq. Sequence bars appear uninterrupted when identical to the reference, whereas sequence alterations are visually displayed. An estimate of the mutation burden (variant allele fraction, VAF) can be ascertained directly by the software. Alignment of more complex sequence variants requires manual evaluation to ensure correct annotation nomenclature. Similar display features are available using the Integrated Genomics Viewer, IGV (www.broadinstitute.org/igv). Programs such as Alamut also include a limited but very useful set of embedded common analytic programs or algorithms to assess missense amino acid substitution predictions or splicing junction alterations. This series of panel images shows (along with specific reference mRNA transcripts): A, Substitution (missense) mutation in *JAK2* c.1849G>T; p.Val617Phe (NM_004972.3); B, deletion/insertion alteration in *BCOR* c.1559_1569delinsGCTCTTT; p.Asn520Serfs*7 (NM_001123385.1); C, deletion variant in *RUNX1* c.1184_1194del; p.Glu395Alafs*174 (NM_001001890.2); D, splicing site mutation in *DNMT3A* c.2082+1G>A (NM_022552.4). The annotation nomenclature follows the criteria of the Human Genome Variation Society standards (http://www.hgvs.org), and the reference sequence data are based on human genome build GRCh 37 or hg19 (https://www.ncbi.nlm.nih.gov/grc).

generated to detail percent and total sequence reads mapped correctly to the regions of interest and to the genome, base quality scores, sequence error rates, failed regions, off target sequences, and many other parameters. The alignment step and variant calling step of the bioinformatics pipeline create BAM files (binary version of a text readable sequence alignment map) and VCF (variant calling format) files, respectively. Either the BAM or VCF file can be uploaded to genome viewer(s) by the end user for detailed visual review, annotation, and interpretations of genetic variants. Popular viewers include the Broad Institute/MIT Integrated Genome Viewer (IGV) (www.broadinstitute.org/igv) and Alamut Visual (www.interactive-biosoftware.com) (Fig. e24.2). Notably, additional pipeline algorithms and processes are required for other specialized applications of NGS, such as RNA sequencing or translocation detection. The most labor- and time-intensive aspect of NGS data interpretation concerns the detailed assembly of knowledge to characterize one to often many detected sequence variants in terms of pathogenicity and clinical significance. A large number of publicly available and subscription databases are present to help guide the interpretation of variants; some key examples include those

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concerned with polymorphic sequence variations (e.g., dbSNP, https://www.ncbi.nlm.nih.gov/projects/SNP; 1000 genomes, http://browser.1000genomes.org/index.html; Exome Variant Server [EVS], http://evs.gs.washington.edu/EVS), cancer genomics (e.g., Catalogue of Somatic Mutations in Cancer [COSMIC], http://cancer.sanger.ac.uk/cosmic; MyCancerGenome, https://www.mycancergenome.org), or genetic variants and phenotypes of human heritable diseases (e.g., Human Gene Mutation Database [HGMD], http://www.hgmd.cf.ac.uk/ac/index.php; ClinVar, https://www.ncbi.nlm.nih.gov/clinvar). However, the accurate curation of findings requires a thorough understanding of the data quality in databases, knowledge of the specific field of pathology or medicine (often supplemented with extensive and ongoing literature review), and the dedicated efforts of a team of individuals including laboratory technologists, bioinformaticians, genetic counselors and pathologists, or clinical laboratory scientists with relevant molecular genetics expertise. Given that each cancer genome may have thousands of somatic mutations, it is clear that rigorous validation of recurrent candidate alterations in large clinical studies or by detailed biochemical and genetic laboratory research will be required to separate true driver mutations from irrelevant changes ("passenger" mutations). To this end, some studies have advocated the use of matched tumor/normal tissue sequencing to distinguish polymorphic from pathogenic changes and to determine the presence of potential germline conditions. A complete description of NGS applications in diagnostic medicine is beyond the scope of this chapter; although the uses of NGS for currently classifying and determining prognosis in hematologic cancers will be described accordingly, it is important to stress that the information accruing from the explosion in this area will continue to shape hematopathology practice for the foreseeable future.

CONTEMPORARY MOLECULAR TESTING IN MYELOID NEOPLASMS: A CASE FOR NGS

As NGS technology becomes more routinely deployed in clinical diagnostic laboratories, the use of purpose-built targeted gene panels offers a high-yield, cost-effective approach for the molecular profiling of myeloid neoplasms, complementing conventional karyotyping, morphology, and clinical data. A targeted approach has some distinct advantages compared to whole exome sequencing (WES) such as (1) a primary focus on well-documented, recurrently mutated genes enriched in myeloid neoplasms providing analysis of highly curated and clinically relevant ("actionable") variants, in contrast to the large datasets of WES containing many lower frequency alterations of uncertain clinical significance; (2) improved depth of sequencing coverage compared to WES, thus improving analytic sensitivity to detect subclonal populations or low variant allele fraction (VAF) neoplastic cell populations in a mixed tumor-normal cell background; and (3) higher sample throughput with reduced cost and turn-around time, in terms of post-sequencing analytics. For myeloid neoplasms encompassing myelodysplastic syndromes (MDS), acute myeloid leukemias (AML), myeloproliferative neoplasms (MPN), and myeloproliferative/myelodysplastic neoplasms (MDS/MPN), overlapping genetic mutation patterns are observed. A targeted pan-myeloid neoplasm panel can be effectively designed by selecting genes according to published clinical relevance. By using larger panel designs, smaller subset analyses can be tailored to focus on individual disease types (e.g., "AML-specific" genes) using bioinformatic masking of extraneous gene regions; this ability further extends the flexibility of using NGS panels for hematologic oncology applications. Mutations in specific single genes (e.g., *SF3B1* and *TP53*) that are now formally recognized in the 2016 WHO classification can be detected by Sanger sequencing technique, and this may be sufficient in many instances to meet diagnostic criteria; however, the power of NGS in this setting resides in its capacity to interrogate many gene regions and patient samples simultaneously. As additional gene mutation patterns demonstrate clinical utility or therapeutic value, the Sanger approach becomes less tenable with its limited sample throughput and lower sensitivity (15% to 20% vs. 5% for NGS). When deciding on the addition of NGS technology to the molecular diagnostic laboratory, many considerations are required, including the scope of the test (e.g., number of gene regions, overall size of panel), analytic framework (e.g., bioinformatics support, critical software, specialized knowledge, management of potential germline findings), cost and reimbursement potential, sample throughput, and the role of competing methodologies, such as Sanger sequencing or PCR-based techniques. Several position papers and guidelines have been published by organizations such as the Association for Molecular Pathology, College of American Pathologists, and American College of Medical Genetics and Genomics to help meet these challenges.

SOUTHERN BLOT HYBRIDIZATION

Southern blot hybridization (SBH) technique occupies a diminishing role in the molecular diagnostic laboratory but continues to have relevance for the analysis of relatively large (kilobase)-scale alterations in genomic DNA. SBH involves digestion of high-molecular-weight DNA with site-specific restriction endonucleases, followed by size separation of the DNA by gel electrophoresis and transfer of the nucleic acid fragments onto a nylon or nitrocellulose membrane. A particular region of immobilized DNA can then be interrogated by stringent hybridization with a specific double-stranded genomic probe, which has been labeled with a radioisotope (e.g., P^{32}) or by a non-isotopic reagent (e.g., for chemiluminescent detection). Exposure of the resultant band pattern on radiographic film reveals the presence of any atypical rearrangement(s) at the genetic locus in question, relative to the expected or "germline" (unaltered) band configuration. SBH has been most widely applied to detect clonotypic rearrangements of the antigen receptor genes in abnormal B- or T-cell lymphoid proliferations, but assays to evaluate specific oncogenes (e.g.,

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KMT2A, BCL2, CCND1) have also been described. Visual interpretation of exposed blots is usually straightforward; however, occasional difficulties may arise owing to degraded DNA quality, incomplete enzymatic digestion of DNA, or when novel pathologic bands co-migrate with germline fragments. The multi-step, technically demanding requirements of SBH also increase the possibility of analytic delays, because errors in procedure are seldom identified before the blot is finally exposed. Of note, sample sources for SBH analysis are limited to fresh or frozen cells, owing to the requirement for high-quality, long-strand genomic DNA. For these reasons and with the advent of more comprehensive fluorescence in situ hybridization (FISH), chromosome microarray, or PCR-based methods, SBH is now less frequently performed in molecular hematopathology laboratories.

MOLECULAR CYTOGENETICS: FLUORESCENCE IN SITU HYBRIDIZATION AND CHROMOSOME MICROARRAYS

Conventional cytogenetic analysis remains the standard method to identify numeric and structural chromosomal aberrations in tumors and to assess the visible genome in its entirety for the presence of these large-scale changes. The cytogenetic karyotype in fact retains its prominence in the current WHO disease classification of hematopoietic tumors. However, the limited resolution of chromosome-specific banding obtained by Giemsa techniques (GTG- or G-banding) makes the recognition and interpretation of smaller, masked, or cryptic chromosome aberrations difficult to ascertain and therefore potentially inaccurate. Fluorescence in situ hybridization (FISH) and more recently, chromosome microarray techniques (e.g., array-based comparative genomic hybridization, A-CGH and single-nucleotide polymorphism array, SNP-A) extend the ability to detect smaller scale changes in chromosomal regions and are an integral aspect of molecular cytogenetics practice. The use of these techniques enhances the resolution of both numeric and structural chromosomal aberrations (especially those that are complex or subtle), bridging the gap between conventional chromosomal band analysis and nucleotide base-level molecular genetic analysis.

FISH essentially involves the base-pairing of fluorescently labeled nucleic acid probes to complementary DNA sequences in cell preparations, followed by direct visualization of probe-specific, intranuclear signals utilizing fluorescence microscopy. A large number of different probes designed to identify specific chromosomes or subregions are commercially available for diagnostic purposes, with the choice of probes dependent on the particular application in question. The most commonly used probes in FISH analysis of hematologic malignancies are (1) repetitive sequence probes and (2) locus-specific probes. So-called whole chromosome paints or multiplex probe methods (M-FISH) have been described as well but are technically challenging for standard diagnostic use. *Repetitive sequence probes* target chromosome-specific satellite sequences of pericentromeric heterochromatin (i.e., centromere-specific probes), or unique DNA sequences at the ends of all chromosomes (i.e., subtelomeric probes). These probes are useful when numeric aberrations carry information of diagnostic or prognostic relevance, as in subgroups of childhood acute lymphoblastic leukemia, or the myelodysplastic syndromes. *Locus-specific probes* target sequences normally present as only one copy per haploid genome. The main utility of locus-specific probes in hematologic malignancies is the detection of deletions, inversions, and translocations that are often disease specific. Locus-specific FISH technique is highly versatile, and many different probe strategies can be designed to optimize the detection of an abnormality, depending on the clinical application. Dual-color/dual-fusion translocation probe (D-FISH) methods have been developed to increase the sensitivity and specificity of FISH detection. Another strategy of particular utility is the use of so-called break-apart probes (BAP) to target genes with multiple possible translocation partners (e.g., the *MLL* gene). In this setting, a typical dual-color/dual-fusion FISH assay directed at one particular translocation would possibly miss a variant translocation. A BAP, on the other hand, can detect the presence of a translocation involving a given gene locus independent of the specific partner gene (although the nature of the translocated partner would not be known without additional investigations). Standard karyotype analysis in hematologic malignancies is sometimes hampered by poor chromosome morphology or a low number of requisite tumor metaphases. The ability of FISH technique to use intact, non-dividing (interphase) cells as DNA targets enables the quantitative analysis of a larger number of cells and has considerable added advantages for assessing hematologic malignancies in which the proliferative activity is low, or when the mitotic cells are reactive in nature and do not represent the neoplastic clone. Since culture of mitotic phase cells is not a requirement, FISH is applicable to a variety of specimen types, including fresh or frozen tissue, cytologic preparations (e.g., fine needle aspiration), air-dried unstained slides, and formalin-fixed paraffin embedded tissues (FFPE). In any FISH assay, nuclear DNA is universally stained with a dim fluorescent dye (e.g., 4′,6-diamidino-2-phenylindole, DAPI) to visualize the individual cell nuclei by microscopy. Experimental controls for FISH analyses are based on the false-positive signal rate threshold. Laboratories performing FISH testing must determine the false-positive rate for each assay individually and, in turn, for each type of tissue or specimen used in that assay. Signals from partially overlapping or truncated nuclei (e.g., from thin FFPE tissue sections) can lead to erroneously high positive signal scoring, particularly with fusion probe sets, leading to potentially serious errors in patient diagnosis and clinical management.

Single-nucleotide polymorphisms represent the most frequent type of variation in the human genome, with an average of 1 per 300 bases of genomic DNA. In addition, the discovery of a relatively large number of kilobase- to megabase-sized variations in regional DNA sequences, known as *copy number variations* (CNVs), is also of significance from the perspective of disease pathogenesis, and these gains

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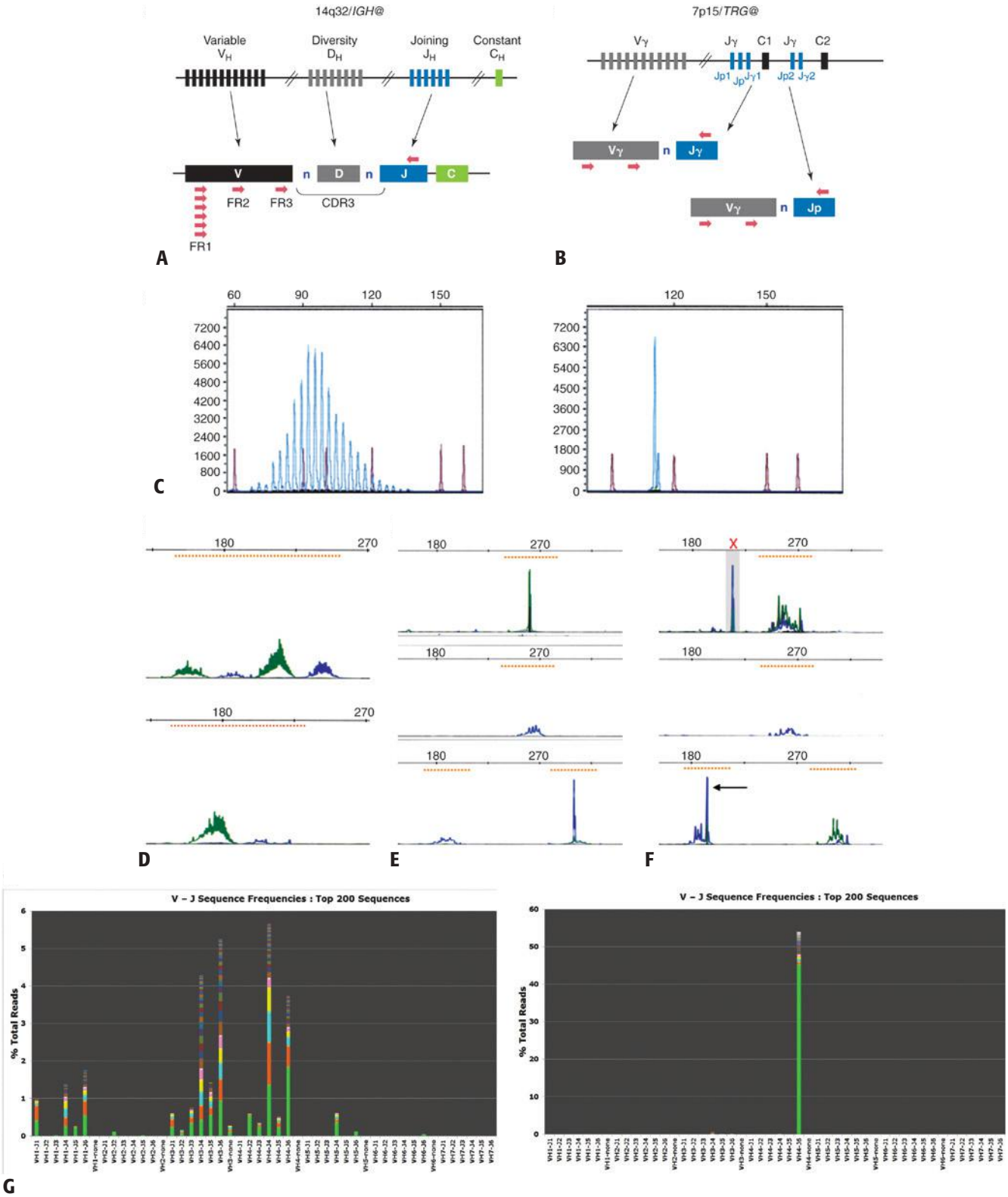
or losses can encompass genomic regions harboring many different genes. Although many CNVs represent polymorphic variants, recent studies have begun to identify recurrent alterations that may be critically involved in disease causation, including acquired CNV events in cancer. The advent of array-based automated technologies for assessing global genome architecture has now enabled more widespread application to a variety of disorders, including hematolymphoid neoplasms. Chromosome array platforms differ in design but are similar in the scope of genomic information provided. Array competitive genomic hybridization uses fluorescently labeled patient and control DNA samples to hybridize to a solid substrate or chip embedded with thousands of small individual DNA probes. The ability to densely tile or "spot" the oligonucleotide probes ensures that a very large proportion of total genomic coverage is potentially obtained. SNP array chips similarly consist of spotted oligonucleotides, but the probes instead are designed to include allelic variants of hundreds of thousands to millions of informative SNP loci distributed throughout the genome. SNP-A powerfully combine both CNV analysis and SNP genotyping, the latter based on probe classification of homozygous versus heterozygous status at the given polymorphic loci. In general, signal differences and intensities between sample and control are measured, and bioinformatic software algorithms analyze the hybridization results to denote copy number changes (gains/losses) in relation to a map of the reference genomic sequence and chromosomal position. A-CGH can detect genome-scale alterations at very high resolution (e.g., below 5–10 kb or even lower, far superior to FISH and standard cytogenetics), but this is critically dependent on the probe density and spacing (coverage). A-CGH delimits the boundaries of CNVs and because a paired control DNA is used, true pathologic CNV changes can be more readily distinguished from common polymorphic DNA regions in the patient sample. A-CGH identifies regional gains (gene amplifications), as well as areas of loss of heterozygosity (LOH) or hemizygosity associated with monoallelic deletion of gene loci. However, A-CGH cannot detect copy number neutral LOH (CN-LOH), also called uniparental disomy (UPD). The latter genomic alteration results from duplication of an allelic region of DNA by homologous recombination with the other (maternal or paternal) allele. CN-LOH can be a result of embryonic autozygosity (i.e., congenital UPD) or can be somatically acquired in tumors. CN-LOH is associated with the presence of two identical haplocopies of a gene (or genomic segment), but without a net loss of DNA content within the affected chromosome regions. CN-LOH is thus not visible to cytogenetic or FISH analyses. A-CGH cannot detect CN-LOH because the method is insensitive to balanced DNA changes, and unlike the related SNP-A technology, A-CGH does not have an additional measure of sequence-level variation.

Using SNP-A, hybridization of the sample genomic DNA to both polymorphic probe variants indicates locus heterozygosity, whereas similar signal intensity for only one allele indicates homozygosity. In addition, the relative intensity of the fluorescence signals allows for the analysis of gene region copy number. SNP-A does not require co-hybridization of normal DNA to generate results but instead uses stored data from reference DNA samples to scale sample fluorescence intensities to a normalized copy number value. Bioinformatic algorithms are used to computationally define regions of copy number loss and gain. In addition, genotype analysis identifies SNP loci as either A, B, or AB (based on hybridization results with probes for allele A, allele B, or both, respectively). For each SNP, comparison with reference DNA profiles will reveal if there is heterozygosity, homozygosity, or LOH at the interrogated site. The results of all individual calls along the chromosome are statistically analyzed, delineating genomic segments showing LOH. SNP-A, like A-CGH, provides a high-resolution technique for detecting unbalanced chromosomal defects such as deletions or gains of chromosomal material undetectable by routine metaphase cytogenetic or FISH techniques. As indicated, a major advantage of SNP-A over A-CGH is its ability to detect CN-LOH (UPD). CN-LOH is established when an apparently homozygous constellation of SNP genotyping calls is observed in a genomic region with overall diploid copy number. Recent studies based on SNP-A technology have shown a high rate of acquired UPD in several hematologic malignancies. This process occurs in polycythemia vera, for example, in which a biallelic *JAK2* V617F gene mutation is produced by an acquired chromosome 9p CN-LOH event; the recognition of this genomic abnormality enabled the initial discovery of the *JAK2* V617F mutation. The non-uniform distribution of SNP markers in human DNA and requirement for sufficient informative heterozygous loci limit the resolution of SNP-A, a drawback when compared to A-CGH, wherein probes can be placed more evenly and can even be targeted to specific regions of interest on individual chromosomes. More recent SNP-A platforms therefore also incorporate additional oligonucleotides to cover SNP-poor regions and increase the sensitivity for detecting CNV, combining the advantages of both approaches. Of note, neither A-CGH, nor SNP-A can resolve balanced chromosomal translocations or low-level mosaicism. The sensitivity of both A-CGH and SNP-A for detecting the presence of genomic abnormalities is variable but typically not better than approximately 20% to 30%, indicating that for somatic changes, normal cells admixed with tumor cells can compromise analysis.

DETECTION OF CLONAL VERSUS NON-CLONAL B-CELL AND T-CELL POPULATIONS IN LYMPHOID PROLIFERATIONS

The concept of "clonality" is central to the cellular definition of cancer and is microscopically represented by characteristic morphologic alterations or by the presence of recurrent or non-random karyotypic abnormalities in a tumor. Lymphoid neoplasms are unique in that the clonal nature of these proliferations can also be readily demonstrated by immunophenotypic or molecular genetic methods. In the primary diagnostic setting, the clonal status of an atypical B-cell

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lymphoid proliferation can usually be determined by deviations in the ratio of expressed surface or cytoplasmic light chains, as assessed by flow cytometry, immunohistochemistry, or RNA in situ hybridization. However, a number of distinct diagnostic situations require the application of molecular genetic clonality analysis. These include lymphoid processes with indeterminate immunophenotypic features for clonality, many T-cell proliferations, or suspected neoplastic lymphoid expansions that pose difficulty for routine immunophenotypic investigation (e.g., small tissue biopsies).

The presence of unique immunoglobulin or T-cell antigen receptor proteins on the surface of mature lymphocytes is a product of the somatic gene rearrangement process occurring early in the development of B and T cells in the bone marrow and thymus, respectively. The antigen receptor genes consist collectively of many variable (V), diversity (D), joining (J), and constant (C) gene segments, each separated by large intervening regions of non-coding DNA. D-segment regions are integral to the immunoglobulin heavy chain (*IGH*) and T-cell receptor beta (*TRB*) loci but are not present in the immunoglobulin light chain (*IGK*, *IGL*) or T-cell receptor gamma (*TRG*) genes. In developing lymphoid cells, site-specific rearrangements bring together V, (D), J, and C gene segments across many kilobases of DNA in the antigen receptor gene loci, by a process of coordinated double-strand DNA breakage and recombination mediated by the recombinase activating gene (RAG) system and a variety of DNA repair enzymes. The resultant relatively compact V(D)JC coding "cassettes" consist of rearranged DNA sequences that are distinct in both size and sequence characteristics in each lymphocyte. The remarkable quantity of unique antigen receptor proteins produced by human lymphocytes is thus initially derived from the combinatorial pool of only a few hundreds of individual V, D, J, and C gene segments. The addition of a variable number of non-templated ("n") nucleotides to the junctional ends of the V and D gene segments (i.e., VnDnJ) by the enzyme terminal deoxynucleotidyl transferase (TdT) during the rearrangement process further dramatically increases sequence diversity (Fig. e24.3). As such, every B or T lymphocyte in a reactive lymphoid population harbors an individual receptor "fingerprint" in both DNA and peptide composition, which in sum comprise a polyclonal population. The expression of heterodimeric antigen receptors on the surface of individual B and T cells (i.e., heavy/light chains on B cells and alpha/beta or gamma/delta chains on T cells) ensures a tremendous range of potential antigen recognition and binding affinities. Although evidently a large spectrum of individual gene rearrangement sequences arise from these recombination events, the coding region segments of the many possible V(D)J recombinations can be reliably

FIG. e24.3

Detection of clonality in lymphoid populations by polymerase chain reaction analysis of antigen receptor gene rearrangements. Panel A schematically demonstrates the basic process of somatic rearrangement of the immunoglobulin heavy chain *(IGH)* locus at chromosome 14(q32). Selection and rearrangement of *IGH* individual variable region (V_H), diversity (D_H) and joining (J_H) exon segments is initiated over a large span of DNA to form a relatively compact VDJ coding unit. The substantial combinatorial diversity of many possible V, D, and J segments are further increased by terminal deoxynucleotidyl transferase (TdT), which inserts a random number of "non-templated" (n) nucleotides at the exon segment junction sites. The constant region (C_H) segment completes the heavy chain gene-coding unit and confers effector specificity to the antibody molecule. It should be noted that each B cell has two *IGH* alleles (as well as two of each of the light chain genes); genomic rearrangements proceed at the second allele, if the first is unsuccessful for protein production. Following a functional *IGH* rearrangement, similar recombination events occur at the immunoglobulin light chain genes (i.e., *IGK* and *IGL*). The T-cell receptor gamma *(TRG)* gene *(panel B)*, located at chromosome 7(p15), is less complex, with only 11 Vγ segments capable of functional rearrangements and no D segments, although there are two J-segment loci (Jp and Jγ) and two constant regions. The T-cell receptor beta locus *(TRB)*, although not shown in the diagram, is highly complex but demonstrates the same structural rearrangement features as *IGH*, *TRG*, and the light chain genes. Although antigen receptor gene rearrangements are unique in any individual B or T cell, regions of nucleic acids in many V- and J-region exon segments show sequence homology, allowing the use of "consensus" oligonucleotide primers that flank the rearrangement junction (i.e., VDJ, or VJ) sites. Thus, gene rearrangements occurring at immunoglobulin and T-cell receptor gene loci in a lymphoid proliferation can be amplified and detected by PCR techniques. This approach is depicted by small *orange arrows* in panels A and B. A limited set of PCR primers directed to consensus sites in the *IGH* framework regions (FR1, FR2, FR3) and a reverse J-exon primer can effectively amplify most rearrangements in B cells by specifically targeting the highly diverse "hypervariable" complementary determining region 3 (CDR3). For *TRG*, a set of Vγ primers in combination with two J-region consensus primers can amplify the majority of gene rearrangements in T cells. By also combining multiple primer PCR strategies for *IGK* and *TRB* loci (not shown), extensive coverage of nearly all possible antigen receptor gene rearrangements can be achieved in lymphoid cell populations. Panel C shows typical data from *IGH* analysis using fluorescently tagged PCR primers and capillary electrophoresis readout (y-axis: relative PCR product fluorescent intensity; x-axis: fragment size in base pairs (bp); small red peaks: size standard markers). The *left image* displays a polyclonal pattern of amplicon fragment lengths producing a normal (bell-shaped) distribution over a defined size range. In contrast, the *right image* shows a single peak population characteristic of a monoclonal B-cell population. In some instances, two monoclonal peaks can be observed, indicative of biallelic clonal rearrangements. Panels D through F illustrate sample T-cell PCR results (*dotted orange lines* indicate expected amplicon size distribution ranges for each primer set amplification). Panel D is an example of polyclonal rearrangements detected at the *TRG* locus with two multiplex primer sets. Panel E demonstrates *TRB* PCR data using three primer cocktails, with two distinct monoclonal peak populations identified *(upper and lower tracings)*. Panel F shows a prominent peak population in a polyclonal T-cell background detected by *TRB* PCR *(arrow, lower tracing)*. Note the polyclonal distributions with the other two primer sets (the upper electropherogram shows an additional peak in shaded area marked by "X," falling outside the expected size range and is owing to non-specific DNA amplification). The situation shown in panel F leads to difficulties in interpretation since the preferentially amplified atypical *TRB* peak may represent the presence of a true monoclonal subpopulation, or a skewed benign population distribution. Such results are not infrequent with T-cell PCR studies and are designated "equivocal" or "not definitive" for a clonal T-cell population; careful clinical and pathologic correlation is needed to avoid overinterpretation. Panel G depicts the most recent iteration of B- and T-cell receptor clonality determination using next-generation sequencing using the Lymphotrack product (Invivoscribe, San Diego, CA) with sequencing performed on the Illumina MiSeq. The left image illustrates a polyclonal B-cell distribution of *IGH* gene rearrangements, whereas the right image shows a single species consistent with a monoclonal *IGH* rearrangement in a B-cell neoplasm. Each colored bar represents a unique sequence of *IGH* rearrangement in the B-cell population, and the vertical size of each bar indicates the relative proportion of that sequence in the total population; the actual sequence data can also be directly displayed as a tabulated rank result. The number of total sequences generated typically exceeds 10^5 per sample. The y-axis in each image indicates the relative percentage of the detected *IGH* rearrangement sequences; in the polyclonal example, the overall proportion of any individual sequence is very small in contrast to the prominent proportion of monoclonal sequence present in the B-cell neoplasm.

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detected by relatively simple PCR techniques using "consensus" PCR primers targeted to relatively conserved foci of DNA sequences within related "families" of V and J segments that flank the rearrangement sites. In this manner, a polyclonal B- or T-cell population containing many unique immunoglobulin or T-cell receptor gene rearrangements can be visualized following PCR as a normal (Gaussian) type distribution of amplified fragments within a defined size range bounded by the relative V(D)J lengths and flanking primers. Conversely, a monoclonal lymphoid proliferation, arising from a single clonal cell proliferation, produces only one or possibly two single size fragments, the latter situation reflecting the occurrence of bi-allelic rearrangements of the particular antigen receptor gene analyzed. These concepts are illustrated in Fig. e24.3.

The immunoglobulin heavy chain gene (*IGH*) located on chromosome 14(q32) and the T-cell receptor gamma gene (*TRG*) on chromosome 7(p15) are the most commonly targeted antigen receptor loci for B- and T-cell clonality assessment by PCR methods. The *IGH* gene consists of 70 to 80 functional V_H-region exons, 20 to 30 D_H-segments, and 6 J_H-segments. Most V_H-segments are characterized by relatively conserved nucleotide sequences encoding three framework (FR) or structural peptide regions of the antibody molecule, which in turn are separated by more diverse intervening sequences encoding three complementarity determining regions (CDR) responsible for specific antigen binding (epitope recognition). Similar to V_H framework regions, nearly all J_H exons also share partial sequence homology. The V_H-FR and J_H gene segments thus constitute templates for binding by consensus PCR primers (see Fig. e24.3). Notably, although these primers are not specific for any particular *IGH* gene rearrangement, this strategy produces short (100–400 base pair) amplicons that include the hypervariable third CDR (CDR3), which contains the greatest sequence and fragment length diversity and gives rise to the normal distribution of PCR products in a polyclonal B-cell process (see Fig. e24.3). The *TRG* locus is by comparison less complex, containing 11 V-region exons (grouped as 4 families) and 5 J-region segments; nonetheless, sequence and fragment length differences remain significant enough (owing to the segmental recombination process and TdT activity) to create a practically wide distribution of rearrangements in polyclonal T-cell populations. Again, a limited set of consensus and V- and J-specific primers can be utilized to efficiently amplify *TRG* gene rearrangements by PCR (see Fig. e24.3). Following PCR amplification, post-PCR analysis of *IGH* or *TRG* products can be achieved by agarose or polyacrylamide gel electrophoresis; however, fluorescent capillary electrophoresis has now become routine in light of its superior resolution and precise fragment-sizing capabilities (see Fig. e24.3). One clear advantage of PCR methodology for antigen-receptor gene studies is the ability to use a variety of tissue sources, including fresh or frozen cells, extracted DNA, cells from glass slides, or paraffin-embedded samples.

Although PCR methods represent a powerful means of determining whether a lymphoid proliferation is monoclonal (i.e., neoplastic) or polyclonal (i.e., benign), the technique is associated with potentially serious errors affecting both clinical sensitivity and specificity. For example, consensus primer PCR methods cannot detect all possible V(D)J rearrangements in the antigen receptor genes. A related problem concerns the failed detection of *IGH* rearrangements in B cells that have experienced a germinal center environment. Germinal center (and post-germinal center) B cells often acquire additional mutations in the immunoglobulin genes resulting from the process of somatic hypermutation (SHM). SHM is mediated by the enzyme activation-induced cytidine deaminase (AID) and its activity increases the affinity of antibody interaction for cognate antigenic epitopes, but during this event, the introduced small genetic mutations may extend into conserved regions (i.e., FR) that are targets for consensus PCR primers. The situations of incomplete gene rearrangement coverage and SHM can lead to false-negative results for true monoclonal *IGH* rearrangements and a corresponding loss of clinical sensitivity, even if the analytic sensitivity of the method is acceptable. False-negative PCR results can be significantly diminished by the use of multiple primers directed to different conserved V_H framework regions or families, in combination with assessment of the *IGK* locus. Similarly, for analysis of T-cell receptor gene rearrangements, targeting both the *TRG* and *TRB* loci can increase the true positive detection rate for monoclonal T-cell populations. Conversely, PCR-based assays can also suffer from false positivity arising from sample contamination (e.g., carryover of tissue fragments or amplified products from an unrelated sample), underscoring the importance of proactively mitigating such events in the molecular diagnostic laboratory. For T-cell receptor PCR studies, another risk of potential false-positive interpretation can occur because of physiologic or iatrogenic circumstances. T-cell repertoire restriction is found in some clinical settings (e.g., autoimmune diseases, post transplantation) and with normal aging. Apparent clonal ("pseudoclonal") or oligoclonal T-cell populations can be identified by the PCR method in these settings, requiring careful correlation with clinical history and other laboratory or morphologic data in order to avoid over-calling such findings. The use of "peak height" criteria has also been proposed to improve confidence for interpreting clonotypic T-cell PCR results; however, considerable overlap between true monoclonal (neoplastic) and atypical reactive amplification patterns is often encountered in practice. Although PCR is a generally sensitive technical modality, the practical lower detection limit of a monoclonal lymphoid population in a tissue sample is nominally 1% to 5% using standard electrophoretic detection methods. However, this level of sensitivity can vary significantly depending on the particular clonal antigen receptor gene rearrangement and the amount of accompanying polyclonal lymphocytic background in a given case. For specialized indications, such as post-therapy MRD monitoring, higher PCR sensitivity has been achieved by designing patient clone-specific primer and oligonucleotide probe reagents, but this is a labor intensive and technically challenging method.

Although consensus PCR with capillary electrophoresis has been the mainstay of lymphoid clonality assessment

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for over two decades, the advent of NGS methodology has brought new opportunities and a profound increase in knowledge and diagnostic ability. Using a similar consensus PCR primer strategy, an amplicon library can be prepared with attached NGS adapters and sample-specific bar codes. Deep sequencing of these PCR products provides not only specific sequence identities of thousands of rearrangements within a lymphoid population but also enables quantitation of relative species abundance and direct determination of somatic mutation status (see Fig. e24.3). NGS applications for antigen receptor gene rearrangements are providing an unprecedented opportunity to refine epitope mapping, explore lymphocyte subsets, and better understand adaptive or cellular immune responses and repertoires. In major part, this has also been made possible by the development of high-quality antigen receptor gene-specific reference sequence database resources, such as the IMGT immunogenetics site (http://www.imgt.org) and IG-BLAST (https://www.ncbi.nlm.nih.gov/igblast). In hematopathology practice, NGS is creating a deeper and richer data set to redefine and explore the concept of "clonality" in lymphoid proliferations. The ability to generate millions of sequence reads from a single sample also provides a powerful advantage for NGS in the direct evaluation of MRD; because each gene rearrangement is unique at the DNA sequence level, an individual population can be identified and quantified in a large background of other sequences.

To complete the discussion on lymphoid molecular clonality evaluation, the merits and drawbacks of Southern blot hybridization should be considered. SBH can identify relatively large-scale rearrangements of single-copy genomic DNA at the antigen receptor gene loci, in contrast to the detection of amplified small rearranged V(D)J region nucleotide fragments by PCR technique. The nature of SBH technique and the ability to interrogate substantially larger regions of genomic DNA for pathologic alterations enables the identification of nearly all possible clonal immunoglobulin and T-cell receptor gene rearrangements in lymphoid tumors; this correspondingly lowers the risk of false-negative results, as long as the assay is performed within its analytic sensitivity range. The *IGH*, *IGK*, and *TRB* loci are the most informative antigen receptor genes for SBH clonality assessment. SBH has become less often employed in the routine molecular diagnostic investigation of the lymphomas and lymphoid leukemias for reasons stated previously. In addition, SBH has an analytic sensitivity of optimally 5% to 10% for the detection of monoclonal B- or T-cell populations, typically inferior to that of standard PCR assays. The more refined development of multi-primer and multi-locus antigen receptor gene PCR approaches, as advocated by the BIOMED-2 consortium and related studies, has largely led to a marked decrease in the routine application of SBH, although some diagnostic laboratories retain the capacity to perform this analysis to occasionally complement PCR-based clonality methods.

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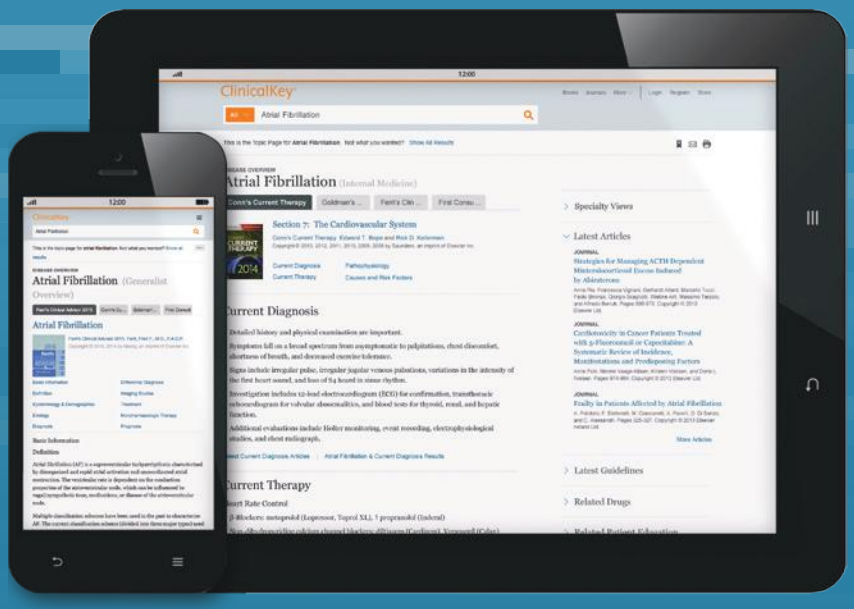
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