Reviews and critical articles covering the entire field of normal anatomy (cytology, histology, cyto- and histochemistry, electron microscopy, macroscopy, experimental morphology and embryology and comparative anatomy) are published in Advances in Anatomy, Embryology and Cell Biology. Papers dealing with anthropology and clinical morphology that aim to encourage cooperation between anatomy and related disciplines will also be accepted. Papers are normally commissioned. Original papers and communications may be submitted and will be considered for publication provided they meet the requirements of a review article and thus fit into the scope of "Advances". English language is preferred, but in exceptional cases French or German papers will be accepted.

It is a fundamental condition that submitted manuscripts have not been and will not simultaneously be submitted or published elsewhere. With the acceptance of a manuscript for publication, the publisher acquires full and exclusive copyright for all languages and countries.

Twenty-five copies of each paper are supplied free of charge.

Advances in Anatomy Embryology and Cell Biology

Vol. 161

Springer-Verlag Berlin Heidelberg GmbH

Z. Kmieć

Cooperation of Liver Cells in Health and Disease

With 16 Figures and 18 Tables

Springer

Zbigniew Kmieć
Medical University of Gdansk
Department of Histology and Immunology
80211 Gdansk, Poland

e-mail: zkmiec@amg.gda.pl

ISSN 0301-5556
ISBN 978-3-540-41887-0

Library of Congress-Cataloging-in-Publication-Data
Kmiec, Z. (Zbigniew), 1952- Cooperation of liver cells in health and disease / Z. Kmiec. p.; cm. — (Advances in anatomy, embryology and cell biology ; vol. 161) includes bibliographical references and index.
ISBN 978-3-540-41887-0 ISBN 978-3-642-56553-3 (eBook)
DOI 10.1007/978-3-642-56553-3
1. Liver cells. 2. Liver—Physiology. 3.Liver—Pathophysiology. I. Title. II. Advances in anatomy, embryology, and cell biology ; v. 161. [DNLM: 1. Hepatocytes—physiology ; 2. Liver—cytology.3.Liver Diseases—physiopathology. WI 700 K66c 2001] QP185 .K556 2001
571 s—dc21 [612.3'5]

http://www.springer.de

Originally published by Springer-Verlag Berlin Heidelberg New York in 2001

Production: PRO EDIT GmbH, 69126 Heidelberg, Germany
Printed on acid-free paper – SPIN: 10834647 27/3136wg - 5 4 3 2 1 0

Acknowledgements

The author is grateful to Prof. Theodor H. Schiebler (Würzburg, Germany) for continuous support, encouragement and critical reading of the manuscript. The helpful comments of Prof. Reinhard Hildebrand (Münster, Germany), Prof. Hartmut Jaeschke (Little Rock, Arkansas, USA), and Dr. Percy A. Knolle (Heidelberg, Germany) are gratefully acknowledged. The author thanks Prof. Bård Smedsrod (Tromso, Norway), Prof. Kenjiro Wake (Tokyo, Japan), and Dr. Kenji Kaneda (Osaka, Japan) for their generous provision of nonparenchymal liver cell photographs.

The work of many authors that could not be cited due to space constraints is also highly acknowledged.

June 2001 ZBIGNIEW KMIEĆ

Contents

Abbreviations

α_2-M	α_2-macroglobulin
ALS	acid-labile subunit
ANP	atrial natriuretic peptide
AP-1	activator protein 1
APC	antigen presenting cell(s)
APP	acute phase protein(s)
ATP	adenosine triphosphate
bFGF	basic fibroblast growth factor
BAL	bioartificial liver
BMP-6	bone morphogenetic protein-6
C5a	C5 component of complement
cAMP	cyclic 3,5-adenosine monophosphate
CD	cluster of differentiation molecule
cGMP	cyclic 3,5-guanosine monophosphate
CINC	cytokine-induced neutrophil chemoattractant
COX	cyclooxygenase
CM	conditioned medium
CRBP I	cellular retinol-binding protein I
CRABP I	cellular retinoic acid-binding protein I
CTGF	connective tissue growth factor
CSF-1	colony stimulating factor
ECE-1	endothelin-converting enzyme
ECM	extracellular matrix
EGF	epidermal growth factor
ET-1	endothelin-1
FAP	fibroblast activation protein
FasL	Fas ligand
FcγR	receptor of the Fc part of immunoglobulin G
FGF	fibroblast growth factor
G6PDH	glucose-6-phosphate dehydrogenase
GM-GSF	granulocyte-macrophage colony-stimulating factor
HC	hepatocyte(s)
HETE	hydroxyeicostetraenoic acid
HGF	hepatocyte growth factor
HO	heme oxygenase
HSC	hepatic stellate cell(s)
ICAM-1	intercellular adhesion molecule-1 (CD54)

IFN	interferon
IGF-I, -II	insulin-like growth factor I, -II
IGFBP	insulin-like growth factor binding protein
IHL	intrahepatic lymphocytes
IL	interleukin
$InsP_3$	inositol-1,2,5-triphosphate
KC	Kupffer cell(s)
LAL	liver-associated lymphocytes
LAP	latency-associated peptide
LO	lipoxygenase
LPS	lipopolysaccharide (endotoxin)
LRAT	lecithin:retinol acyltransferase
LT	leukotriene(s)
LTBP	latent TGF-β1 binding protein
MAP kinase	mitogen-activated protein kinase
M-CSF	macrophage colony-stimulating factor
MCP-1	monocyte chemotactic protein-1
MHC	major histocompatibility complex
MIP	macrophage inflammatory protein
MMP	matrix metalloproteinase
MT1-MMP	membrane-type 1 matrix metalloproteinase
NFκB	transcription factor nuclear factor κB
NGF	nerve growth factor
NK cells	natural killer cells
NOS	nitric oxide synthase
PAF	platelet-activating factor
PAI	plasminogen activator inhibitor
PBL	peripheral blood lymphocytes
PDGF	platelet-derived growth factor
PECAM	platelet-endothelial cell adhesion molecule
PG	prostaglandin(s)
PI-3 kinase	phosphoinositide 3-kinase
PMN	polymorphonuclear granulocyte (neutrophil)
PPAR	peroxisome proliferator-activated receptor
RA	retinoic acid
RAR	retinoic acid receptor
RBP	retinol-binding protein
RDH	retinal dehydrogenase
REH	retinyl ester hydrolase
ROS	reactive oxygen species
rMF	rat myofibroblast(s)
RT-PCR	reverse transcription-polymerase chain reaction
RXR	retinoic X receptor
SEC	sinusoidal endothelial cell(s)
SPARC	secreted protein acidic and rich in cysteine
TCR	T-cell receptor
TGF-α	transforming growth factor α
TGF-β	transforming growth factor β

1 Introduction – Morphology of the Liver Lobule

The liver plays a unique role as a metabolic center of the body, and also performs other important functions (Table 1). The macroscopic and microscopic structure of the mammalian liver has been recognized relatively early. In 1833, Kiernan proposed that lobes of the pig liver organized around main branches of the portal vein were built up of small polyhedron morphological units of parenchyma, called lobules, with boundaries made of connective tissue (Kiernan 1833). This classical liver lobule is characterized by the presence of a central vein (terminal hepatic vein) located approximately in the middle of the unit, and of areas of connective tissue at its corners, called portal tracts, that contain interlobular branches of hepatic artery and portal vein, biliary ductules, lymphatic vessels and nerves. Portal tracts are bridged by narrow stripes of connective tissue which accompany terminal afferent arterial and venous branches running between the lobules to supply the sinusoids that lead the blood into a central vein draining the lobule. However, in man and rodents, classical lobules cannot be easily recognized because only a sparse amount of connective tissue septa lies between portal tracts.

In 1954, Rappaport and co-workers proposed that the functional unit of the liver parenchyma may be represented by the smallest hepatic microcirculatory unit called simple acinus. Acinus contains the area of liver parenchyma supplied by terminal afferent blood vessels, portal venule and hepatic arteriole, present in the connective tissue septa between adjacent classical lobules (Rappaport et al. 1954; Rappaport 1976). Terminal afferent vessels, together with bile ductules, form the axis of the acinus, from which it is supplied with blood. Following the bloodstream, at least two different zones can be discerned in the acinus: the periportal zone perfused with blood rich in oxygen, substrates and hormones, and the perivenous zone that received blood with low oxygen content; moreover, a transitional, intermediate zone had also been described (Rappaport 1976). At the periphery of the acinus, the terminal hepatic venule (central vein of the classical lobule) drains blood from sinusoids belonging to several adjacent acini. The concept of liver acinus as the basic morphological liver unit seemed to explain many pathological lesions of the liver and apparent hepatocyte heterogeneity that can be demonstrated in the mammalian liver by the use of histochemical, microchemical, or immunohistochemical techniques (e.g., Novikoff 1959; Teutsch 1981). However, data accumulated over the past 20 years have shown that the idea of a simple acinus cannot be correctly applied to the human or rat liver, which have a different vascular supply of hepatic lobules than pig or dog liver (Matsumoto and Kawakami 1982; Teutsch et al. 1999), and show distribution of some enzymatic activities that contradicts the acinus concept (Hildebrand et al. 1986; Teutsch 1988).

Table 1. Main functions of the liver (modified from Jungermann 1995)

1.	Service functions for non-hepatic organs
1.1	Center of metabolism
	Energy supply of the organism
	Glucose uptake and release
	Amino acids uptake and release
	Urea formation
	Lipid processing and synthesis
	Ketone bodies synthesis
	Biosynthesis and biodegradation
	Plasma protein synthesis and degradation
	Bile formation (excretion of endobiotics and xenobiotics)
1.2	Center of defense
	Xenobiotic metabolism, scavenging of reactive oxygen species
	Phagocytosis
	Uptake and destruction of bacteria, viruses, parasites, particulate material, and macromolecules
	Elimination of tumor cells
	Acute phase reaction
1.3	Control station of the hormonal system
	Inactivation and elimination of hormones and mediators
	Synthesis and release of (pro)hormones
	Synthesis of hormone-binding proteins
1.4	Blood reservoir: passive and active blood storage
1.5	Involvement in the regulation of blood pH
1.6	Hematopoietic function in fetal and probably postnatal life
1.7	Immunoregulatory functions
	Antigen presentation to lymphocytes
	Clearance of apoptotic lymphocytes
	Tolerance induction towards oral antigens
1.8.	Formation and maintenance of organ structure
	Synthesis and degradation of extracellular matrix components

The understanding of many specific functions of the liver, initially forwarded by biochemical studies, has greatly expanded during the last 30 years by the introduction of the effective methods of the isolation of parenchymal liver cells, i.e., hepatocytes

(Berry and Friend 1969; Seglen 1976), that allowed culturing of these cells for many days. Although the maintenance of all specific functions of parenchymal cells in vitro was difficult to obtain, primary cultures of hepatocytes had been found to provide vast amounts of information about factors that control different liver functions.

The advent of methods for the successful isolation and culture of nonparenchymal liver cells (Eyhorn et al. 1988; Friedman and Roll 1987; Knook and Sleyster 1977; Knook et al. 1982; Smedsrod et al. 1985) has enabled detailed characterization of their structure and functions, which was not possible earlier in biochemical studies of liver homogenates. The use of the co-culture system of hepatocytes and nonparenchymal cells, or co-cultures of different types of nonparenchymal liver cells, has provided evidence for the existence of intercellular communication mediated mainly in the paracrine way via the release of various mediators. This way of intercellular cooperation between different cells that build the liver lobule is becoming increasingly recognized as an important mechanism for the control of diverse liver functions. The aim of this monograph is to present the current understanding of the signalling networks between nonparenchymal liver cells and hepatocytes that maintain homeostasis both in normal liver and in some pathological conditions. The short description of the hepatic sinusoids, and a more detailed presentation of the structural and functional features of hepatocytes and nonparenchymal liver cells, will be first presented. Because the scope of this review is limited to the cross-talk of various cells that build classical liver lobule, the participation of other liver cells, such as biliary epithelial cells, dendritic cells, fibroblasts, mast cells, cells building the extrasinusoidal vessels, and nerves, in the regulation of liver functions will be omitted.

The liver tissue is organized at the microscopic level in lobules that consist of muralium made of anatomizing plates one cell thick, which extend from the portal tract in linear fashion to the central vein, and transmural spaces that contain tortuous hepatic sinusoids that are separated from hepatocytes by the perisinusoidal space of Disse (Fig. 1). Hepatic sinusoids, of a mean diameter of 5–7 μm (MacPhee et al. 1995), lead the mixed blood of the terminal branches of hepatic artery and portal vein to the terminal branch of the hepatic vein, i.e., central vein. The sinusoid represents a unique form of capillary characterized by continuous, but fenestrated endothelial lining, the presence of hepatic macrophages, i.e., Kupffer cells (KC), inside the lumen or as a part of sinusoid lining, the lack of genuine basement lamina, and the presence of perisinusoidal space (Disse's space) at its external aspect. There are no larger gaps between sinusoidal endothelial cells (SEC), and the only communication between the space of Disse and sinusoids is through the fenestrations of SEC. The absence of basement lamina facilitates the rapid exchange of acellular components of the blood with hepatocytes. Kupffer cells, which usually lie over the endothelium, are attached to the latter with cytoplasmic processes that sometimes pass through the fenestrations of endothelial cells extending into the perisinusoidal space. As seen by scanning electron microscopy, the perisinusoidal space of Disse appears as a continuous three-dimensional labyrinth of intercellular and pericapillary microlacunae, in which hepatocytes are suspended (Motta 1984). The perisinusoidal space contains many cytoplasmic dendritic projections and cell body of hepatic stellate cells (HSC; synonyms: fat-storing cells, Ito cells, lipocytes, perisinusoidal cells, vitamin A-rich cells), abundant microvilli of hepatocytes, nerve endings, and a non-electron dense complex extracellular matrix (ECM). The ECM of the perisinusoidal space contains (Rojkind and Greenwel 1994) few both collagen type I and procollagen type III striated collagen fibrils,

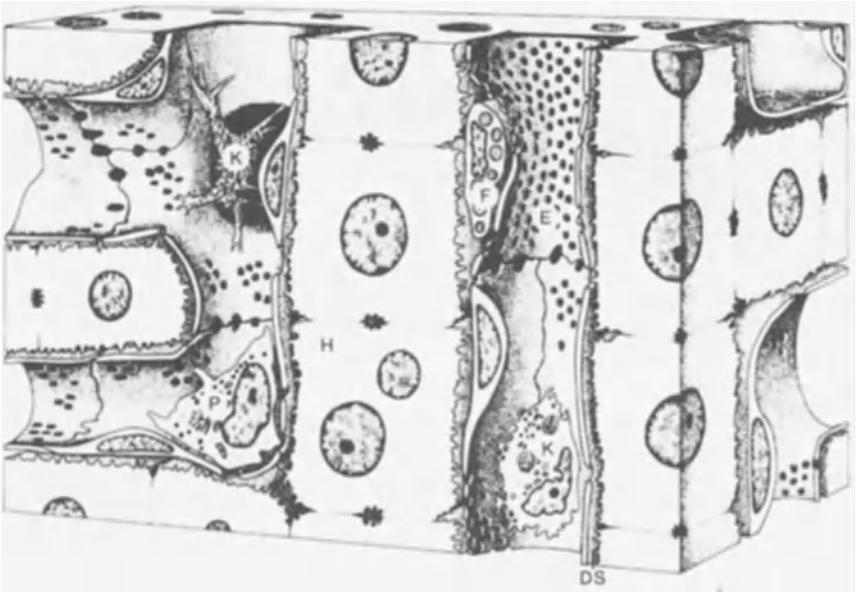

Fig. 1. Schematic drawing of liver tissue. The parenchyma is built up of trabecular network of cell plates made of hepatocytes (*H*) that have di- or polyploid nuclei, some cells are binucleated. The wall of sinusoid (*S*), formed by fenestrated endothelial cells (*E*) and Kupffer cells (*K*), does not show basal membrane; however, stellate cells (*F*) are present in the perisinusoidal space of Disse (*SD*). In some places Kupffer and pit cells (*P*) bulge into the sinusoidal lumen. Bile canaliculi (*BC*) are located on the lateral surfaces of adjoining hepatocytes. (Reproduced with permission from Sasse et al. 1992)

deposits of nonfibrillar collagen types IV, VI, XIV, XVIII (Musso et al. 1998), components of the basal membrane (collagen type IV, glycoprotein laminin, proteoglycan perlecan), as well as other macromolecules such as glycoproteins (fibronectin, tenascin, undulin, entactin), proteoglycans (heparan, dermatan and chondroitin sulphates, hyaluronic acid, syndecan, biglycan and decorin; Gallai et al. 1996), and osteonectin, also known as SPARC (Frizell et al. 1995). Besides SEC and Kupffer cells, liver sinusoids also harbor resident dendritic cells (Sato et al. 1998; Drakes et al. 2000), and a heterogeneous population of intrahepatic lymphocytes (IHL), which contains the subpopulation of pit cells representing liver-specific natural killer (NK) cells (Wisse et al. 1976; Kaneda and Wake 1983).

Bile canaliculi, made up of longitudinal plasma membrane invaginations of two adjacent hepatocytes, continue to the periphery of the lobule until they reach canalicular-ductular junctions, previously known as canals of Hering (Motta 1984). The walls of these terminal canaliculi are formed both by hepatocytes and by a small number of flat epithelial cells that are closely associated with the surrounding hepatocytes. On leaving the lobule, Hering canals pass over into bile ductules; however, frequently bile canaliculi open directly into the large portal bile ducts (Motta 1984). Both parasympathetic and sympathetic nerves, which influence hepatocyte metabo-

Table 2. Composition of rat liver (from Blouin et al. 1977)

	Volume	Relative number of cells
Extracellular space compartment		
Sinusoidal lumen	10.6%	
Disse space	4.9%	
Biliary canaliculi	0.4%	
Relative part of cells in total liver volume		
Hepatocytes	78.0%	60%
Non-hepatocytes	6.3%	
Sinusoidal endothelial cells	2.8%	19%
Kupffer cells	2.1%	15%
Stellate cells	1.4%	6%

lism and blood flow (Jungermann and Stumpel 1999), emanate from portal tracts to the centrolobular spaces being located mainly in the space of Disse close to stellate cells, hepatocytes, and sinusoidal endothelial cells (Bioulac-Sage et al. 1990; Tiniakos et al. 1996; Ueno and Tanikawa 1997).

The results of detailed stereological morphometric investigations, which disclosed relative volumes of different cellular and intercellular compartments in the rat liver, reflect the importance of individual tissue components (Table 2). Lobular parenchyma, i.e., hepatocytes, sinusoids, Disse space, and billiary canaliculi, makes up 96% of rat liver volume; the remaining 4% include portal triads, and hepatic and central veins (Weibel et al. 1969). The bulk of liver parenchyma is made up of hepatocytes that occupy more than ten times the volume of non-hepatocytes; however, the number of hepatocytes is only by half bigger than that of nonparenchymal liver cells (Table 2). Despite small contribution to the total volume of the liver, nonparenchymal liver cells contribute (Blouin et al. 1977) 26.5% to the total cell membrane surface (mainly endothelial cells), 58% to the volume of pinocytotic vesicles (mainly endothelial cells), 43% to the total lysosomal volume (mainly Kupffer and endothelial cells), and 55% to the total fat droplet volume (mainly stellate cells). Besides endocytic and phagocytic functions of endothelial and Kupffer cells, and storage of vitamin A in stellate cells, apparent from morphometric investigations of the liver, nonparenchymal liver cells have many other functions that will be characterized in following chapters.

It has only been recently realized that many functions of the liver depend on the cooperation of nonparenchymal and parenchymal liver cells. The communication between cells of the liver lobule takes place via three basic mechanisms: (1) gap junctional, (2) paracrine (through the release of mediators that act on target cells localized in close proximity to the secreting cell), and (3) juxtacrine (similar to

paracrine communication but requires close apposition of cell membranes, e.g., between leukocytes and endothelial cells).

Only the two latter phenomena, i.e., paracrine and juxtacrine cellular interactions, which result in the multiplicity of signalling networks within the liver lobule, have been chosen as subjects of this review. Gap junctions allow direct transfer of small molecules between neighboring cells via intercellular channels composed of two hemichannels, each of which is formed by the two adjacent cells (Spray et al. 1994). Gap junctions function as low-resistance intercellular pathways that connect cytoplasms of neighboring cells and play an important role in the exchange of ions and nucleotides, propagation of calcium waves (Dupont et al. 2000; Tordjmann et al. 1997), and adrenergic stimulation between parenchymal cells of the liver lobule (Saez 1997; Seseke et al. 1992; Stumpel et al. 1998), and also in bile secretion (Nathanson et al. 1999). Because gap junctional communication involves only one type of liver cells (hepatocytes), its description is beyond the scope of this monograph. Endocytic and phagocytic properties of sinusoidal endothelial cells and Kupffer cells, which enable intracellular processing, secretion and uptake of the ligands processed further by neighboring hepatocytes (e.g., lipoproteins or hemoglobin), will also not be described in detail, since they are not specific for the liver cells, but rather reflect common features of endothelial cells and macrophages.

Part I
Cells of the Liver Lobule

2 Hepatocytes

Parenchymal liver cells, i.e., hepatocytes, constitute the major cellular compartment of the liver and may be regarded as key hepatic effector cells since the majority of liver functions could be attributed to their activity. Hepatocytes are also the main target of liver-damaging agents (biological and chemical), and their injury leads to acute or chronic liver disease. The aim of this monograph is to show that the activities and interactions of nonparenchymal liver cells with hepatocytes and non-hepatocytes constitute important factors that may regulate many aspects of liver physiology and pathology. Therefore, and because of space constraints, only basic information about the structure and function of hepatocytes will be presented. For in-depth reviews about the role of liver parenchymal cells in health and disease, the reader should consult textbooks of hepatology and specialized monographs.

2.1 Morphology of Parenchymal Liver Cells

The hepatocyte is a polyhedral multifaceted cell with eight or more surfaces. Its diameter varies between 25–30 μm in length, and 20–25 μm in width (Motta 1984). Several structural surface components participate in two or three major domains of the hepatocyte: (a) the basolateral surface (perisinusoidal and pericellular), which is highly enlarged through the presence of many short microvilli; (b) the straight or contiguous domain between adjacent cells; and (c) the bile canalicular domain.

The structure and function of hepatocyte organelles are similar to that of other cells; however, parenchymal liver cells are endowed with vast numbers of organelles. Stereological measurements of rat liver revealed that the single mononuclear rat hepatocyte has an average volume of 5,000 μm^3 (Loud 1968; Weibel et al. 1969). The hepatocyte cytoplasm occupies slightly more than 50%, the nucleus 7%, mitochondria 22% (ca. 1,650 per cell), smooth endoplasmic reticulum and Golgi apparatus 12%, rough endoplasmic reticulum, which contains approximately 12 million attached ribosomes, 6% of the cell volume; the amount and volume of lysosomal compartment is highly variable, there are also about 1,000 peroxisomes in one hepatocyte (Blouin et al. 1977; Loud 1968; Weibel et al. 1969). The volume of glycogen granules depends on the nutritional status of an animal. Many hepatocytes of the mammalian liver may be binucleated or polyploid. There are some differences in hepatocyte ultrastructure that are related to the location of the cell in the liver lobule, e.g., greater number and dimensions of mitochondria in periportal hepatocytes as compared with perivenous hepatocytes are assumed to reflect their higher metabolic activity (Blouin et al. 1977).

2.2
Functions of Hepatocytes

Parenchymal liver cells participate almost in all functions that have been attributed to the liver (Table 1). They confer to the liver an important function of the body's glucostat by releasing glucose through the process of glycogenolysis, and producing glucose from noncarbohydrate substrates via the pathway of gluconeogenesis. Hepatocytes are the only cells that inactivate toxic ammonia in the urea cycle. Parenchymal liver cells synthesize the bulk of serum proteins, including components of the complement system and acute-phase proteins, important compounds of the innate immunological system. Hepatocytes play a substantial role in the metabolism of exogenous and endogenous lipids through the synthesis of many classes of lipoproteins and catabolism of blood-derived cholesterol-enriched proteins. Parenchymal liver cells are well equipped for the defense against oxidative stress, and are responsible for the detoxification of numerous endo- and exogenous substances. The production of bile components such as bile acids, cholesterol, phospholipids and conjugated bilirubin, takes place also in hepatocytes. The reactivity of parenchymal cells of the liver towards the multiplicity of hormonal, paracrine, and autocrine signals is of utmost importance for the control of liver functions. The liver represents probably the most intensively studied organ of the body in respect to the mechanisms of hormonal signal transduction, and plasma membrane and intracellular hormonal receptors have been identified in hepatocytes for many hormones and other signalling molecules. Moreover, hepatocytes synthesize and release some (pro)hormones, and many intercellular mediators into the circulation. Some of the substances synthesized in hepatocytes, which may play a role in the intercellular cross-talk inside the liver lobule, have been listed in Table 3.

Besides hormones and signalling molecules released from extrahepatic sources or by nonparenchymal liver cells, hepatocytes within one lobule respond to neurotransmitters released from both sympathetic and parasympathetic nerve endings located either directly on hepatocytes or in close proximity to the cells of liver sinusoids (Bioulac-Sage et al. 1990; Tiniakos et al. 1996; Ueno and Tanikawa 1997). In normal liver, parenchymal cells rarely divide; however, upon resection of a part of the liver, or in some forms of severe liver injury, hepatocytes proliferate rapidly to restore the organ mass. This phenomenon of hepatic regeneration is regulated not only by extracellular factors but also by many substances released from neighboring nonparenchymal liver cells that act on hepatocytes via paracrine interactions (Fausto 2000). Although parenchymal liver cells are mostly presented as targets of action of multiple mediators generated inside intra- and extrahepatic regulatory networks, hepatocytes secrete tens of biologically active substances that affect many cell types in the liver and throughout the body. Remarkably, the effects of mediators released by hepatocytes on nonparenchymal cells of the liver lobule have been relatively poorly characterized (see Table 11).

Table 3. Mediators released from hepatocytes that may affect nonparenchymal liver cells via paracrine interactions

	References
Cytokines	
EGF	Mullhaupt et al. 1994
IGF-I	Dong et al. 1998; Saile et al. 1995; Scott and Baxter 1986
Interleukin-6	Saad et al. 1995
Interleukin-8/CINC	Dong et al. 1998; Shiratori et al. 1994; Thornton et al. 1990
MCP-1	Dong et al. 1998
MIP-2	Dong et al. 1998
TNF-α	Gonzales-Amaro et al. 1994a; Saad et al. 1995
VEGF	Mochida et al. 1996; Yamane et al. 1994
Other mediators	
Nitric oxide	Geller et al. 1993; Saad et al. 1995
Carbon monoxide	Bauer et al. 1998b; Goda et al. 1998
Reactive oxygen species (ROS)	Bailey and Cunningham 1998
Cysteinyl leukotrienes (LTC_4 and LTD_4)	Fukai et al. 1996; Shimada et al. 1998; Titos et al. 2000
ATP	Schlosser et al. 1996

a Expressed in diseased human liver, and at low level in normal liver.

2.3 Heterogeneity of Hepatocytes and Nonparenchymal Liver Cells Inside the Liver Lobule

The structure of liver plates that build the liver lobule, homogenous by H&E staining, appears heterogenous by the use of histochemical techniques. The metabolic heterogeneity of hepatocytes may be explained through their distance from the terminal portal and arterial branches that determines their relation to the nutritional and oxygen gradients (Jungermann 1995). Parenchymal liver cells localized at the periphery of the classical liver lobule (i.e., in the periportal zone of Rappaport's simple acinus), which can be termed as periportal hepatocytes (Sasse et al. 1992), function in the microenvironment rich in oxygen, substrates, and hormones contained in the blood of terminal afferent vessels. In contrast, parenchymal cells located around the central vein (i.e., terminal hepatic venule of Rappaport's simple acinus), called perivenous hepatocytes (Sasse et al. 1992), contact the perfusate of blood partially depleted of oxygen and substrates, but enriched in CO_2, and other products of metabolism (Rappaport 1976). Thus, the enzymatic phenotype of a hepatocyte is to a great extent dependent on its location on the porto-central axis. Metabolic zonation ex-

pressed, however, to various degrees for different processes, was described for carbohydrate, oxidative, xenobiotic, amino acid and ammonia metabolism, cholesterol synthesis, and bile formation (Jungermann 1995). The development of methods for the isolation of periportal and perivenous hepatocyte subpopulations (Lindros and Pentilla 1985) has demonstrated the existence of the intralobular metabolic cooperation in relation to ammonia (Haussinger 1990) and glucose metabolism (Jungermann 1995). For example, it was found that periportal hepatocytes are predominantly gluconeogenic, but perivenous hepatocytes are glycolytic (Ikezawa et al. 1998; Quistorff 1985; Teutsch 1988).

The concept of functional zonation relates mainly to hepatocytes, although some zonal differences in the distribution and characteristics of nonparenchymal liver cells have also been observed (Jungerman and Kietzmann 1996). For example, it has been shown that Kupffer cells are more numerous around portal vein branches, and that these periportal cells are larger and more active than centrolobular liver macrophages (Kaneda and Wake 1983; Sleyster and Knook 1982). A gradient of mediators released from periportal liver macrophages into the circulation probably exists between perivenous cells (which would first come into contact with the mediators released into sinusoidal blood and quickly be washed out along the porto-central axis of the classical lobule) and periportal cells (both nonparenchymal and parenchymal), which would react later to much smaller (if any) amounts of mediators delivered through the systemic circulation (Jungermann and Kietzmann 1996). A zonal heterogeneity of the sinusoidal endothelial lining has been reported with regard to filtering (fenestrations are more numerous and larger in periportal SEC) capacity and binding of lectins (Wisse et al. 1999), distribution of plasma membrane proteins (Scozaec et al. 1994), and ability to endocytose apoptotic lymphocytes (Dini and Carla 1998). Hepatic stellate cells are more numerous in perivenous than periportal areas (Jezequel et al. 1984). Perivenular HSCS have longer cytoplasmic processes, contain less retinoid stores (Wake and Sato 1993), express less desmin (Ballardini et al. 1994) and fewer endothelin-1 receptors (Gondo et al. 1993) than periportal stellate cells. Similar to Kupffer cells, pit cells are also numerically predominant in the periportal region of the liver lobule (Kaneda and Wake 1983).

3 Sinusoidal Endothelial Cells

Endothelial cells of liver sinusoids (SEC) differ in many structural and functional aspects from other endothelial cells of the body. They do not have a regular basement membrane, and are often embraced by the cytoplasmic processes of underlying hepatic stellate cells. SEC constitute an important filtration barrier between macromolecules and blood cells present in the sinusoidal lumen and hepatocytes that prevents their direct contact, and determines the exchange of various substances. SEC possess a large pinocytotic and endocytic capacity due to the presence of numerous plasma membrane receptors. They also actively participate in the immunological functions of the liver.

3.1 Morphology

Liver sinusoidal endothelial cells form a continuous lining of the liver sinusoids, separating parenchymal cells and hepatic stellate cells from sinusoidal blood. These flat, elongated cells with a small cell body and numerous thin cytoplasmic processes form the lining of the sinusoidal wall (Fig. 2). The fenestrations, of an average diameter of 160 nm in rat liver (Wisse 1972), are distributed in clusters of 10–50 pores, referred to as sieve plates, and occupy 6%–8% of endothelial surface, which means that ca. 93% of the sinusoidal lining is represented by continuous endothelium. Fenestrae have a cytoskeletal ring consisting of actin, myosin, and actin-binding proteins (Wisse et al. 1999). Contraction of fenestrae has been observed in cultured cells in response to serotonin, nicotine, and other agonists; however, the mechanisms that regulate the number and diameter of fenestrae have not been well recognized (Fraser et al. 1995). The nuclei of SEC are relatively small, containing aggregates of heterochromatin and a prominent nucleolus (Wisse 1972). Besides the usual cell organelles, such as mitochondria, endoplasmic reticulum, microtubules, microfilaments, Golgi apparatus, and a few peroxisomes, there are abundant lysosomes, phagosomes, coated pits, micro- and macropinocytotic (diameter 700–1000 nm) vesicles (Wisse 1972), so that components of the lysosomal and endosomal system contain 12.4% of the cell volume (Blouin et al. 1977). The specialization of SEC in endocytosis is emphasized by the morphometric data, showing that SEC contribute 45% to the total hepatic volume of the pinocytotic vesicles although these cells occupy only about 3% of total liver volume; lysosomes contribute 17% to the total lysosomal volume of the liver (Blouin et al. 1977). In contrast to other endothelia, Weibel-Palade bodies could not be seen in situ in rat liver SEC (Wisse 1972); however, these structures were observed in cultures

of human (Harrison and Boudreau 1989) and rat (Hellman et al. 1989) SEC. Although liver sinusoidal endothelial cells lack a morphologically defined basement membrane, they secrete components of the basement membrane-like material to the space of Disse (see Table 5).

3.2 Cellular Functions

The main function of SEC is believed to be formation of a general barrier against pathogenic agents and they serve as a selective sieve for substances passing from the blood to parenchymal and stellate cells, and in the opposite direction. Blood cells that flow through the sinusoids are excluded from the entry into Disse space. The filtering effect is greatly enhanced by a huge transport and clearance capacity of SEC based on the receptor-mediated endocytosis and intercellular metabolism of a wide range of macromolecules, including glycoproteins, lipoproteins, extracellular matrix components, and inert colloids. Liver sinusoidal endothelial cells constitute an important component of the complex network of cellular interactions in the liver through the secretion of many substances and reactivity to many signals received form neighboring cells.

3.2.1 Filtration

The sieving function of SEC has a major effect on the hepatic uptake of particles or rigid droplets, such as lipoproteins, of which chylomicrons and their remnants are involved in the bulk transport of lipids. Chylomicrons up to the size of fenestrae are present in the space of Disse; however, larger chylomicrons carrying alimentary lipid must first be metabolized by the action of lipase, which is localized on the surface of endothelial cells (Sanan et al. 1997), to be able to pass through the fenestrae (Wisse et al. 1985). Through the dynamic changes of the fenestrae size, SEC are supposed to control the admission of cholesterol-enriched, vitamin A-containing chylomicron remnants to hepatocytes and stellate cells, and play an important role in controlling the distribution of lipids, cholesterol, and vitamin A between the liver and other organs (Bakkeren et al. 1990; Fraser et al. 1995).

3.2.2 Endocytosis, Pinocytosis, Phagocytosis, and Transcytosis

The ultrastructural features indicate that SEC possess a high pinocytotic and endocytic capacity for fluids, molecules, and small particles (Wisse 1972). Receptor-mediated endocytosis (Table 4) of circulating collagen, hyaluronic acid, fibronectin, laminin, nidogen (entactin), and chondroitin sulphate proteoglycan is a major physiological role of SEC (Smedsrod et al. 1994). Hyaluronic acid, a major extracellular matrix component, is rapidly removed by SEC from the circulation, so that its serum

Table 4. Receptors of sinusoidal endothelial cells

	References
1, Receptors for connective tissue components	
Hyaluronic acid	McCourt et al. 1999; Smedsrod et al. 1984
(Pro)collagen, collagen α-chain receptor	Smedsrod et al. 1990
Fibronectin	Johansson et al. 1987
2. Other receptors active in endocytosis and phagocytosis	
Scavenger receptor class A	Smedsrod et al. 1994
Scavenger receptor specific for LPS	van Oosten et al. 1998
Mannose/*N*-acetylglucosamine receptor	Magnusson and Berg 1989; Praaning et al. 1987
Fc-receptor for immune complexes	Johansson et al. 1996; Muro et al. 1987
CD14 (LPS-binding protein receptor)	Scoazec et al. 1994
Ceruloplasmin receptor	Omoto and Tavasoli 1990
Transferrin receptor	Tavasoli et al. 1986
3. Cytokines and growth factor receptors	
IGF-I, type 1	Zimmermann et al. 2000
Interferon-γ	Volpes et al. 1991
IL-1	Anasagasti et al. 1996
IL-6	Deaciuc et al. 1994a
PDGF-α and -β	Heldin et al. 1991
TGF-β1	Rieder et al. 1993
TNF-α	Deaciuc et al. 1995
Thrombopoietin	Cardier and Dempsey 1998
VEGF (Flt-1 and KDR/flk-1)	Mochida et al. 1996; Yamane et al. 1994
4. Prostanoid receptors	
PGD_2, PGE_2, PGI_2, TXA_2	Fennekohl et al. 1999
TXA_2	Ishiguro et al. 1994
5. Hormonal receptors	
ANP (guanylate cyclase)	Bilzer et al. 1999
Endothelin type A and B receptorsa	Diamantis et al. 1998
Endothelin, only type B receptorb	Housset et al. 1993
Estrogen	Vickers and Lucier 1996
Glucagon	Watanabe et al. 1988

Table 4 continued. Receptors of sinusoidal endothelial cells

Glucocorticoid	Raddatz 1996
Growth hormonea	Zimmermann et al. 2000
Lutropin	Fiete et al. 1992
P_1 and P_2 purinergic receptors	Takemura et al. 1994
6. Other receptors	
Complement C5a (anaphylotoxin)	Schlaf et al. 1999
Thrombin	Marra et al. 1998a

a Detected through the presence of receptor's mRNA by in situ RT-PCR.
b Demonstrated by ligand-binding assay and mRNA levels.

level is used as an indicator of sinusoidal endothelial cell function (Deaciuc et al. 1994b).

Scavenger receptors bind modified forms of low-density proteins (LDL), thus forming a protection system that may prevent uptake of modified LDL with subsequent foam cell forming by infiltrating macrophages (Krieger 1997). Scavenger receptor class A of the endothelial cell takes up a wide array of macromolecular waste molecules, a function reflected by the uptake of unphysiologically modified serum proteins with a net negative charge, such as acetylated low density lipoprotein (Smedsrod et al. 1994). It has been shown that advanced glycation end products, e.g., α_2-macroglobulin, were eliminated from blood through the scavenger receptor-mediated endocytosis both by hepatic endothelial and Kupffer cells (Smedsrod et al. 1997; van Dijk et al. 1992).

Rat liver endothelial cells have a greater capacity than Kupffer cells to endocytose *N*-acetylglucosamine- and mannose-terminated glycoproteins (Praaning et al. 1987). Besides many nonphysiological ligands (e.g., ovalbumin, mannan, invertase, horseradish peroxidase) and several lysosomal enzymes (that may enter the portal vein from the gut), which bind to the mannose receptor, tissue-type plasminogen activator and circulating C-terminal propeptide (of type I procollagen) were the first physiological ligands shown to bind in vivo to this receptor on liver endothelial cells (Smedsrod et al. 1994). Rat and human SEC recognize and internalize apoptotic peripheral blood lymphocytes through their binding to the mannose receptor (Dini and Carla 1998).

The Fc receptor of endothelial cells is involved in the clearance of soluble antigen-antibody complexes through the interaction with the Fc part of immunoglobulin G (Johansson et al. 1996, 2000; Kosugi et al. 1992). This feature enables SEC to store and metabolize serum immunoglobulins (Chroneos et al. 1995; Iwamura et al. 1995). The quantitative estimation of Kupffer cells and SEC immune-complexes binding capacity showed that liver endothelial cells may constitute an important reserve capacity in

addition to KC which are normally the most important cells in the elimination of circulating immune complexes (Bogers et al. 1992; Johansson et al. 2000).

Transcytosis comprises receptor-mediated endocytosis of some ligands and secretion on the other side of the epithelium. This mechanism has been demonstrated in SEC for the transport of ceruloplasmin (Irie and Tavasoli 1985), transferrin (Irie and Tavasoli 1989), and transcobalamin (Soda and Tavasoli 1985). During transcytosis these ligands are partially digested or desialylated, and transferred to parenchymal cells through the interaction with the galactose receptor on hepatocytes (Irie and Tavasoli 1989; Soda and Tavasoli 1989).

3.2.3 Immunological Function

Vast amounts of particulate and soluble material generated in the gut, such as food antigens and bacterial products, continuously enter the liver through the portal vein. Due to the presence of Kupffer cells, and sinusoidal endothelial cells that show prominent endocytic and phagocytic capabilities, the liver removes many of the foreign molecules both in nonspecific and specific ways. However, it has recently become clear that many exogenous antigens arriving in the liver may induce specific immunological reactions that lead to the development of immune tolerance rather than immune responses. Liver sinusoidal endothelial cells have the capacity to function as antigen-presenting cells (APC) as they constitutively express MHC class I and II (Rubinstein et al. 1986), CD4 (Scoazec and Feldman 1990), CD11 (Knolle et al. 1999), CD54 (ICAM-1; Essani et al. 1995), CD106 (VCAM-1; Wong 1997) molecules, and also costimulatory molecules CD80 (Lohse et al. 1996), CD40 and CD86 (Knolle et al. 1999), necessary for the presentation of antigens to T cells. In contrast to endothelial cells from other organs, SEC do not need prestimulation with proinflammatory cytokines (such as IFN-γ or TNF-α) to induce effective antigen presentation to CD4+ T cells (Lohse et al. 1996). Liver SEC can activate naive CD4+ T cells into IL-4/IL-10-expressing cells through the MHC-class II restricted antigen presentation; however, in contrast to conventional APC populations, they do not induce the differentiation of T cells into T_{h1} cells, but rather generate regulatory T cells that can be engaged in antigen-specific downregulation of immune responses (Knolle et al. 1999). Thus, the induction of cytokine production in activated T cells by SEC, acting as antigen-presenting cells, may serve to enhance nonspecific effector mechanisms such as endocytosis and phagocytosis; hepatocellular damage will be avoided as induction of effector T_{h1} will not occur. Moreover, it was shown that sinusoidal endothelial cells can efficiently present exogenous, soluble antigen on MHC class I molecules to CD8+ T cells, in a process of cross-presentation, with the resulting development of antigen-specific T cell tolerance (Limmer et al. 2000). The activation of T cells by sinusoidal endothelial cells was shown to be downregulated by IL-10, PGE_2, and TNF-α, mediators released from LPS-elicited Kupffer cells, demonstrating paracrine control mechanism for antigen presentation in SEC (Knolle and Gerken 2000).

Another role of SEC in the modulation of the immunological responses appears from the selective retention in the liver of activated, but neither rested nor apoptosing, CD8+ T cells, which occurs via mechanism mediated primarily by ICAM-1 (CD54) constitutively expressed on SEC and Kupffer cells (Mehal et al. 1999). T cells selectively

trapped in the liver became apoptotic (Mehal et al. 1999) in a process related to the expression of Fas ligand on Kupffer and sinusoidal endothelial cells, and the release of cytotoxic mediators from these cells (Muschen et al. 1998). In contrast to other organs, the adhesion of T cells to liver sinusoidal epithelium does not require the expression of selectins to induce leukocyte rolling but depends upon the expression of vascular adhesion protein 1 (McNab et al. 1996).

Thus, the normal liver may both destroy foreign or self antigens by nonspecific phagocytosis, induce immune tolerance towards other "nonharmful" antigens through the APC function of its endothelial cells, and may also play the role of a "sink" for activated T cells that traffic through the liver. The participation of pit cells and intrahepatic lymphocytes in the regulation of hepatic immune responses will be further described in this chapter.

3.2.4
Secretory Activity

Sinusoidal endothelial cells synthesize many substances that affect neighboring cells in a paracrine way, or, after release into the circulation, may influence distant cells. Some of these substances are produced constitutively, whereas others are released after induction by specific factors. To the main secretory products of endothelial cells belong prostanoids, nitric oxide, endothelin-1, and various cytokines (Table 5 and further chapters). The short-lived mediators, such as prostacyclin, thromboxane, and nitric oxide, seem to act in a paracrine way on hepatic stellate cells, Kupffer cells, and hepatocytes rather than on distant cells.

Similarly to other endothelial cells, SEC produce endothelin (Eakes et al. 1997; Rieder 1991). In the normal liver only nonsinusoidal endothelial cells express ET-1 mRNA (Eakes et al. 1997); however, during liver injury ET-1 has been shown to be overproduced by both SEC and stellate cells (Clemens and Zhang 1999).

Although sinusoidal endothelial cells, in contrast to other endothelial cells, do not rest on the complete basement membrane, they express transcripts and immunoprecipitates of some of the main basal lamina protein components (Table 5); the increased synthesis of basal lamina material in acute and chronic liver injury (Neubauer et al. 1999) may be involved in the formation of typical electron-dense basement membrane, the so-called capillarization of liver sinusoids, one of the characteristics of liver fibrosis (Schaffner and Popper 1967).

3.2.5
Other Functions

Endothelial cells of the liver sinusoids were shown to participate in the control of hemostasis, coagulation (through the production of PAI-1; Table 5), sinusoidal blood flow (secretion of endothelin-1, NO, and prostanoids), tissue proliferation and repair via the production of growth factors (HGF, TGF-β), and antitumor activity (Umansky et al. 1996; Vidal-Vanaclocha et al. 2000). Some of these functions, controlled by the cooperation with other nonparenchymal liver cell types, will be reviewed in further chapters.

Table 5. Substances synthesized and secreted by cultured sinusoidal endothelial cells

	References
1. Components of the ECM	
Basement membrane components	
Laminin	Neubauer et al. 1999a,b
Collagen type IV	Geerts et al. 1993a; Herbst et al. 1997a
Entactin (nidogen)	Neubauer et al.1999a,b; Schwoegler et al. 1994
Perlecan (proteoglycan)	Rescan et al. 1993
Other ECM components	
Collagen type I and III	Irving et al. 1984
Fibronectin	Neubauer et al. 1999a; Rieder et al. 1987
Tenascin	Neubauer et al. 1999; Ramadori et al. 1991b
Undulin (collagen type XIV)	Knittel et al. 1992
Syndecan 1 (proteoglycan)	Roskams et al. 1995
2. Cytokines and growth factor binding proteins	
HGF	Maher 1993
IGFBP-2 and -3	Zimmerman et al. 2000
IL-lα, IL-1β	Feder et al. 1993; Lohse et al. 1996
IL-1 receptor antagonist	Rokita et al. 1997
IL-6	Feder et al. 1993; Knolle et al. 1997; Rieder et al. 1990
IL-18	Vidal-Vanaclocha et al. 2000
Basic FGF	Rosenbaum et al. 1989
Thrombopoietin	Cardier and Dempsey 1998
TGF-β1	Rieder et al. 1993
TGF-β2	Bissel et al. 1995, De Bleser et al. 1997a
TNF-α	Vidal-Vanaclocha et al. 2000
3. Other substances	
Endothelin-1	Eakes et al. 1997; Rieder et al. 1991
Leukotriene C_4	Shimada et al. 1998
Nitric oxide	Shah et al. 1997
Prostanoids: PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, TXA_2	Eyhorn et al. 1988
Plasminogen activator inhibitor-1 (PAI-1)	Kuiper et al. 1989; Rieder et al. 1993
von Willebrand's factor (factor VIII)c	Harrison and Boudreau 1989; Hellman et al. 1989

a Transcripts present in freshly isolated SEC.
b Secretion stimulated by TGF-β1.
c Some authors localized factor VIII also in hepatocytes.

4 Kupffer Cells

Kupffer cells, the resident macrophages of the liver, are located within the lumen of the sinusoids overlapping the thinned endothelial wall, and their cellular extensions contribute to a kind of double lining of the sinusoid (Motta 1984). Kupffer cells represent the largest population of macrophages in the mammalian body (Bouwens et al. 1986), and are largely responsible for clearing the post-mesenteric blood of gut-derived bacteria and potent bacterial toxins such as endotoxins or peptidoglucans. Besides high phagocytic and endocytic activity, hepatic macrophages secrete a host of mediators which interact in a paracrine way with neighboring cells, mainly hepatocytes and stellate cells.

4.1 Morphology

Kupffer cells closely resemble other macrophages: their cell body shows numerous microvillous projections, blebs, lamellipodia and occasional fillipodia. In many instances their cytoplasmic processes penetrate through the fenestrae of endothelial cells and reach out into the subendothelial Disse's space being in direct contact with hepatic stellate cells and hepatocytes (Wisse 1974). Kupffer cells have a bean-shaped nucleus and abundant cytoplasm containing many well-developed heterogeneous lysosomes and pinocytotic vesicles (Fig. 2). These ultrastructural features reflect the prominent role of these cells in the degradation of particles and substances taken up from the bloodstream. Stereological analysis of Kupffer cells in perfusion-fixed rat liver revealed the following volumetric composition: nuclei 19%, cytoplasm 61%, mitochondria 4.5%, lysosomes 13.5%, and pinocytic vesicles 2% (Blouin et al. 1977).

Morphologically, Kupffer cells are distinct from other macrophages as they show a characteristic pattern of endogenous peroxidase staining of the rough endoplasmic reticulum and perinuclear envelope (Wisse 1974). Kupffer cells express a number of antigens characteristic of monocytes/macrophages such as CD68 antigen; however, they possess also cell-specific antigens, e.g. galactose/fucose receptor (Hoyle and Hill 1988).

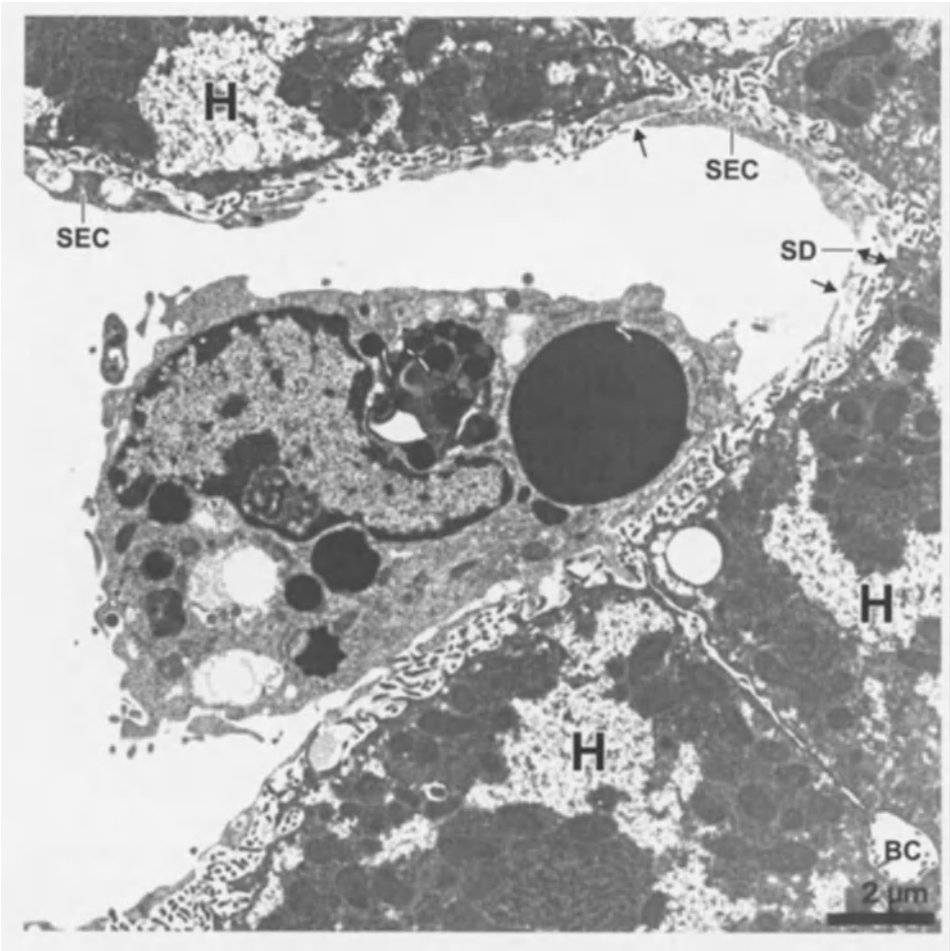

Fig. 2. Transmission electron microscopy of rat liver sinusoid with a Kupffer cell in its lumen and sinusoidal endothelial cell (*SEC*) lining. *Arrows* indicate open fenestrations in the cytoplasmic processes of SEC. Irregularly shaped nucleus, and multiple vacuoles with endocytosed and phagocytosed material are present in the cytoplasm of Kupffer cell. Two large vacuoles represent heterophagosome and phagosome, the latter possibly resulting from phagocytosis of effete red blood cells. *H*, hepatocytes with short microvilli protruding into the space of Disse (*SD*). Bile canaliculus (*BC*) can be seen between adjacent hepatocytes. *Scale bar*, 2 µm. (Courtesy of Professor Bård Smedsrod, University of Tromso, Norway)

4.2 Distribution and Origin

Kupffer cells are concentrated in the periportal region of the liver (Wake et al. 1989), which is an advantageous position to monitor the blood entering the liver. Within the rat liver lobule 43% of Kupffer cells are periportal, 32% mid-zonal, and 25% are found in the perivenous zone (Sleyster and Knook 1982). Periportal Kupffer cells are larger

and show both a higher endocytic activity and higher lysosomal enzyme activity per cell than midzonal and perivenous Kupffer cells (Sleyster and Knook 1982). The smaller KC in the perivenular zones were found to be more active in cytokine production, and have a higher cytotoxic capacity (Hardonk et al. 1989). Heterogeneity of Kupffer cells in respect to lysozyme (Bardadin et al. 1991) and Fcγ receptor (Tomita et al. 1994) expression, and differences in the secretory properties of isolated rat liver macrophages (Hoedemaker et al. 1995) have also been described.

Hepatic macrophages are the end-stage cells in the mononuclear phagocytic series, which may be derived from monocytes or stem cells in the bone marrow (Naito et al. 1997). It was shown in vivo in mice that under normal conditions bone marrow cells accumulated in the liver and rapidly differentiated into liver macrophages (Takezawa et al. 1995).

4.3 Isolation

Centrifugal elutriation is the method of choice for the purification of nonparenchymal liver cell suspensions (Knook and Sleyster 1977); however, the difference in the adherence properties of KC and other nonparenchymal cells may be used for successful and efficient isolation because KC rapidly adhere in vitro to various types of tissue culture dishes (Smedsrod and Pertoft 1985).

4.4 Activation of Kupffer Cells

Macrophage activation, a term originally used to describe the enhanced bactericidal properties of macrophages exposed to intracellular bacteria, has taken on a broader definition to cover all the functions of in vivo or in vitro stimulated cells. However, macrophage effector mechanisms are often differentially activated in response to various signals so that different secretory products may be released (Decker 1990; Table 7).

Kupffer cells, by virtue of their location in the mainstream of splanchnic blood flow, are positioned to receive a constant exposure to gut-derived mediators known to activate macrophages. Of the many substances that activate Kupffer cells the most important physiologically are gut bacterial endotoxins (lipopolysaccharides) that reach the liver through the portal vein system. It is possible that Kupffer cells, constantly exposed to low levels of the gut-derived bacterial products (Lumsden et al. 1988), may be in a semiactivated state even in control animals or healthy individuals. Under pathological conditions, bacteria that break the intestinal barrier and invade the liver are the most important activators of Kupffer cells. To other activators of liver macrophages, often used in experimental studies, belong zymosan, bacterie Calmette-Guerin, IFN-γ, colony-stimulating factor, macrophage-activating factor, platelet-activating factor, arachidonic acid, and many others (Decker 1990). During phagocytosis Kupffer cells undergo so-called oxidative burst, which leads to the production of toxic reactive oxygen species and other biologically active compounds (Table 7).

Table 6. Receptors of Kupffer cells

	References
1. Receptors involved in KC endocytosis and phagocytosis	
Galactose/fucose receptor (77–88 kDa)	Hoyle and Hill 1988
Mannose/*N*-acetylglucosamine receptor	Praaning et al. 1987
Low-density lipoprotein receptor	Kamps et al. 1991
Scavenger receptor class A, types I and II	Smedsrod et al. 1997
Macrosialin, a 95-kDa receptor for oxidized lipoproteins	van Oosten et al. 1998; van Velzen et al. 1997
Fibronectin receptors	Cardarelli et al. 1990
IgG receptors (FcγRII and -III)	Johansson et al. 1996, 2000; Tomita et al. 1994
IgA receptor (FcαRI)a	van Egmond et al. 2000
Complement receptors CR1, CR3, and CR4	Hinglais et al. 1989; Yan et al. 2000
Anaphylatoxin C5a receptor	Schlaf et al. 1999
Carcinoembryonic antigen (CEA) receptor	Thomas et al. 1992
Scavenger receptor specific for LPS	van Oosten et al. 1998
Toll-like receptor 4	Su et al. 2000
2. Cytokine receptors	
IL-1	Decker et al. 1995
IL-2β	Decker et al. 1995; Funaki et al. 1996
IL-6	Deaciuc et al. 1994a; Hoffmann et al. 1994
PAF	Chao et al. 1989
TGF-β type I	Kossmann et al. 1992
TNFα-R1 (type 1, 55 kDa)	Zhang et al. 1994a
VEGF (Flt-1 and KDR/flk-1)	Yamaguchi et al. 2000
3. Hormonal receptors	
ANP (guanylate cyclase receptor)	Bilzer et al. 1999; Gerbes et al. 1998
Endothelin, type B receptor	Housset et al. 1993
Glucagon	Watanabe et al. 1988
Glucocorticoid	Raddatz et al. 1996
Growth hormone	Zimmermann et al. 2000
4. Other receptors	
Beta-adrenergic	Liao et al. 1995
Fas (APO-1/CD95)	Muschen et al. 1998
P_2 purinergic	Hashimoto et al. 1995

Table 6 ***(continued).*** Receptors of Kupffer cells

	References
Retinoic acid (RARα, -β, -γ, and RXRα, -β, -γ)	Ohata et al. 2000
Peroxisome proliferator-activated receptor γ (PPARγ)	Peters et al. 2000

a Present in inflammatory human liver.
b Present on rat KC but low expression on human KC.

Table 7. Secretory products of activated Kupffer cellsa

	References
1. Factors involved in protein degradation and tissue remodeling	
Lysosomal enzymes	Knook and Sleyster 1977
95-kDa type IV collagenase/gelatinase B (MMP-9)	Knittel et al. 1999c; Winwood et al. 1995
Interstitial collagenase (MMP-13)	Hironaka et al. 2000
TIMP-1 and TIMP-2	Knittel et al. 1999c
Sulfated proteoglycan	Laskin et al. 1991
2. Intercellular mediators and modulators of cell function	
Eicosanoids: prostanoids and leukotrienes	Decker 1990; Kmiec et al. 1997
NO	Gaillard et al. 1991
CO	Bauer et al. 1998b; Goda et al. 1998
Platelet-activating factor	Chao et al. 1989
Cytokines	
IL-1α, IL-1β	Armbrust et al. 1995; Shirahama et al. 1988
IL-1 receptor antagonist	Rokita et al. 1997
IL-6	Busam et al. 1990; Knolle et al. 1997
IL-10	Knolle et al. 1995
IL-12	Dobashi et al. 1999; Takahashi et al. 1996
IL-18 (IFNγ-inducing factor)	Okamura et al. 1995
TNF-α	Karck et al. 1988
TGF-α	Decker et al. 1995
TGF-β1, TGF-β2	Bissell et al. 1995; Meyer et al. 1990a

Table 7 (*continued*). Secretory products of activated Kupffer cellsa

	References
Reactive oxygen species, such as superoxide anion, hydrogen peroxide, hypochlorous acid, singlet oxygen, hydroxyl radicals, induced in KC by	
PAF	Bautista and Spitzer 1992
LPS	Arthur et al. 1988
Endocytosis of soluble IgG-immune complexes	Johansson et al. 2000
3. Defense mechanisms and cytotoxicity	
C1q subcomponent of complement	Armbrust et al. 1997
Lysozyme	Miyauchi et al. 1985
4. Growth factor-binding proteins	
IGFBP-2 and -3	Arany et al. 1994; Scharf et al. 1996; Zimmerman et al. 2000
Latent TGF-β-binding proteins (LTBPs)	Roth et al. 1998a
5. Other proteins and enzymes	
Apolipoprotein E	Dawson et al. 1989
Erythropoietin	Paul et al. 1984
Osteopontin	Kawashima et al. 1999
Bone morphogenetic protein-6 (BMP-6)	Knittel et al. 1997a

a Different stimuli were used, usually endotoxin (LPS), but also ATP and adenosine (Hashimoto et al. 1995), calcium ionophore, zymosan, phorbol esters, PAF, and muramyl dipeptide (Decker 1990).

4.5 Functions

Similarly to other macrophages, Kupffer cells are very active in endocytosis and phagocytosis due to the presence of numerous cell membrane receptors (Table 6). Activated liver macrophages release many substances (listed in Table 7) that have paracrine and autocrine effects within the liver, and endocrine effects throughout the body (see later chapters). In contrast to liver SEC that take up soluble molecules from the circulation, Kupffer cells play an essential role in the elimination of both soluble and blood-borne particulate material derived from portal circulation. For example, in the normal liver Kupffer cells clear blood from IgG-immune complexes through their binding to the Fc receptors (Johansson et al. 2000). The presence of complement receptors on KC enhances phagocytosis of opsonized immune complexes. Oxidized and acetylated lipoproteins are mainly taken up by the scavenger receptors of KC (Terpstra et al. 2000).

Table 8. Kupffer cell functions

	References
1. Endocytosis and phagocytosis	
Bacteria, viruses, yeasts, parasites, old and foreign cells, particulate debris	Wake et al. 1989
Endotoxins	Fox et al. 1989
Lipoproteins	Kleinherenbrink-Stins et al. 1991
Glycoproteins	
α_2-Macroglobulin	van Dijk et al. 1992
N-acetylglucosamine- and mannose-terminated glycoproteins	Praaning et al. 1987; Sano et al. 1990
Fibronectin	Cardarelli et al. 1990
Fibrin degradation products	Emeis et al. 1981
Tissue-type plasminogen activator t-PA	Otter et al. 1992; Stang et al. 1992
Hyaluronian fragments	Rockey et al. 1998
Apoptotic lymphocytes	Falasca et al. 1996; Mehal et al. 1999
Tumor cells	Gardner et al. 1991; Gjoen et al. 1989
Neutrophils	Shi et al. 1998
2. Modulation of immune and inflammatory responses	
Secretion of immunomodulating and inflammatory mediators: cytokines, eicosanoids, PAF, NO (see Table 7)	Lohse et al. 1996; Rubinstein et al. 1986;
Antigen presentation toward intrahepatic lymphocytes due to the presence of abundant HLA-DR antigen (Funaki 1996) and APC activity	Squiers et al. 1993
Participation in the development of oral tolerance to bacterial superantigens	Terabe et al. 2000
3. Tissue and matrix remodeling (see Table 7)	
4. Paracrine effects on neighboring cells (see later chapters)	
5. Metabolism of iron and bilirubin as a result of erythrophagocytosis	Sibille et al. 1988; Terpstra and van Berkel 2000; Yan et al. 2000
6. Cytotoxicity against tumor cells	Rushfeldt et al. 1999; Schuurman et al. 1995; Thomas et al. 1995
7. Motility, i.e., capability to migrate	MacPhee et al. 1992

Besides typical macrophage activities, Kupffer cells play an important role in the clearance of senescent and damaged erythrocytes by binding them to the members of the scavenger receptor family (Terpstra and van Berkel 2000) and in subsequent

degradation of hemoglobin and recirculation of iron (Bissell et al. 1972; Sibille et al. 1988). Heme oxygenase (HO) is the first and rate-limiting enzyme of heme catabolism that degrades protoheme IX into carbon monoxide (CO), free Fe2+ ion, and biliwerdin IXα (Maines 1997). HO-1, also known as heat shock protein 32, the inducible form of the enzyme, is strongly expressed in the rat liver in Kupffer cells, but not in hepatocytes (Bauer et al. 1998b; Goda et al. 1998; Immenschuh et al. 1999). In contrast, the constitutive form, HO-2, was shown to be strongly expressed in hepatocytes (Goda et al. 1998), and less strongly in SEC and hepatic stellate cells (Bauer et al. 1998b). It has been shown that under oxidative stress conditions HO-1 expression increased in both nonparenchymal and parenchymal liver cells. In the normal liver CO can induce relaxation of stellate cells, and thus affect sinusoidal perfusion (Suematsu et al. 1995). Moreover, CO may function as a gaseous regulator of hepatocyte cytochrome P450-dependent biotransformation including bile acid synthesis and xenobiotic catabolism (Suematsu and Ishimura 2000).

Kupffer cells may play an important role in intrahepatic immunosuppression and induction of immunological tolerance (Sato et al. 1996; Terabe et al. 2000). Because activated Kupffer cells also act as antigen-presenting cells toward intrahepatic lymphocytes due to the expression of high levels of class I (Ramadori et al. 1986) and class II MHC (Rubinstein et al. 1986) molecules, and CD80 and CD40 co-stimulatory molecules (Lohse et al. 1996; Squiers et al. 1993), they may play a role in the regulation of hepatic immune responses. Similarly to other macrophages, Kupffer cells have strong cytotoxic activities and may also function in anti-tumor defenses (Table 8).

5 Hepatic Stellate Cells

Carl Kupffer, by the use of a gold chloride staining method, detected in mammalian livers a population of stellate-shaped cells that were located perisinusoidally, always attached to the sinusoidal capillaries and also to the parenchymal cells (Kupffer 1876, cited by Wake 1980). Although originally Kupffer thought that these cells belonged to perivascular cells of connective tissue, after 20 years he changed his mind and suggested that the cells were a special kind of phagocytic endothelial cells (see discussion by Wake 1980). The cells he described, however, appear to be the perisinusoidal cells, which express quite different functions than liver macrophages (Wake 1971; Wake et al. 1989). These cells had been rediscovered after almost 80 years and named Ito cells after one of the persons who described them (Ito and Nemoto 1952). Ito cells are known by a variety of synonyms (vitamin A-storing cells, lipocytes, fat-storing cells, liver-specific pericytes, perisinusoidal cells) reflecting their functions; however, the term "hepatic stellate cells" (HSC) has been recently most often used.

5.1 Morphology of Stellate Cells in Liver Sections

Hepatic stellate cells (HSC) are located in the perisinusoidal (Disse) space in direct vicinity to the endothelial cell layer with their cell bodies often compressed into the recesses between hepatocytes. Stellate cells are not engulfed by a true basement membrane unlike typical pericytes in blood capillaries (Wake 1980; Wisse et al. 1985). They have two types of cytoplasmic extensions: the intersinusoidal or interhepatocellular processes (Fig. 3), which penetrate the hepatic cell plates and may reach the nearby sinusoids, and shorter perisinusoidal or subendothelial processes that encircle the sinusoid, thus reinforcing its wall (Wake 1988). The processes of HSC contain a prominent cytoskeleton oriented along their long axes, and several ultrastructures like mitochondria, vesicles, rough endoplasmic reticulum, and glycogen particles (Geerts et al. 1990). A single stellate cell may provide such processes to more than one neighboring sinusoid and make contact with a great number of hepatocytes (Wake 1980).

The most characteristic structural feature of stellate cells is the presence in the cytoplasm of numerous, large (up to 8 μm in diameter) lipid droplets, which exist in membrane bound and nonmembrane bound forms (Wake 1980), and are composed of retinoids (mainly retinyl palmitate), triglycerides, cholesterol, and free fatty acids (Vogel et al. 1999). The formation, size, and number of lipid droplets depend on physiological circumstances, species, and dietary vitamin A intake (Vogel et al. 1999).

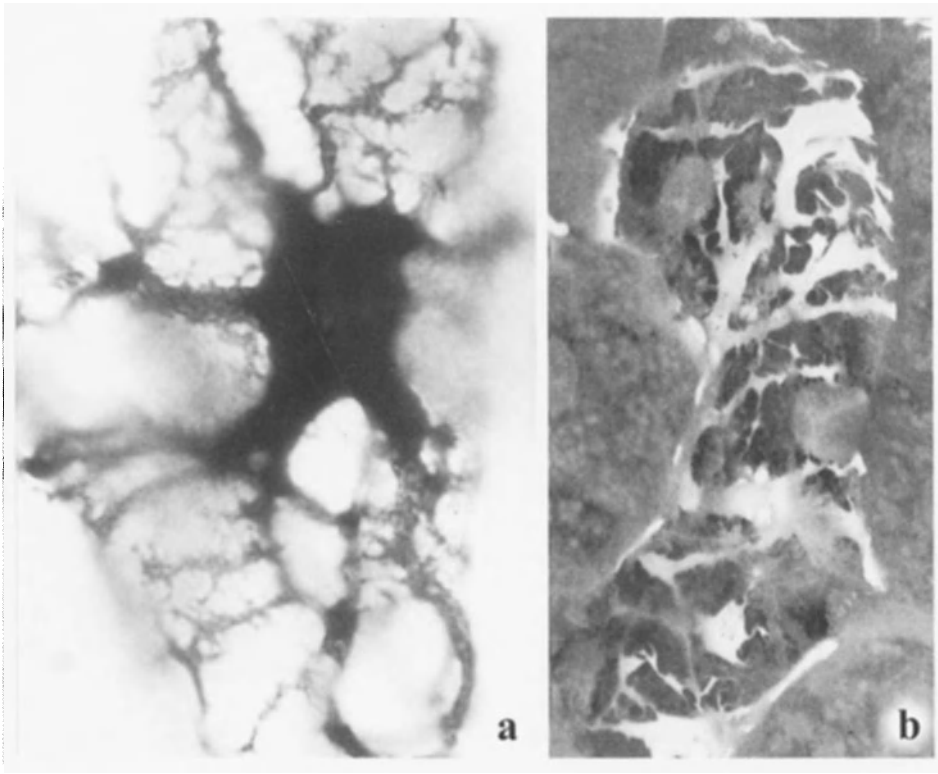

Fig. 3. a Hepatic stellate cell in the liver of a 5-week-old rat. Dendritic branches of perisinusoidal processes are decorated with many thorn-like microprojections, stained with the Golgi silver method, ×1800. **b** Back scattered scanning electron-microscopic image of Golgi-stained cell extensions of a hepatic stellate cell of porcine liver. [Courtesy of Professor Kenjiro Wake (Tokyo)]

In normal human liver lipid droplets contribute 20.5% to the stellate cell volume (Sztark et al. 1986). The autofluorescence of stored vitamin A allows for in vivo localization of HSC by the use of epifluorescence microscopy (Suematsu et al. 1993).

The ultrastructure of hepatic stellate cells has been well characterized. The nucleus is oval and often compressed by the lipid droplets. Cytoplasm is characterized by the presence of a large Golgi apparatus, numerous micropinocytic vesicles, well developed rough endoplasmic reticulum, and poorly developed smooth endoplasmic reticulum, small numbers of mitochondria and lysosomes, rare peroxisomes, a few glycogen particles, and a distinct centriole (Geerts et al. 1990; Wake 1980). The cytoplasmic matrix contains bundles of microfilaments and microtubules, including actin filaments and intermediate filaments (Geerts et al. 1990). The presence of smooth muscle α-actin (Ramadori et al. 1990), intermediate filaments, such as desmin (Yokoi et al. 1984), nestin (Niki et al. 1999), and vimentin (de Leeuw et al. 1984), and of proteins characteristic of cells of neural origin such as glial fibrillary acidic protein (Buniatian et al. 1996; Gard et al. 1985; Neubauer et al. 1996), NGF, and other neurotrophins (Cassiman et al. 2001), synaptophysin (Cassiman et al. 1999) and RhoN protein

(Nishi et al. 1999) were found to be specific markers for hepatic stellate cells in vivo and in vitro.

5.2 Isolation

Stellate cells have been isolated from rodent (de Leeuw et al. 1984) or human (Friedman et al. 1992) liver by digesting the organ with collagenase and pronase, followed by differential centrifugation of the resultant cell suspension through various density media (de Leeuw et al. 1984; Friedman and Roll 1987; Knook et al. 1982). Because of their high lipid content and concomitant low density, stellate cells can be separated from other nonparenchymal liver cells. A method of isolating virtually pure cell preparations based on cell sorting using high side scatter of incident light has been recently reported (Geerts et al. 1998).

5.3 Morphology and Functions of Stellate Cells in Early Cultures

In vivo, hepatic stellate cells exhibit a dual phenotype: a quiescent one in normal liver, and an activated phenotype in chronically diseased liver, especially in liver fibrosis. In the quiescent state the cells show abundant lipid droplets, low proliferative rate, and low synthetic capacity. The activated or myofibroblast-like phenotype is characterized by the loss of lipid vacuoles, increased cell proliferation, and enhanced synthesis of extracellular matrix components (Friedman 1993). Interestingly, similar phenotypic transformation from quiescent to activated phenotype could be observed in cultures of stellate cells. Cultured on plastic, the cells spread within 48–72 h expressing activity of stellate cells can be normally measured in vitro after culturing the cells for at least a few days, procollagen I and III, collagen IV, and laminin transcripts (Gressner 1998). Although the synthetic activity of stellate cells can be normally measured in vitro after culturing the cells for at least a few days, the use of sensitive techniques, such as Northern blot, RNAase protection, and polymerase chain reaction, made possible the demonstration of messenger RNAs for different ECM components and cytokines in HSC shortly after isolation (Geerts et al. 1993). In early culture, up to 3 days, so-called quiescent stellate cells showed migratory activity, which was accelerated by PDGF, and the presence of Kupffer cells (Ikeda et al. 1999). The following functions can be attributed to quiescent (nonactivated) stellate cells.

1. **Retinoid metabolism**
   More than 85% of vitamin A in the body is stored in the liver, and 80–90% of the retinods in the liver are stored in the lipid droplets of hepatic stellate cells (Hendriks et al. 1985). The cooperation of stellate cells and hepatocytes in the regulation of vitamin A metabolism will be described in Chap. 10.
2. **Modulation of sinusoidal blood flow**
   As a result of contractility, which was demonstrated in vitro (Kawada et al. 1992) and in vivo (Bauer et al. 1994; Zhang et al. 1994b), hepatic stellate cells might influence sinusoidal blood flow through the contraction and dilation of the sinusoidal lumen (see Chap. 12).

3. **Synthesis of extracellular matrix components**
   Under physiological conditions stellate cells are considered major producers of ECM macromolecules and ECM-degrading enzymes (Arthur 2000; Friedman 1993). The interactions between HSC, other liver cell types, and ECM components play a central role in liver tissue repair resulting from acute or chronic liver damage (see Chap. 14).
4. **Intercellular communication through the synthesis of cytokines and growth factors**
   In normal liver stellate cells express all major isoforms of hepatocyte growth factor, transforming growth factor β, insulin-like growth factors, and TGFβ- and IGF-binding proteins, and synthesize other cytokines that interact with all types of neighboring cells, including hepatocytes (Chap. 11).
5. **Erythropoietin synthesis**
   In the liver the presence of erythropoietin was identified in stellate cells and hepatocytes (Eckardt et al. 1994). Both renal peritubular cells and hepatic stellate cells contain ecto-5'-nucleotidase, the activity of which may be involved in the oxygen sensing mechanism via a hydrolysis of AMP to adenosine, which in turn may stimulate EPO synthesis (Bachmann 1997).
6. **Synthesis of plasminogen activation system**
   Human HSC in culture produce all the components of the plasminogen activation system (urokinase-type plasminogen, u-PA, receptor of u-PA, and plasminogen-activator inhibitor 1, PAI-1); their expression is differentially regulated in paracrine and autocrine ways by various growth factors such as PDGF and basic FGF (Fibbi et al. 1999; Leyland et al. 1996).

5.4 Morpholgy and Functions of Activated Hepatic Stellate Cells

5.4.1 Activation of Hepatic Stellate Cells

During primary culture on uncoated tissue culture plastic for more than 7 days hepatic stellate cells acquire an "activated" phenotype characteristic of myofibroblast-like cells. This process of phenotypic transition from the "resting" or "quiescent" cell phenotype has been called "activation" or "transformation" of hepatic stellate cells. In their activated phenotype, stellate cells are characterized by pronounced synthetic, proliferative, chemotactic, and contractile properties (Friedman 1993; Gressner 1998). The spontaneous proliferation of cultured stellate cells may be attributed to the presence of growth factors present in serum supplementing the culture media. Several structural changes characterize the transdifferentiation of liver stellate cells into myofibroblast-like cells. They include loss of vitamin A-containing fat droplets (Friedman et al. 1992), and increased cell spreading accompanied by the formation of cellular processes expressing prominent actin microfilaments, i.e., "stress-fibers" and focal adhesions (Yee 1998). The enhanced expression of smooth muscle α-actin, the main myofibroblastic marker protein (Ramadori et al. 1990), desmin (Friedman et al. 1992), class VI intermediate filament nestin (Niki et al. 1999), RhoN protein (Nishi et

al. 1999), and synaptophysin (Cassiman et al. 1999) has been recognized as morphological markers of activated hepatic stellate cells. In contrast to quiescent cells, activated stellate cells express neural cell adhesion molecule 1 (Nakatani et al. 1996); however, the expression of glial fibrillary acidic protein was downregulated during prolonged culture of HSC (Buniatian et al. 1996). Interestingly, prion protein, another protein characteristic mainly of nervous cells, has been demonstrated only in activated rat (Ikeda et al. 1998) and human stellate cells in chronic liver disease (Kitada et al. 2000). The expression in HSC of many proteins associated with the central nervous system suggests that they may derive from neural crest cells.

The in vitro activation of stellate cells has been associated with enhanced synthesis of various collagenous and noncollagenous extracellular matrix components, and other biologically active mediators (Table 10). The use of powerful proteomics technique enabled comparison of protein profiles expressed in activated vs. quiescent rat stellate cells, identifying 312 proteins that were up- or downregulated (Bach Kristensen et al. 2000). Some of the phenotype changes that accompany HSC activation will be shortly described below.

During the course of the phenotypic transition cells show altered expression of many receptors. Decreased expression of peroxisome proliferator-activated receptor γ (PPAR-γ), which belongs to a group of nuclear receptors regulating the "fat phenotype" of several cell types, may be involved in the disappearance of lipid droplets during the transformation of HSC (Galli et al. 2000). Activated HSC were shown to lose the expression of the main subunits of soluble guanylate cyclase, i.e., nitric oxide receptor (Failli et al. 2000); however, they also express new receptors that are absent in quiescent cells, e.g., for a potent mitogen, PDGF-β (Friedman and Arthur 1989), TGF-β (Friedman et al. 1994), or ferritin (Ramm et al. 1994). The sensitivity of TGF-β1 (Friedman et al. 1994), endothelin-1 (Reinehr et al. 1998), and thrombin (Marra et al. 1998a) cognate receptors was found to be upregulated proportionally to the progress of HSC activation.

The expression of several cytokines was found to be altered during stellate cell transformation. In activated HSC a strong increase of TGF-β2 and TGF-β3 expression was found, while TGF-β1 remained moderate, in contrast to the normal liver, where TGF-β1 constitutes the predominant isoform (De Bleser et al. 1997a). Cytokines such as activin A (De Bleser 1997b), connective tissue growth factor (Williams et al. 2000), leptin (Potter et al. 1998), IGF-I and IGF-binding proteins (Scharf et al. 1998), absent in quiescent cells in normal liver, were detected at the mRNA and protein level in activated HSC. However, the expression of hepatocyte growth factor, present in quiescent HSC, disappeared during their activation (Ramadori et al. 1992b).

In contrast to unstimulated HSC that are resistant to Fas-ligand-induced apoptosis, myofibroblast-like stellate cells become sensitive to this pathway of programmed cell death (Gressner 1998). Similarly to myofibroblasts in other organs, activated HSC show chemotactic activity in response to soluble mediators such as PDGF (Ikeda et al. 1999), MCP-1 (Marra et al. 1999), basic FGF (Fibbi et al. 1999), or IGF-I (Pinzani et al. 1990), which may be locally released from neighboring cells.

Activation of HSC results also in the alterations of enzyme activities and function of ion transporters. The expression of glutamine synthetase protein and activity was found in all cultured stellate cells (Bode 1998), whereas in the normal liver expression of this enzyme was observed only in a small subpopulation of perivenous hepatocytes (Gebhardt and Mecke 1983). Stellate cells, proportionally to the level of their activa-

tion, lose most of the activities of principal forms of glutathione-*S*-transferases, which may suppress their resistance to oxidative stress (Whalen et al. 1999). Activated stellate cells express L-type voltage-operated calcium channels (Oide et al. 1999), and the Na+/Ca2+ exchanger (Nakamura et al. 1998), absent in quiescent cells. Enhanced osmolyte transport activity and induction of the BGT1 transporters have been proposed to be another activation marker of HSC (Peters-Regehr et al. 1999).

The up-regulation of collagen synthesis during activation, one of the most important responses of HSC to injury, is mediated both by transcriptional and post-transcriptional mechanisms. Transcriptional activation of the type I collagen in activated HSC has been extensively studied (Friedman 2000). The half-life of collagen α(I)1 mRNA was shown to be increased 20-fold in activated compared to quiescent stellate cells (Stefanovic et al. 1997).

Despite abundant characteristics of activated stellate cells, cellular events that initiate HSC activation are poorly understood. Several factors have been shown to play an important role in the promotion of the full picture of activated HSC. They include extensive changes in the composition and organization of the extracellular matrix, and the establishment of paracrine and autocrine loops for several growth factors, cytokines, chemokines, products of oxidative stress, and other soluble factors released mainly from Kupffer (Table 7) and stellate cells (Table 10). Profound phenotypic alterations observed during the transformation of HSC have been associated with the activation of a number of transcription factors such as AP-1 (Armendariz et al. 1994), SP-1 (Rippe et al. 1995), *c*-myb (Lee et al. 1995), Kruppel-like zinc finger protein KLF6 (Ratziu et al. 1998), and nuclear factor-κB (NFκB). NFκB, the key transcription factor that activates multiple genes in response to inflammation, infection, and stress, was induced upon the transformation of quiescent to activated HSC (Lee et al. 1995), which were shown to express a variety of NFκB-responsive genes (Elsharkaw et al. 1999; Hellerbrand et al. 1998). Persistent activation of NFκB in activated HSC could be required for the expression of specific genes associated with the activated phenotype, and may also play an anti-apoptotic role for stellate cells (Elsharkaw et al. 1999). Moreover, stress-activated protein kinases, such as p38 MAP kinase, were also found to be involved in the phenotypic transition of HSC (Reeves et al. 2000).

It is widely believed that the in vitro activation of HSC corresponds to the stellate cell changes observed in the liver following its acute or chronic damage (Chap. 14). However, only the confrontation of the results obtained from cell culture or animal studies with the observations made during the natural course of human liver disease can justify the extrapolation of the results obtained from experimental investigations to the in vivo situation in humans.

5.4.2 Functions

In the liver damage-activated stellate cells or liver myofibroblasts play a pivotal role in the initiation and progression of liver fibrosis. This topic has been described in Chap. 14. Moreover, in the liver injury HSC show increased contractile activity, and in this way may affect sinusoidal blood flow (Chap. 12). The secretion of leptin by activated HSC (Potter et al. 1998) may be involved in the modulation of action of many proinflammatory cytokines (Kaplan 1998).

5.4.3
Receptors and Secretory Products

The responses of stellate cells to extracellular mediators are mediated through the cell membrane receptors that have been listed in Table 9. The extended list of substances released by activated HSC is shown in Table 10. The presence of both the effector synthesis and its receptors on stellate cells strongly suggests the existence of autocrine regulation of HSC functions.

Table 9. Receptors of hepatic stellate cells

	References
1. Receptors for extracellular matrix components	
Integrins ($\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_v\beta_1$, $\alpha_6\beta_4$)a	Carloni et al. 1997; Racine-Samson et al. 1997
2. Receptors for cytokines and growth factors	
Basic FGF	Rosenbaum and Blazejewski 1995
IFN-γ	Rockey et al. 1992
IGF-I	Brenzel and Gressner 1996; Scharf et al. 1998
IGF-II/mannose-6-phosphate receptor	De Bleser et al. 1995
Neutrophins, including NGF, low affinity p75 receptor	Cassiman et al. 2001; Trim et al. 2000
PDGF-α and -β receptors	Friedman and Arthur 1989; Pinzani et al. 1996b
TGF-β type II, III, and TGF-β/activin type I receptors	Bedossa et al. 1995
TNF-α, type I and II receptors	Knittel et al. 1997b
Urokinase-type plasminogen activator (u-PA) receptor	Fibbi et al. 1999
VEGF	Ankoma-Sey et al. 1998
3. Receptors for vasoactive substances	
Angiotensin-II	Pinzani et al. 1992c
ANP	Gorbig et al. 1999
Endothelin-1, type A and B receptors	Housset et al. 1993
V_1-arginine-vasopressin receptor	Bataller et al. 1997
Natriuretic peptide (C-type)	Tao et al. 1999
Prostaglandins	Fennekohl et al. 1999
Thromboxane	Fennekohl et al. 1999
Thrombin	Marra et al. 1998a
Purinergic receptors	Takemura et al. 1994

Table 9 (*continued*). Receptors of hepatic stellate cells

	References
α_1-Adrenergic receptor	Reinehr et al. 1998
4. Receptors for other ligands	
Anaphylatoxin C5a	Schlaf et al. 1999
Fas (CD95)	Saile et al. 1997
Ferritin	Ramm et al. 1994
Glucocorticoid	Raddatz et al. 1996
PPAR-γ	Galli et al. 2000
Retinoic acid (RARα, -β, -γ and RXRα, -β)	Friedman et al. 1993; Weiner et al. 1992
Somatostatin	Reynaert et al. 2000

a Receptors for type IV collagen, type I collagen, fibronectin and laminin.

5.5 Heterogeneity of Hepatic Stellate Cells and Liver Myofibroblasts

Hepatic stellate cells constitute a heterogeneous population of cells that differ in their cytoskeletal filament phenotype (Ballardini et al. 1994), retinoid content (Ramm et al. 1995), and/or production of extracellular matrix components (Greenwel et al. 1993). A part of these phenotypic differences may reflect alterations in gene expression across the acinar gradient similar to that demonstrated for hepatocytes (see Sect. 2.3).

By the use of an isolation technique that destroys vitamin A-rich stellate cells, Knittel et al. (1999a) established from normal rat liver cultures of fibroblastic cells termed "rat liver myofibroblasts" (rMF). In contrast to HSC, rat liver myofibroblasts lacked the expression of glial fibrillary acidic protein, desmin, and vascular cell adhesion molecule 1; however, both cell types displayed mostly overlapping characteristics with only a few differences. Immunohistochemical techniques and in situ hybridization allowed to localize stellate cells exclusively in hepatic parenchyma, whereas rMF were located in the portal fields, the walls of central veins, and only occasionally in parenchyma (Knittel 1999b). During acute liver injury the number of HSC increased prominently, while the number of rMF was nearly unchanged. In contrast, in chronically injured livers not only stellate cells but also rMF were involved in scar formation (Knittel 1999b). These data demonstrate that functionally different fibroblast populations are present in different compartments of normal liver, respond differentially to an acute injury, and may be detected in distinct areas of fibrotic liver. Apart from stellate cells, rat liver myofibroblasts can therefore be regarded as an important cell population involved in liver fibrogenesis, at least in this species (Knittel 1999b).

Table 10. Products synthesized and secreted by cultures of activated stellate cells

	References
1. Extracellular matrix components, enzymes and molecules involved in ECM degradation	
Proteins	
Collagen types I and III–VI	Friedman et al. 1985; Kawase et al. 1986
Collagen type XVIII (nonfibrillar)	Musso et al. 1998
Entactin (nidogen)	Schwoegler et al. 1994
Fibronectin	Geerts et al. 1993; Ramadori et al. 1992a
Laminin	Milani et al. 1989; Schwoegler et al. 1994
Tenascin	Ramadori et al. 1991b
Undulin	Knittel et al. 1992
ECM-degrading enzymes	
Interstitial collagenase (MMP-1 in humans, MMP-13 in rat) 72-kDa type IV collagenase/gelatinase A (MMP-2)	Iredale et al. 1995; Milani et al. 1994 Arthur et al. 1992; Milani et al. 1994
Stromelysin-1 (transin, MMP-3) and -2	Knittel et al. 1999c; Vyas et al. 1995
MT1-MMP (progelatinase A, MMP-14)	Benyon et al. 1999; Theret et al. 1997
Fibroblast activation protein (FAP)	Levy et al. 1999
Tissue inhibitors of metalloproteinases (TIMPs)	
TIMP-1	Iredale et al. 1992; Knittel et al. 1999c
TIMP-2	Herbst et al. 1997b
Key components of plasminogen-activating system	
Urokinase-type plasminogen activator (uPA)	Fibbi et al. 1999; Leyland et al. 1996
Plasminogen activator inhibitor type I (PAI-1)	Fibbi et al. 1999; Leyland et al. 1996
C4 complement component	Fimmel et al. 1996
C1-esterase inhibitor	Schwogler et al. 1992
Glycosaminoglycans and proteoglycans	
Biglycan	Meyer et al. 1992
Decorin	Meyer et al. 1992
Chondroitin, heparan, and dermatan sulfate	Gressner 1991
Hyaluronic acid	Gressner and Haarmann 1988
Syndecan	Meyer et al. 1992
2. Cytokines and growth factor-binding proteins	
Activin	De Bleser et al. 1997b
Connective tissue growth factor	Williams et al. 2000
Connective tissue stem cell factor	Gaca et al. 1999
CINC	Maher et al. 1998

Table 10 *(continued)*. Products synthesized and secreted by cultures of activated stellate cells

	References
Epidermal growth factora	Mullhaupt et al. 1994
Fibroblast growth factor	Kan et al. 1989
Hepatocyte growth factor	Ramadori et al. 1992b; Skrtic et al. 1997
Insulin-like growth factor-I (IGF-I)	Pinzani et al. 1990; Scharf et al. 1998
IGF-II	Zindy et al. 1992
IGFBP-2, -3, -4, -5, -6b	Gentilini et al. 1998; Scharf et al. 1998
Interleukin-1	Sakamoto et al. 1997
Interleukin-6c	Tiggelman et al. 1995
Interleukin-10	Thompson et al. 1998; Wang et al. 1998b
Monocyte chemotactic protein-1	Marra et al. 1993; Xu et al.1996
Macrophage colony-stimulating factor	Pinzani et al. 1992a
Macrophage inflammatory protein-2	Sprenger et al. 1997
NGF and other neurotrophins	Cassiman et al. 2001
PDGF	Marra et al. 1994
TGF-α	Bachem et al. 1992
TGF-β1, TGF-β2, TGF-β3	Casini et al. 1993; De Bleser et al. 1997a
Latent TGF-β-binding proteins (LTBPs)	Gong et al. 1998; Roth et al. 1997
3. Other substances	
PAF	Pinzani et al. 1994
$PGF_{2\alpha}$ and PGD_2d	Athari et al. 1994
NOe	Helyar et al. 1994; Rockey and Chung 1995
Endothelin-1	Eakes et al. 1997; Rieder et al. 1991
Leptin	Potter et al. 1998
Apolipoprotein	Friedman et al. 1991; Ramadori et al. 1989
α_2-Macroglobulin	Andus et al. 1992; Ramadori et al. 1991a
Complement-activating protease P100	Knittel et al. 1999a
Epimorphine	Hirose et al. 1996
BMP-6	Knittel et al. 1997a
Osteopontin	Kawashima et al. 1999

a EGF mRNA was localized in the intact rat liver, stellate cells, and hepatocytes.

b IGFBP-6 was detected at the mRNA but not protein level.

c IL-6 was detectable almost exclusively in rat liver myofibroblasts (Knittel et al. 1999a).

d Cells were stimulated by ATP and noradrenaline. e HSC were stimulated by mixture of cytokines.

5.6
Differences Between Human and Rat Hepatic Stellate Cells

The relevance of the results of many studies performed on cultured rat stellate cells should be evaluated for the human situation with caution since prominent gender-related differences have been reported. For example, while quiescent rat stellate cells express desmin (Tsutsumi et al. 1987), it was found to be minimally expressed or even absent in human stellate cells (Schmitt-Graaf et al. 1991). Rat HSC express α-smooth muscle actin only upon activation, while it is constitutively expressed in normal human liver (Schmitt-Graaf et al. 1991). Predominance of type B over type A endothelin receptors was found on rat stellate cells (Gabriel et al. 1999), whereas exactly the opposite situation was reported for human cells (Pinzani et al. 1996a). In contrast to rat cells, in human stellate cell cultures nitric oxide donors did not stimulate guanylate cyclase activity; however, rapid and sustained synthesis of PGE_2 and cyclic AMP was observed (Failli et al. 2000). The presence of these interspecies variations provides an additional rationale for performing more investigations on human stellate cells isolated from normal donor livers.

6 Pit Cells and Other Intrahepatic Lymphocytes

Morphological and phenotypical data indicate that liver sinusoids contain a very large and heterogeneous population of resident lymphocytes which comprise liver-specific natural killer (NK) cells, and different subpopulations of T cells (Jonsson et al. 1997; Winnock et al. 1995). It has been suggested that the term "liver-associated lymphocytes" (LAL), which encompasses all sinusoidal lymphocytes, can be used for this type of sinusoidal cells. LAL are defined as those lymphocytes that remain in sinusoids after liver perfusion with physiological solution at physiological pressure to remove circulating hematopoietic cells (Winnock et al. 1995). However, because liver contains also numerous extrasinusoidal lymphocytes that reside in portal tracts or between parenchymal cells, terms such as "intrahepatic lymphocytes" (IHL) or "liver-resident lymphocytes" that encompass many subpopulations of liver lymphocytes have recently been introduced (Crispie and Mehal 1996). The main groups of liver lymphocytes include: (1) pit cells, i.e., large-granular lymphocytes (LGL) that functionally correspond to the natural killer (NK) cells; (2) unconventional γδT lymphocytes; (3) T cells with conventional levels of T cell receptor (αβT cells) expressing either CD4 or CD8 molecules; (4) unconventional αβT cells (TCRintIL-2Rβ+) expressing IL-2β receptor and lower (intermediate) levels of CD3 than conventional T cells; (5) TCRintIL-2Rβ+ cells that exhibit dual T cell and NK cell phenotype (NK1.1+) and function (cytotoxicity), denoted NK T cells; (6) small population of c-Kit+ cells (stem cells) lacking lineage markers; and (7) small numbers of B lymphocytes and monocytes.

6.1 Isolation

Intrahepatic lymphocytes can be isolated by two methods: enzymatic digestion of liver tissue (Hata et al. 1990), and sinusoidal lavage (Bouwens et al. 1987). The latter method is based on the principle that the force which attaches IHL to sinusoidal endothelial cells or Kupffer cells in liver sinusoids is strong enough to resist the physiological pressure of portal blood flow, but can not resist a higher perfusion pressure, unharmful to resident sinusoidal cells. The mean yield of lymphocytes from human and mouse livers was 107/g tissue, which suggests that the average human liver contains a lymphocyte population of 109–1010 cells, i.e., 15%–20% of lymphoid cell number in the spleen (Mehal et al. 1999).

(Vanderkerken et al. 1990). LD pit cells may be regarded as the NK cells that are activated in vivo.

6.4 Morphology of Pit Cells

Pit cells can often be seen in the sinusoidal lumen (Fig. 4). They are in contact with Kupffer cell fillipodia or with sinusoidal endothelial cells; however, no junctional complexes have been observed (Bioulac-Sage et al. 1996). Wisse, Kaneda and Bioulac-Sage with their collaborators (e.g., Bioulac-Sage et al. 1996; Kaneda et al. 1994; Vanderkerken et al. 1990; Wisse et al. 1976) have provided detailed description of the pit cell ultrastructure. They are characterized by a low nucleo-cytoplasmic ratio and a cytoplasm containing few and often polarized organelles. Their shape can vary: round with short, small pseudopodia, elongated or with uropodes devoid of organelles. The chromatin-dense nucleus is often indented and displaced (Fig. 4). Well-developed Golgi apparatus with numerous vesicles is often seen in close association with an extensive microtubular network radiating from centrioles. The other main characteristics of liver LGL (Fig. 5) are electron dense granules that can be subdivided into

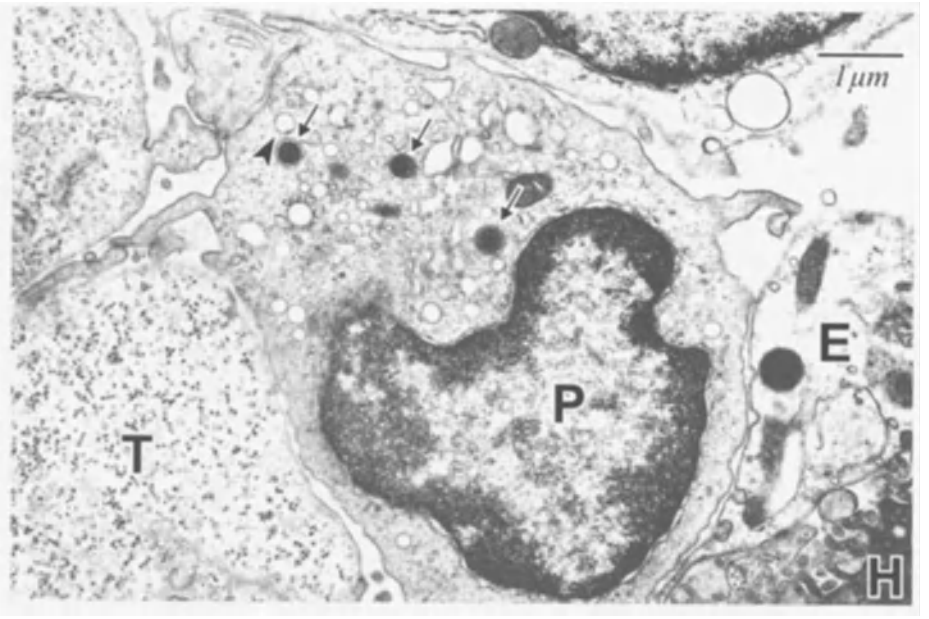

Fig. 4. Transmission electron microscopic photograph of a pit cell (*P*) that conjugates with ascite hepatoma tumor cells (*T*) AH130 in the lumen of rat hepatic sinusoid. Dense granules (*arrows*) and rod-cored vesicles (*arrowhead*) are present in the cytoplasm of pit cell. Close apposition of pit cell to endothelial cell (*E*). *H*, small part of hepatocyte's cytoplasm with short microvilli protruding into the space of Disse. *Space bar*, 1 μm. [Courtesy of Dr. Kenji Kaneda (Department of Anatomy, Osaka City University Medical School, Japan)]

(Norris et al. 1998). Activated liver NK T cells can secrete high levels of interleukin-4, but also IL-2, TNF-α, and interferon-γ (Bendelac et al. 1997). They also exhibit perforin and/or Fas ligand cytotoxicity, especially when activated by IL-12, which results in potent antitumor activity (Kawamura et al. 1999). Liver NK T cells may also play an important immunoregulatory role in the induction of immune tolerance (Trop et al. 1999), and act in the defense against metastatic tumor cells (Takeda et al. 1996).

6. The liver is the most important hematopoietic organ during fetal life. Although this function is drastically reduced after birth, a normal human adult liver contains pluripotent stem cells, i.e., c-kit+ cells, which under certain experimental conditions give rise to multiple cell lineages including thymic and extrathymic T-cells (Crispe and Mehal 1996; Crosbie et al. 1999). However, it has not yet been shown that extrathymic T cell development can function in the normal liver.
   [The term "stem cells" may also denote in the liver so-called oval cells. Oval cells are the progeny of bipotential hepatic stem cells derived from a few biliary epithelial cells, located probably in the smaller interlobular bile ducts and canals of Hering (Theisse et al. 1999). These cells intensively proliferate under the conditions of severe hepatocellular injury, and differentiate into cells of either hepatocyte or biliary cell lineages (Strain and Crosby 2000)].

The presence of many subpopulations of intrahepatic lymphocytes with multiple effector functions including abundant unconventional T lymphocyte subpopulations in the normal adult human liver suggests that they may have specialized functions in regional immune responses. Many data suggest that mammalian liver is an important site of immune surveillance, early regulation of adaptive immunity, and peripheral tolerance induction (Knolle and Gerken 2000).

Because of their distinct morphology and relatively well-characterized functions, only the pit cells, i.e., liver-specific NK cells, will be more extensively characterized in this chapter. However, it has to be noted that intensive studies of intrahepatic lymphocytes rapidly reveal new information about the role of the liver in the functioning of the immunological system in health and disease.

6.3 Origin and Development of Pit Cells

Pit cells derive from large-granular lymphocytes that originate from the bone marrow, circulate in blood, and marginate in the liver, where they lower their density and increase the number of granules. These cells first differentiate into high-density (HD) and subsequently into low-density (LD) pit cells (Vanderkerken et al. 1993). The process of pit cell maturation takes about 2 weeks (Bioulac-Sage et al. 1996), and may be influenced by factors released from neighboring nonparenchymal liver cells, most probably Kupffer and endothelial cells (Dobashi et al. 1999; Vanderkerken et al. 1995). Pit cells may proliferate locally when stimulated with interleukin-2 or other agents (Bouwens et al. 1990). LD pit cells have more but smaller granules than HD cells. HD cells show a stronger positive reaction for asialo-GM1, whereas the cytotoxicity of LD cells is about twice as strong as HD and five times as strong as the one of blood LGL

(Vanderkerken et al. 1990). LD pit cells may be regarded as the NK cells that are activated in vivo.

6.4 Morphology of Pit Cells

Pit cells can often be seen in the sinusoidal lumen (Fig. 4). They are in contact with Kupffer cell fillipodia or with sinusoidal endothelial cells; however, no junctional complexes have been observed (Bioulac-Sage et al. 1996). Wisse, Kaneda and Bioulac-Sage with their collaborators (e.g., Bioulac-Sage et al. 1996; Kaneda et al. 1994; Vanderkerken et al. 1990; Wisse et al. 1976) have provided detailed description of the pit cell ultrastructure. They are characterized by a low nucleo-cytoplasmic ratio and a cytoplasm containing few and often polarized organelles. Their shape can vary: round with short, small pseudopodia, elongated or with uropodes devoid of organelles. The chromatin-dense nucleus is often indented and displaced (Fig. 4). Well-developed Golgi apparatus with numerous vesicles is often seen in close association with an extensive microtubular network radiating from centrioles. The other main characteristics of liver LGL (Fig. 5) are electron dense granules that can be subdivided into

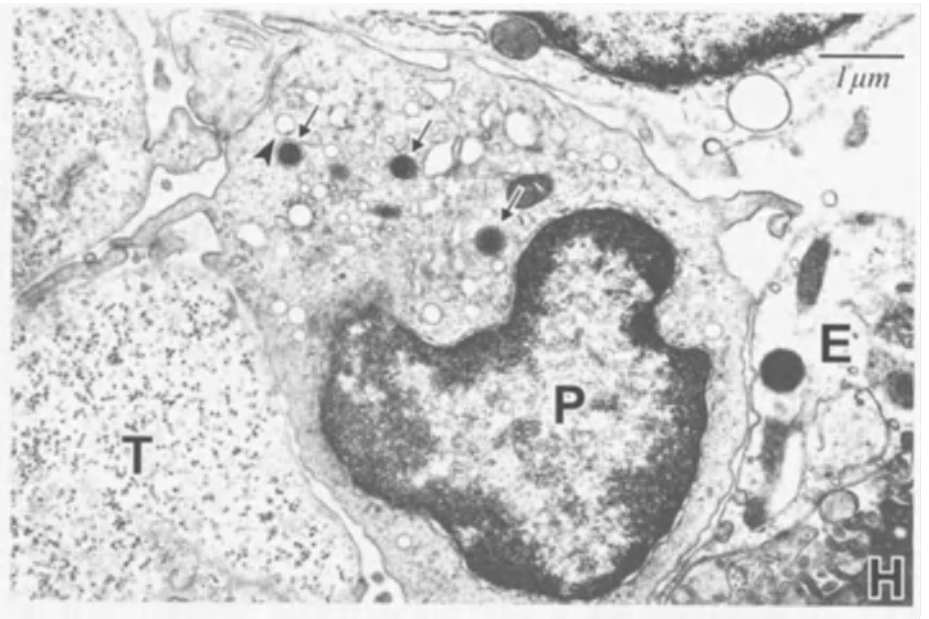

Fig. 4. Transmission electron microscopic photograph of a pit cell (*P*) that conjugates with ascite hepatoma tumor cells (*T*) AH130 in the lumen of rat hepatic sinusoid. Dense granules (*arrows*) and rod-cored vesicles (*arrowhead*) are present in the cytoplasm of pit cell. Close apposition of pit cell to endothelial cell (*E*). *H*, small part of hepatocyte's cytoplasm with short microvilli protruding into the space of Disse. *Space bar*, 1 μm. [Courtesy of Dr. Kenji Kaneda (Department of Anatomy, Osaka City University Medical School, Japan)]

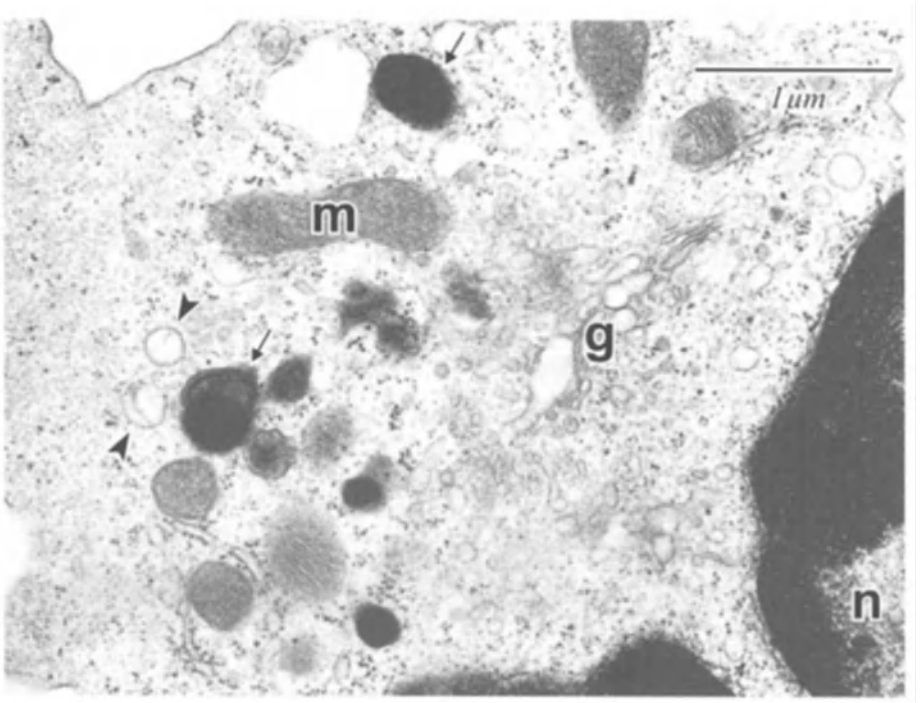

Fig. 5. Higher magnification of the part of pit cell. Well-developed Golgi apparatus (*g*), dense granules (*arrows*), rod-cored vesicles (*arrowheads*), mitochondria (*m*), and small part of nucleus (*n*) can be recognized. *Space bar*, 1 μm. [Courtesy of Dr. Kenji Kaneda (Department of Anatomy, Osaka City University Medical School, Japan)]

(1) typical dense granules and heterogeneous granules of a larger diameter with a dense core made up of proteins including a pore-forming protein (perforin or cytolysin), granzyme, and serine esterases, (2) numerous small uncoated vesicles, multivesicular bodies, and (3) rod-cored vesicles (Bioulac-Sage et al. 1996; Kaneda et al. 1994). The presence of significant numbers of many small coated or uncoated vesicles may indicate that pit cells may be very active in both endocytosis and exocytosis.

6.5 Functions of Pit Cells

Pit cells are liver-specific NK cells that possess a high level of natural cytotoxic activity against a variety of tumor cell lines, which is comparable to the cytotoxicity level of lymphokine-activated killer (LAK) cells (Kaneda and Wake 1983; Wisse et al. 1997). They spontaneously kill a variety of tumor cells in an MHC-unrestricted way, that is, without the involvement of antibodies or without the activation by cytokines or other signals (Hata et al. 1990). Pit cells are more cytolytic than peripheral blood NK cells against certain tumor cells (Vanderkerken et al. 1990; Vermijlen et al 1999), probably due to a stronger expression of CD11a/CD18 (LFA-1) adhesion molecule (Luo et al.

1999). Pit cells were also shown to induce apoptosis of rat colon carcinoma cell line (CC531) through the Ca2+-dependent perforin/granzyme pathway (Vermijlen et al. 1999). The role of pit cells in the antitumor defense was supported by the demonstration that administration of antibodies against liver NK cells prominently enhanced the metastasis of colon carcinoma cells in the rat liver (Rushfeldt et al. 1999; Shiratori et al. 1992). Pit cells may enhance antitumor activity through the secretion of interferon-γ, a multifunctional cytokine that exerts its cytotoxic activity directly on tumor cells or indirectly via stimulation of effector cells such as macrophages (Rushfeldt et al. 1999; Schuurman et al. 1995). Indeed, tumor cell killing was synergistically augmented when pit cells attacked tumor cells together with Kupffer cells (Thomas et al. 1995).

Apart from cytotoxic activity, liver NK cells may participate in the homing of thymic CD4+NK T cells to the liver through the expression of the LFA-1 molecule (Miyamoto et al. 2000). In this way pit cells may additionally increase the cytotoxic potential of the liver. Moreover, pit cells may participate in the hepatocyte damage as it has been shown that interleukin-2-activated natural killer cells can induce both apoptosis and necrosis in rat hepatocytes (Blom et al. 1999).

The majority of liver NK cells was shown to secrete IFN-γ; however, less than 10% produced IL-2, TNF-α, or IL-4 (Jonsson et al. 1997, 2000). Under specific conditions IL-1, IL-3, GM-CSF, and M-CSF were also secreted by hepatic NK cells (Bioulac-Sage 1996). The latter probably participates in the hematopoietic activity of the adult liver (Crosbie et al. 1999). Cultured pit cells also exhibit strong locomotory activity, which responds to chemotactic attraction (Bouwens et al. 1992).

PART II
Cross-Talk of Liver Cells in Physiology and Pathology

The data presented in the first part of this monograph show that there are tens of biologically active factors synthesized and released by nonparenchymal liver cells that may exert profound effects on neighboring cells (paracrine effects) or on themselves (autocrine action). The impact of these mediators on liver function has been defined to a great extent in experimental models of liver injury and in observations made in human liver disease. Paradoxically, the role of cell interactions under normal conditions is far less understood than in pathological ones. The reason for this is the lack of precise methods for the investigation of normal liver cell function in vivo. Most of the data documenting cooperation of liver cells derive from experiments performed on the cultures of different liver cell types which enabled studying the effects of various mediators such as cytokines, growth factors, hormones, active oxygen derivates or various biological response modifiers. Isolated cell systems can rarely serve as models of complete organisms or organs, but through the use of an in vitro test model, endogenic factors (e.g., hormonal or nervous effects) or experimental stress in laboratory animals can be eliminated as variables affecting hepatocellular responses. Parenchymal liver cells partially de-differentiate in culture by losing some of their important functions such as the potential for biotransformation (Guillouzo 1998). Hepatic stellate cells spontaneously change their phenotype in vitro undergoing transformation into myofibroblasts, a process that closely resembles the activation of stellate cells in vivo under conditions of acute or chronic liver injury (see Chap. 5). An obvious drawback of any cell culture system is the absence of neighboring cells that usually affect cells under investigation through the release of many more mediators than the few ones that can be tested in vitro. To overcome this problem, many authors have used the co-culture system of two liver cell types (or liver cells and extrahepatic cells like blood neutrophils, epithelial or tumor cell lines), to compare the results with those obtained in separate cell-type cultures. Alternatively, one type of liver cells was cultured in the presence of conditioned media from another liver cell type culture. Although the inherent complexity of the co-culture systems often did not allow to draw definitive conclusions, such methodological approaches helped to obtain valuable results that became starting points for targeted detailed studies. The results of some experiments demonstrating the effects of conditioned media of one liver cell type (or mediators identified in conditioned media) on the functions of co-cultured liver cells have been summarized in Table 11. However, it has to be remembered that the responses of target cells were often obtained by using concentrations of elicitors that were many times higher than those present in the natural milieu, and, hence,

Table 11. Effects of mediators released by different types of liver cells on other liver cells

Mediators released by "source" cell	Effects in "target" cells	References
"Source": Kupffer cells; "target": hepatocytes		
CMa,b	Modulation of protein synthesis	West et al. 1989
CM	Inhibition of cytochrome P-450 activity	Milosevic et al. 1999
CM	Increased NO synthesis	Curran et al. 1989
CMb, NO	Decrease of albumin synthesis	Kowalski et al. 1992
NOb	Inhibition of protein synthesis	Billiar et al. 1990a
NOb,c	Mitochondrial dysfunction	Kurose et al. 1996
CM, IL-1β	Increase of CINC expression and production	Mawet et al. 1996
IL-1βb	Induction of heme oxygenase 1 synthesis	Rizzardini et al. 1998
CMb, IL-1, TNF-α	Decreased canalicular anion transporter expression	Nakamura et al. 1999
IL-1β, TNF-α	Induction of IL-8 synthesis	Thornton et al. 1991
CMb, TNF-α	Induction of i-NOS mRNA and NO synthesis	Shiratori et al. 1998
CMb, TNF-α	Induction of hepatocyte apoptosis	Hamada et al. 1999
Various cytokines	Increased NO synthesis	Gaillard et al. 1991
CM, prostaglandins	Increased glycogenolysis	Casteleijn et al. 1988
Leukotriene A_4	Transformation into leukotriene C_4	Fukai et al. 1996; Titos et al. 2000
T_3 and T_4	Increased activity of lipogenesis and pentose cycle enzymes after thyroglobulin endocytosis by KC	Brix et al. 1997
"Source": Kupffer cells; "target": stellate cells		
CM	Regulation of hyaluronate synthesis	Gressner et al. 1988
CM, TGF-βb,c	Increased proteoglycan and collagen synthesis	Matsuoka and Tsukamoto 1990; Meyer et al. 1990a
CM	Induction of PDGF receptors resulting in increased matrix synthesis and stimulation of proliferation	Friedman and Arthur 1989
CM	Induction of cell proliferation	Meyer et al. 1990a
CM, PDGF	Tenfold increase in retinol release	Friedman et al. 1993
"Source": Kupffer cells; "target": sinusoidal endothelial cells		
CMb,c, TGF-β	Induction of ET-1 mRNA and protein synthesis	Eakes and Olson 1998
TGF-βb,c	Increased ET synthesis	Rieder et al. 1991

Table 11 *(continued).* Effects of mediators released by different types of liver cells on other liver cells

Mediators released by "source" cell	Effects in "target" cells	References
"Source": Kupffer cells; "target": liver NK cells		
CM, PGE_2	Suppression of activity	Tzung et al. 1990
CM, IL-12	Activation of liver NK and NK1.1+ cells	Dobashi et al. 1999
"Source": cultured hepatocytes; "target": stellate cells		
IGF-1	Induction of HGF synthesis	Skrtic et al. 1997
CM	IGF-I-independent HGF synthesis	Skrtic et al. 1999
CM	Activation of latent form of MMP-2	Theret et al. 1997
CM	Proliferation induced by factor(s) released from normal or damaged hepatocytes	Gressner et al. 1993b; Skrtic et al. 2000
"Source": cultured hepatocytes; "target": Kupffer or endothelial cells		
CM	Modulation of PGE_2 synthesis in KC	Billiar et al. 1990b
CMd	Induction of CINC synthesis in KC	Maher 1995
CM, VEGF	Stimulation of SEC growth in culture	Krause et al. 2000
"Source": SEC; "target": cultured hepatocytes		
IL-6	Induction of acute phase protein synthesis	Rieder et al. 1990
CM, prostaglandins	Increased glycogenolysis	Casteleijn et al. 1988
CMb, NO	Inhibition of α_1-acid glycoprotein and albumin secretion, partially mediated by NO	Itoh et al. 1994
"Source" cells: hepatic stellate cells; "target": cultured hepatocytes		
TGF-βe	Inhibition of proliferation	Meyer et al. 1990b
TGF-βc	Decreased albumin production and secretion	Koda et al. 1996

a CM, conditioned medium.
b After challenge of source cells with endotoxin.
c Co-culture experiment.
d Rat hepatocytes were incubated with ethanol, but did not produce CINC.
e Transformed (activated) stellate cells in culture. Control cultured cells generally showed low levels of activity, in contrast to activated ones.

might represent nonphysiological reactions. In the next chapters, examples of the interactions of liver cells in physiology and in some relatively well-characterized pathological situations will be reviewed with the emphasis placed on the complexity of the revealed relationships. First, the cellular cross-talk mediated by two different types of signaling molecules synthesized by cells of the hepatic lobule, eicosanoids, and nitric oxide will be presented. Then, the role of hepatic intercellular communica-

tion in the regulation of some important physiological functions of the liver, like vitamin A storage and glucose production, will be described. Functioning of the liver as an endocrine gland secreting growth factors and respective binding proteins will be shown to depend on the interactions between different kinds of liver cells. Regulation of sinusoidal blood flow will be demonstrated to result from the interplay of vasocontracting and vasodilating substances released from different types of liver cells that act on hepatic stellate cells. The functioning of intercellular signal networks under pathological conditions will be exemplified through the description of endotoxin effects on liver cells. Activation of hepatic stellate cells by plethora of signals generated by all main cell types of the liver lobule and recruited blood cells will be shown as the key event that leads to the development of liver fibrosis.

7 Cooperation of Liver Cells in the Synthesis and Degradation of Eicosanoids

Eicosanoids, the oxygenated derivates of 20-carbon unsaturated fatty acids (*eicosa*, "20" in Greek) comprising prostaglandins (PG), thromboxanes (TX), leukotrienes (LT), lipoxins and hydroxyeicosanoid acids (HETE), are immediately released after synthesis by a variety of cells in response to physiological and pathological stimuli. Since the biological activity of eicosanoids is rapidly destroyed both in tissues and in the circulation, they are regarded as mediators that maintain local homeostasis. In normal rat liver, eicosanoids are produced only by nonparenchymal cells (mostly Kupffer cells), whereas hepatocytes respond to the arachidonic acid metabolites, metabolize and excrete them into bile (Keppler 1994).

7.1 Synthesis and Catabolism of Eicosanoids

Synthesis of prostanoids and leukotrienes requires the net liberation of arachidonate, catalyzed mainly by phospholipase A_2 (Fig. 6). Prostaglandin-endoperoxide synthase, which contains both cyclooxygenase (COX) and peroxidase activity, catalyzes the initial reactions in prostanoid biosynthesis, i.e., formation of hydroperoxy-endoperoxide PGG_2, and its rapid peroxidation to the hydroxyendoperoxide PGH_2. Two COX isozymes (COX-1 and -2) are known. COX-1 is a constitutive enzyme that functions in normal cell physiology and is expressed in nearly all tissues under basal conditions. COX-2 is an inducible enzyme expressed in many cell types such as fibroblasts, macrophages, epithelial and endothelial cells, upon treatment with inflammatory cytokines, growth factors, and tumor promoters (Smith et al. 2000). Immunohistochemical studies revealed that under normal conditions COX-1 was present in the liver on the membranes of the nucleus and endoplasmic reticulum of sinusoidal endothelial cells and Kupffer cells; however, inducible COX-2 was not detected in normal liver (Suzuki-Yamamoto et al. 1999).

PGD_2 is formed by enzymatic or nonenzymatic isomerization of PGH_2, whereas PGE_2 is generated from PGH_2 through the action of prostaglandin E synthase. Both PGD_2 and PGE_2 belong to major eicosanoids secreted by Kupffer and sinusoidal endothelial cells (Casteleijn et al. 1988; Eyhorn et al. 1988; Hashimoto et al. 1995).

$PGF_{2\alpha}$ may be derived from PGH_2 by reduction of the endoperoxide or from PGE_2 by 9-keto reduction. $PGF_{2\alpha}$ is a vasoconstrictor in various vascular beds, including the liver (Iwai et al. 1988a). In normal rat liver PGF synthase was detected immunohistochemically only in SEC, which suggests that these cells are the main site of hepatic

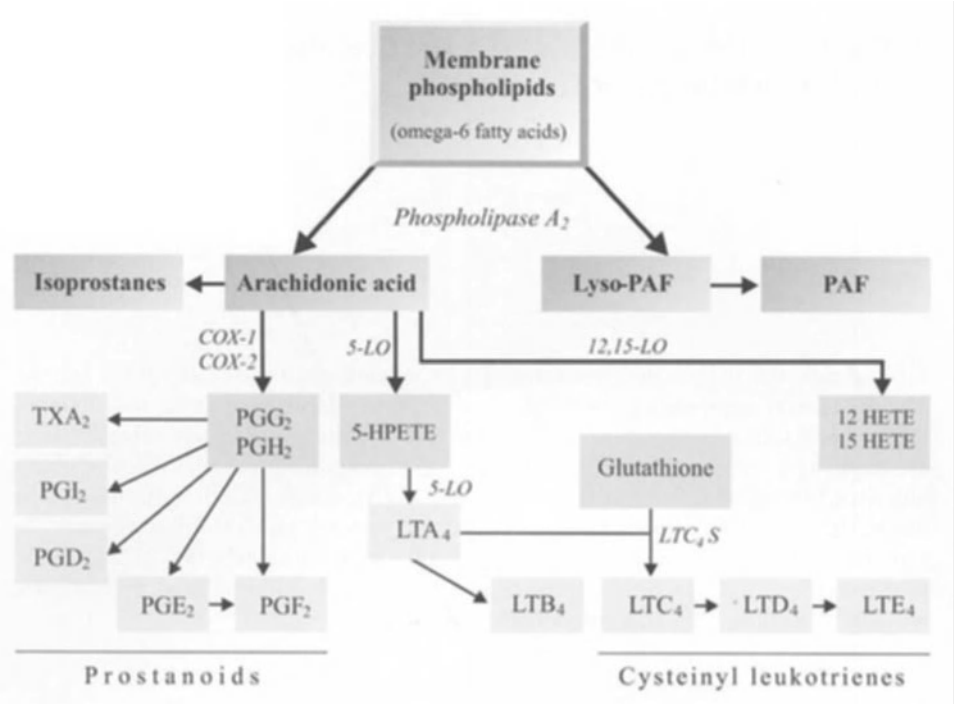

Fig. 6. Arachidonic acid metabolism. Eicosanoid synthesis pathways are shown with major metabolites and relevant enzymes (*italics*), abbreviations as described in text. *COX*, cyclooxygenase; *HETE*, hydroxyeicosatetraenoic acid; *LO*, lipooxygenase; *LTC_4S*, LTC_4 synthase

$PGF_{2\alpha}$ formation, since only in these liver cells a complete synthetic pathway from arachidonic acid to $PGF_{2\alpha}$ exists (Suzuki-Yamamoto et al. 1999).

Prostacyclin (PGI_2) is synthesized from PGH_2 by the microsomal PGI_2 synthase. PGI_2, which is mainly generated by endothelial and smooth muscle cells of blood vessels, inhibits platelet aggregation and relaxes blood vessels. Within seconds this prostanoid hydrolyzes to 6-keto-$PGF_{1\alpha}$.

Formation of thromboxane, TxA_2, the principal prostanoid generated by blood platelets, but synthesized also in many other cell types including Kupffer cells (Decker 1990), from PGH_2, is catalyzed by thromboxane A synthase, a cytochrome P-450 enzyme. TxA_2 spontaneously and rapidly hydrolyzes in water to its stable metabolite TxB_2. TxA_2 is a potent inducer of platelet aggregation and smooth muscle contractions.

After binding to specific receptors of parenchymal liver cells and exerting their actions (see below), prostanoids are rapidly and efficiently degraded by hepatocytes (Keppler 1994).

In contrast to prostanoids, cellular sources for leukotriene generation comprise a limited number of cell types that express the enzymatic activities for the 5-lipooxygenase pathway. The liver plays a major role in metabolism and elimination of leukotrienes. It produces cysteinyl leukotrienes (cLT) that have been implicated in the

regulation of the tone of hepatic sinusoids, and hepatocellular toxicity in several models of endotoxin-associated liver injury.

Leukotriene synthesis is initiated by 5-lipoxygenase that forms 5-hydroperoxyeicosatetraenoic acid (5-HPETE) from arachidonate, and then converts 5-HPETE to the 5,6-epoxide LTA_4 (Ford-Hutchinson et al. 1994). LTA_4 is either hydrolyzed to LTB_4 by a specific LTA_4 hydrolase or converted to LTC_4 through the conjugation with the tripeptide glutathione by a specific microsomal LTC_4-synthase. Removal of the γ-glutamyl moiety from LTC_4 by γ-glutamyl-transferase yields LTD_4. Irreversible hydrolysis of LTD_4 by the ectoenzyme LTD_4-dipeptidase leads to the formation of LTE_4 and glycine (Keppler 1994). Cysteinyl leukotrienes, LTC_4, LTD_4, and LTE_4, are rapidly eliminated from circulation and taken up mostly by the liver and kidney. Leukotrienes are secreted into bile by an ATP-dependent organic anion transporter localized in the canalicular domain of hepatocyte plasma membrane (Keppler et al. 1997).

7.2 Cellular Sources of Eicosanoids in the Liver

Different types of hepatic cells were shown to synthesize eicosanoids in the liver. It was demonstrated that activated Kupffer cells (Decker 1990) and sinusoidal endothelial cells (Eyhorn et al. 1988) produced significant amounts of prostanoids, mainly PGD_2 and PGE_2. The secretion of prostanoids by hepatic stellate cells seems to be limited to specific situations (Table 12). The production of eicosanoids by parenchymal liver cells was for many years a matter of controversy, as the conventional cultures of hepatocytes usually contained small amounts of nonparenchymal liver cells known to secrete prostanoids. However, it was shown that highly purified hepatocyte preparations, not contaminated by nonparenchymal cells, did not produce any cyclooxygenase products (Johnston and Kroenig 1996). Thus, hepatocytes do not synthesize eicosanoids; however, they respond to, metabolize, inactivate, and excrete eicosanoid degradation products into bile.

Cysteinyl leukotrienes found in the liver have long been presumed to be synthesized by Kupffer cells (and also by mast cells; Hagmann et al. 1992), since peripheral blood monocytes and exudate macrophages are capable of producing cysteinyl LT. However, it was demonstrated that in the rat liver the majority of the LTC_4 synthesizing activity was not localized in Kupffer cells but in hepatocytes (Fukai et al. 1993). It was next shown that in vitro hepatocytes possessed the capacity to synthesize LTC_4 from exogenous LTA_4, but not from arachidonic acid (Fukai et al. 1996). Therefore it has been proposed that the Kupffer cell-hepatocyte transcellular system plays an important role in cysteinyl LT production in rat liver (Fukai et al. 1996). Further studies have supported this hypothesis by the demonstration of 5-lipooxygenase gene expression in Kupffer cells and SEC, but not in hepatocytes, and by the detection of LTC_4-synthase mRNA in hepatocytes and SEC but not in Kupffer cells (Shimada et al. 1998). Thus, in the rat liver transcellular synthesis of leukotrienes takes place (Fig. 7).ig. 7).

In summary, hepatocytes are target cells for the action of eicosanoids and site of their transformation and degradation, but can not directly oxidate arachidonic acid to eicosanoids.

Table 12. Inducers of eicosanoid synthesis in nonparenchymal liver cells

	References
Prostanoid synthesis in Kupffer cells[a]	
Endotoxin (LPS)	Casteleijn et al. 1988; Gaillard et al. 1991
TNF-α	Decker 1990
PAF	Gandhi et al. 1992
Interferon-γ	Kawada et al. 1990
Anaphylatoxins C3a and C5a	Hespeling et al. 1995b; Schlaf et al. 1999
Glucagon	Hespeling et al. 1995a
Endothelin-1	Gandhi et al. 1992
Ethanol	Qu et al. 1996
ATP	Hashimoto et al. 1995
Calcium ionophore, phorbol esters, zymosan	Decker et al. 1990
Prostanoid synthesis in sinusoidal endothelial cells	
ATP	Hashimoto et al. 1995
Adenosine	Hashimoto et al. 1995
Endotoxin	Eyhorn et al. 1988
Prostanoid synthesis in hepatic stellate cells	
Anaphylatoxin C5a	Schieferdecker et al. 1998
ATP	Athari et al. 1994
Nitric oxide	Failli et al. 2000
Noradrenaline	Athari et al. 1994
Leukotriene synthesis in the liver	
Endotoxin (LPS)	Hagmann et al. 1985
TNF-α	Fukai et al. 1996
Viral infection	Hagmann et al. 1987
Several types of tissue trauma	Denzlinger 1996

[a] Prostanoids: PGD_2, PGE_2, $PGF_{2\alpha}$, TXA_2.

7.3 Expression of Prostanoid Receptors on Different Types of Liver Cells

Prostanoid actions in the body are mediated by specific receptors on plasma membranes that have been characterized pharmacologically, biochemically, and structurally (Coleman et al. 1994). These receptors are classified into five basic types, termed DP, EP, FP, IP, and TP, on the basis of their sensitivity to the five primary prostanoids,

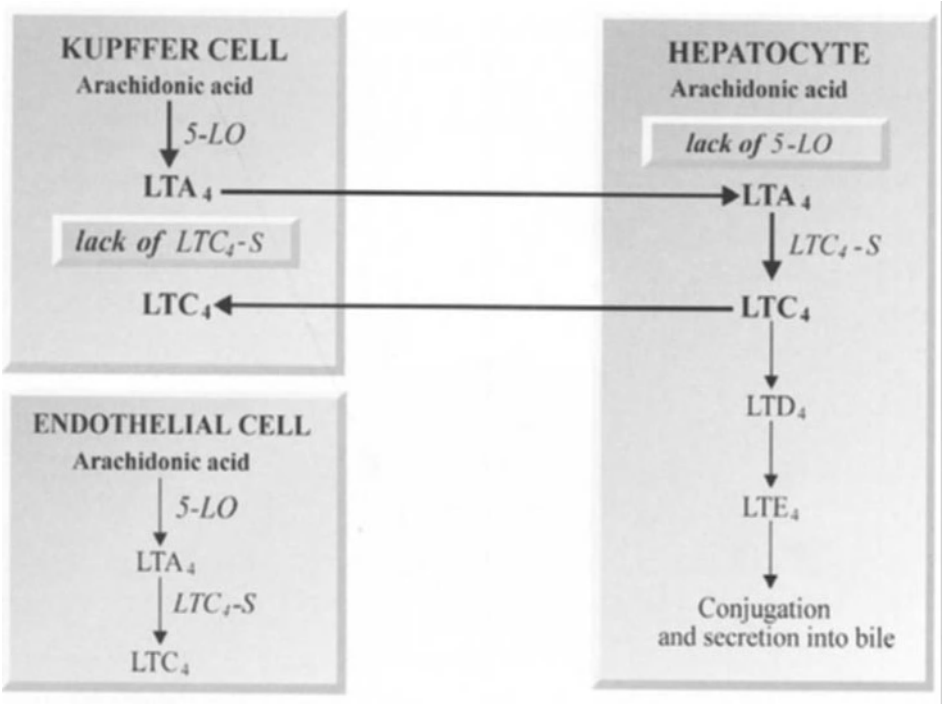

Fig. 7. Transcellular synthesis of leukotrienes between Kupffer cell and hepatocyte system (see text). *LO*, lipooxygenase; *LTC4 S*, LTC_4 synthase

PGD_2, PGE_2, PGF_2, PGI_2, and TXA_2, respectively. Furthermore, EP is subdivided into four subtypes, EP1, EP2, EP3, and EP4, on the basis of their responses to various agonists and antagonists. The cloning of TXA_2 receptor DNA and homology screening of mouse cDNA libraries subsequently identified the structure of all the eight types and subtypes of prostanoid receptors (Negishi et al. 1995). It was shown that prostanoids transmit their signal via G-protein-coupled receptors through the changes in second messenger levels. In addition, approaches based on Northern blot analysis, in situ hybridization, and RT-PCR have shown that each prostanoid receptor is specifically distributed in the body, and that expression levels are variable among tissues (Sugimoto et al. 2000).

The distribution of prostanoid receptors on various types of liver cells (Table 13), determined by the use of sensitive RT-PCR method (Fennekohl et al. 1999), may partially explain some controversies about the involvement of various prostanoids in the control of liver metabolism, and inflammatory mediator release from Kupffer cells. For example, PGE_2 has been reported both to stimulate glycogen-phosphorylase activity (glycogenolytic effect), and to inhibit the glucagon-stimulated glycogen-phosphorylase activity (antiglycogenolytic effect) in rat hepatocytes (Puschel et al. 1993). This apparent contradiction may be explained by PGE_2 action on type 1 receptor (stimulation of glucose output via an increase in the intracellular level of inositol triphosphate), and type 3 PGE_2 receptor (inhibition of cAMP-dependent glucose output). The subtype 2 PGE_2 receptor present on Kupffer cells can restrain the inflam-

Table 13. Expression of prostanoid receptor mRNAs on various types of rat liver cells

Receptor type	Natural ligand	G-protein	Second messenger	Relative amounts of prostanoid receptor mRNAs on different types of liver cells			
				Hepatocyte	KC	SEC	HSC
DP-R	PGD_2	G_s	cAMP ↑	–	(+)a	++b	+++
EP1-R	PGE_2	G_q	$InsP_3$ ↑	+++	+++	+++b	+++
EP2-R	PGE_2	G_s	cAMP ↑	–	+++	++	–
EP3-R	PGE_2	G_ic	cAMP ↓ or ↑	++	++b	++b	++
EP4-R	PGE_2	G_s	cAMP ↑	–	(+)	++	(+)
FP-R	$PGF_{2\alpha}$	G_q	$InsP_3$ ↑	+	–	–	–
IP-R	PGI_2	G_sc	cAMP ↑	–	(+)	++	+++
TP-R	Thromb oxane A_2	G_qc	$InsP_3$ ↑ $_{cAMP}$ ↑, ↓c	–	++b	+++	++

a +, ++, +++ describe relative abundancy of receptor mRNA in different types of liver cells, (+) very low levels in some cell preparations, ↑ and ↓ denote, respectively, increase or decrease of the intracellular concentration of the second messenger.

b Lack of functional evidence for the receptor presence on Kupffer cells, modified from Fennekohl et al. (1999).

c Involvement of other G proteins and different signal transducing mechanisms have also been described (Narumiya et al. 1999).

matory response of liver macrophages via an increase of intracellular cAMP level, leading to the suppression of TNF-α (Grewe et al. 1994) and IL-6 release (Callery et al. 1990). Moreover, a negative-feedback loop mediated by prostanoids may exist between Kupffer cells and proinflammatory cytokine-elicited hepatocytes. It has been shown that in vivo and in vitro administration of IL-6, the key acute phase cytokine, efficiently and rapidly upregulated hepatocyte G_S-coupled prostaglandin receptors (EP2-R, EP4-R, and DP-R) that are normally not expressed on these cells (Fennekohl et al. 2000). IL-6-stimulated cultured hepatocytes reacted to exogenous PGE_2 with the suppression of acute phase reaction (Fennekohl et al. 2000). Thus, it is possible that endotoxin-stimulated Kupffer cells that release both IL-6 and PGE_2 may limit the extent of acute-phase reaction in neighboring hepatocytes by rendering them sensitive to the negative control of their acute phase response by PGE_2 (see also Sect. 13.5.1). In the basal state hepatocytes may not exhibit cAMP-dependent responses to PGE_2 (Puschel et al. 1993) because of the absence of respective types of prostanoid receptors (Fennekohl et al. 1999).

7.4
Functions of Eicosanoids in the Liver

The hepatic actions of eicosanoids, molecules that may be generated both outside and inside the liver, can be grouped into vascular, metabolic, bile-secretory, and pro- or anti-inflammatory effects. In the intraorgan cell-cell communication Kupffer cells, sinusoidal endothelial cells, and hepatic stellate cells are sources of eicosanoids, and the intrahepatic vasculature, hepatocytes, and stellate cells are responding elements.

The hemodynamic actions of eicosanoids were studied in the preparations of isolated perfused liver of rat and other mammals. Portal pressure was shown to be elevated by the vasoconstricting activities of thromboxane A_2, PGD_2, and $PGF_{2\alpha}$ (Iwai et al. 1988a), whereas the vasodilatory effect of PGE_2 and prostacyclin (PGI_2) resulted in the decrease of portal pressure and liver blood flow (Kawada et al. 1992). Part of the PGE_2 action could be attributed to its relaxing effect on hepatic stellate cells (see Chap. 12). Cysteinyl leukotrienes were shown to profoundly decrease hepatic blood flow and increase portal pressure through their vasoconstrictory effects exerted at least partially at the level of hepatic stellate cells (Cincu et al. 1997; Iwai et al. 1988b; Titos et al. 2000). Thus, the sinusoidal blood flow may be controlled by hepatic stellate cells through the paracrine action of both prostanoids released from Kupffer and endothelial cells, and cysteinyl leukotrienes released from hepatocytes.

Beside hemodynamic actions prostaglandins have been implicated in the modulation of hepatocyte proliferation (Kimura et al. 2000) and liver regeneration (Skouteris et al. 1988), control of carbohydrate metabolism (Chap. 10), downregulation of proinflammatory cytokine formation in Kupffer cells (Chap. 13), inhibition of hepatic stellate cell proliferation (Chap. 14), and protection against liver or hepatocyte injury (see further sections in this chapter). Both prostaglandins and leukotrienes take part in the regulation of bile secretion (Beckh et al. 1994; Titos et al. 2000).

Apart from acting as mediators of the effects of many substances on the liver (Table 12), prostaglandins are formed in nonparenchymal liver cells in response to noradrenaline and adrenaline that are released in the liver after the stimulation of sympathetic nerves, and are thought to transpose many neural effects on metabolic and hemodynamic functions of the whole liver (Iwai et al. 1988a; Jungermann and Stumpel 1999).

7.4.1
Prostaglandins

Prostanoids produced in the liver may affect the activation status of both parenchymal and nonparenchymal liver cells, especially under pathological conditions. For example, Kupffer cells stimulated in vitro with bacterial endotoxin were shown to release prostanoids (and IL-10) that suppressed leukocyte adhesion to liver sinusoidal endothelial cells through the downregulation of the adhesion molecule (CD54, i.e., ICAM-1, and CD106, i.e., VCAM-1) expression on SEC (Knolle and Gerken 2000). Moreover, constitutive antigen presenting activity of SEC reflected by the activation of CD4+ T cells was shown to be inhibited by endogenously produced prostanoids (Knolle and Gerken 2000). PGE_2 was shown to suppress nitric oxide synthesis and the release of reactive oxygen intermediates from Kupffer cells (Harbrecht et al. 1995);

however, the opposite PGE_2 effect was also reported (Gaillard et al. 1992). Moreover, PGE_2 suppressed cytokine-stimulated nitric oxide synthase type 2 expression in isolated rat hepatocytes (Harbrecht et al. 1996).

It was found that PGE_2 stimulated O_2 consumption in cultured hepatocytes and in perfused rat liver through the stimulation of mitochondrial respiration via a cAMP-mediated mechanism (Qu et al. 1999). Thus, PGE_2 released from endotoxin-primed Kupffer cells may stimulate in a paracrine way ATP synthesis in hepatocytes, which would be beneficial for cellular protection against endotoxemia.

Prostaglandin E_2, the main prostanoid of blood and tissue macrophages including activated Kupffer cells, was shown to inhibit inflammatory cytokine synthesis in LPS-elicited Kupffer cells. Interestingly, this effect, apart from the immunosuppressive action of PGE_2 on blood leukocytes, may represent the means for autoregulation of PGE_2 production in liver macrophages. IL-6, and TNF-α, key proinflammatory cytokines secreted by activated Kupffer cells, were shown to elicit PGE_2 formation in Kupffer cells; however, PGE_2 attenuated release of these cytokines from Kupffer cells, and in this way suppressed its own production (Callery et al. 1990; Karck et al. 1988; Peters et al. 1990). Thus, the interrelationships between the release from activated Kupffer cells of pro- or anti-inflammatory mediators depend on the delicate balance of reciprocally acting substances that may be rapidly induced upon the effects of many endogenous or exogenous factors.

It has been shown that the inhibition of cytokine release by PGE_2 was initiated by its binding to the EP2 and EP4 receptor subtypes on Kupffer cells, which led to the increase of cAMP intracellular concentration (Grewe et al. 1994; Table 13). Elevated levels of cAMP inhibited the activation of nuclear factor NFκB (via retarded degradation of the inhibitory factor IκB), and stimulated activating transcription factor/cAMP response element (ATF/CRE) site-mediated gene transcription. In this way the production of cytokines, the genes of which contain an NFκB site in the 5' regulatory region such as TNF-α and IL-12, is inhibited by cAMP-elevating agents (such as PGE_2), and the expression of cytokine genes containing an ACF/CRE response element-1-like site, such as IL-10, is upregulated by the cAMP-dependent pathway (Grewe et al. 1994).

In many models of liver or hepatocyte injury prostaglandins such as PGE_2, PGI_2, and their synthetic derivates, have been shown to exert a cytoprotective action (e.g., Gove et al. 1990; Kmiec 1994; Stachura et al. 1979). The mechanisms underlying the beneficial effects of prostaglandins are probably different depending on the specific model of liver injury. The cytoprotective action of prostaglandin E_1 has been used for the protection of donor livers during hepatic transplantation in humans (Klein et al. 1996). This effect can be partially related to the suppression of sinusoidal endothelial cell activation in the donor liver (Mizoguchi et al. 1991; Yamaoka 1997).

7.4.2 Leukotrienes

Contrary to the cytoprotective effects of prostaglandins, cysteinyl leukotrienes, specifically LTC_4 and LTD_4, have been associated, through the beneficial effects of leukotriene antagonists, with the induction of liver injury in the galactosamine/endotoxin model of fulminant hepatitis (Tiegs and Wendel 1988) and endotoxic shock (Hag-

mann et al. 1985). Since no direct damage of freshly isolated rat hepatocytes by exogenously added LTC_4 or LTD_4 could be observed (Kmiec et al. 1992), these cytotoxic effects had to be mediated by factors released from nonparenchymal liver cells and extrahepatic cells, most probably TNF-α (Tiegs et al. 1989). One mechanism of deleterious action of leukotrienes released from in vivo LPS-elicited Kupffer cells may involve suppression of sinusoidal endothelial cell function reflected by diminished hyaluronan uptake (Deaciuc et al. 1994b)

8 Nitric Oxide as a Mediator of Intercellular Communication in the Liver

Nitric oxide (NO), a short-lived free radical first identified as a potent endothelium-derived relaxing factor released from vascular endothelial cells, is synthesized by different cell types and plays an important role in many processes, such as blood pressure regulation, neurotransmission, tumor cell killing, immunity, and inflammation (Moncada et al. 1991). Its role in the regulation of cell and organ function under physiological and pathological conditions is complex, especially in the liver, where complicated relationships exist between different cell types that may be both sources of nitric oxide and targets of its action.

8.1 Nitric Oxide Synthesis in Liver Cells

NO is formed from l-arginine and molecular oxygen by the l-arginine:NO pathway catalyzed by the enzyme NO synthase (NOS) yielding citrulline as a coproduct. Nitric oxide synthase has three distinguishable isoforms: (1) NOS-1 (ncNOS), a constitutive isoform originally isolated from neuronal sources and dependent on Ca2+-calmodulin; (2) an inducible Ca2+-independent form of the enzyme, NOS-2 or iNOS, that can be increased in response to inflammation and oxidative stress, and has been identified in the liver in many cell types (Kupffer cells, sinusoidal endothelial cells, hepatocytes, stellate cells, cholangiocytes); (3) NOS-3 (eNOS), a constitutive Ca2+-dependent isoform located in endothelial cells (Clemens 1999). Under normal conditions, only the constitutive eNOS is present in the liver, and the low level of NO produced by eNOS contributes to the regulation of hepatic blood flow (Clemens 1999). NOS-2, however, is readily upregulated in the liver under a number of conditions, including endotoxemia, hemorrhagic shock, ischemia-reperfusion, sepsis, infection, hepatitis, ozone exposure, and liver regeneration (Clemens 1999). Besides cells of hepatic parenchyma, in the liver NO may be released from other cell types, such as hepatic vascular endothelium, cholangiocytes, nerve endings, mast cells, platelets, or blood mononuclear cells, in a response to various stimuli. However, it is believed that under pathological conditions Kupffer cells and hepatocytes are the main source of NO (Clemens 1999).

The cytokine-mediated upregulation of iNOS gene transcription occurs via the activation of the transcription factor NF-κB (de Vera et al. 1997). In most cell types the action of NO is largely dependent on the stimulation of the soluble form of guanylate cyclase that catalyzes the synthesis of cyclic GMP (Moncada et al. 1991); however, cGMP-independent effects of nitric oxide have also been reported (Clemens 1999).

8.1.1
Regulation of NO Synthesis in Hepatocytes

NO production by cultured hepatocytes was not significantly enhanced by endotoxin (Shiratori et al. 1998); however, in vivo endotoxin (LPS) administration or in vitro combination of cytokines (IL-1β, TNF-α, and INF-γ) were shown to induce iNOS synthase activity in human (Liu et al. 1996), pig (Monshouwer et al. 1996), and rat (Curran et al. 1990; Kitade et al. 1996; Laskin et al. 1995; Shiratori et al. 1998) hepatocyte cultures. Although IL-1β, TNF-α, and INF-γ acted synergistically to induce iNOS expression (Geller et al. 1993), only IL-lβ alone, in the absence of other cytokines or LPS, was shown to be an effective stimulator of iNOS in primary cultured rat hepatocytes (Kitade et al. 1996; Shiratori et al. 1998). These data strongly suggest that in endotoxemia cytokines released by activated Kupffer cells are potent inducers of nitric oxide synthesis in hepatocytes.

The classical method of hepatocyte isolation by the collagenase perfusion of the liver was shown to transiently induce NOS-2 in these cells (Wang et al. 1998a). The effect was endotoxin- and serum-independent, and resulted from de novo protein synthesis (Wang et al. 1998a). The response was triggered by a complex interaction between several different factors including Kupffer cell activation, reactive oxygen species generation, and endotoxin contamination of some collagenase preparations (Tirmenstein et al. 2000).

Nitric oxide synthesis and iNOS expression in human hepatocytes were shown to be inhibited by hepatic mitogens, HGF, EGF, and TGF-α, which also suppressed and partially prevented NO-induced inhibition of DNA and protein synthesis (Liu et al. 1996). Such effects of mitogenic cytokines may be important for promoting the regeneration of liver tissue in various forms of inflammation. Dexamethasone negatively regulated iNOS expression in hepatocytes by inhibiting transcription factor NF-κB through the upregulation of inhibitory factor IκB (De Vera et al. 1997; Shiratori et al. 1998), which indicates that glucocorticoids may express their anti-inflammatory actions also at this level.

8.1.2
Regulation of NO Synthesis in Kupffer Cells

Many factors and conditions, some listed in Table 14, stimulate or suppress the expression of iNOS gene in liver macrophages that are thought to represent the major hepatic source of nitric oxide under pathological conditions (Clemens 1999). Attenuation of LPS-induced iNOS formation in Kupffer cells by elevated intracellular cAMP levels due to PGE_2 action (Harbrecht et al. 1995) was shown to occur by preventing the degradation of inhibitory factor IκBα, which suppressed the activation of the nuclear factor NFκB, and inhibited the transcription of the iNOS gene (Mustafa and Olson 1998).

Table 14. Factors eliciting and inhibiting NO synthesis in Kupffer cells

	References
Inducers and stimulators	
LPS, LPS, and IFN-γ	Gaillard et al. 1991; Shiratori et al. 1998
TNF-α	Gaillard et al. 1991
PAF, also in LPS-elicited KC	Mustafa et al. 1996
Interferon-γ	Gaillard et al. 1992; Rockey et al. 1998
Hyaluronan fragments	Rockey et al. 1998
Endothelin-1	Stephenson et al. 1997
IL-1β	Kitade 1996; Shiratori et al. 1998
Inhibitors of NO synthesis in LPS- or cytokine-stimulated Kupffer cells	
Glucocorticoids	Grewe et al. 1994
PGE_2	Gaillard et al. 1992; Harbrecht et al. 1995, 1996

8.1.3 Regulation of NO Synthesis in Sinusoidal Endothelial Cells and Hepatic Stellate Cells

In the liver only sinusoidal endothelial cells (Rockey and Chung 1998) express the constitutive form of nitric oxide synthase (eNOS), produce NO basally, and increase its synthesis in response to blood flow (Shah et al. 1997). Similarly to Kupffer cells the inducible form of NOS was upregulated by endocytosed hyaluronan fragments (Rockey et al. 1998), interferon-γ, and LPS (Rockey and Chung 1996); however, in contrast to Kupffer cells, LPS alone only transiently induced iNOS mRNA, whereas the combination of IFN-γ and TNF-α resulted in the prolonged induction of iNOS mRNA in SEC (Rockey and Chung 1996). Thus, through the secretion of many cytokines (Table 17) endotoxin-activated Kupffer cells may induce NOS-2 expression not only in hepatocytes, but also in sinusoidal endothelial cells.

In hepatic stellate cells LPS alone (Kawada et al. 1998), interferon-γ, with or without LPS or TNF-α (Helyar et al. 1994; Rockey and Chung 1995), were shown to induce NO synthesis, which may lead to the relaxation of hepatic sinusoids, and inhibition of stellate cell proliferation (Kawada et al. 1998). These in vitro findings may be of clinical significance, because leukocytes that infiltrate portal areas in patients with chronic liver disease may stimulate, through the release of proinflammatory cytokines, adjacent stellate cells to produce NO, and thus be involved in the pathomechanisms of disturbed hepatic circulation in these patients.

8.2
Nitric Oxide Effects on Liver Functions

Similarly to eicosanoids NO exerts in the liver hemodynamic, metabolic, and pro- or anti-inflammatory effects. Under basal conditions, nitric oxide is postulated to modulate hepatic vascular tone in the normal liver (Clemens 1999), affecting sinusoidal blood flow through its relaxing effect on hepatic stellate cells and other segments of liver vasculature (Chap. 12). Nitric oxide may regulate hepatic metabolism directly, by causing alterations in the functions of hepatocytes and Kupffer cells, or indirectly, as a result of its vasodilatory properties. NO is involved in the regulation of hepatic carbohydrate metabolism (Chap. 10). Under physiological conditions NO was shown to increase bile-acid-independent canalicular bile secretion (Trauner et al. 1998).

Nitric oxide displays cytotoxic and cytoprotective effects depending on the site and quantity produced (Clemens 1999). Excessive NO generated by hepatic cells in response to endotoxin and inflammatory substances (e.g., PAF) prominently contributes to the pathophysiological outcomes observed in the liver during sepsis (Chap. 13). Oxidative stress was shown to increase induction of hepatocyte NOS by the proinflammatory cytokine interleukin-1 (Kuo et al. 2000), which may enhance NO-mediated protection against reactive oxygen-related liver injury (Sect. 13.4).

Although high levels of NO induce apoptosis in a number of cell types, relatively low levels of NO can block it in others (Moncada et al. 1991). In cultured hepatocytes NO suppressed apoptosis via the interruption of caspase activation and mitochondrial dysfunction (Li et al. 1999).

Another role for nitric oxide in the liver emerges from the induction by NO of heme oxygenase activity and the production of carbon monoxide through the cGMP-related mechanisms; this effect may be involved in the cytoprotective actions of both gaseous monoxides (Polte et al. 2000).

9 Cooperation of Hepatocytes and Stellate Cells in the Metabolism and Storage of Retinoids

All retinoids (vitamin A and its analogs) must be provided to higher animals in the diet, either as preformed vitamin A or as provitamin A carotenoids, since they are necessary for embryonic development, growth, vision, and survival of vertebrates (Vogel et al. 1999). Vitamin A active substances are compounds, other than carotenoids, that exhibit qualitatively the biological activities of all-*trans*-retinol (free-alcohol form of vitamin A). Retinol can be reversibly converted by enzymatic activity to the visually active form of vitamin A, retinal, in a variety of tissues. Retinoic acid (RA), the oxidized form of retinal, has been shown to be the active derivative of vitamin A, being a potent transcription factor. Retinoic acid transduces its pleiotropic effects by two families of nuclear receptors, the RARs and the RXRs, which function as nuclear transcription factors (Mangelsdorf and Evans 1995). Whereas retinol, retinal, and retinoic acid are physiologically active forms of vitamin A, all are toxic in high concentrations, so that surplus of vitamin A must be stored. Long-chain fatty esters of the vitamin, manufactured in the liver by the action of acyltransferase enzymes, are the primary storage form. The majority (50%–80%) of the total body vitamin A is stored in the liver as long-chain fatty acid esters of retinol (Vogel et al. 1999). Hepatic retinoid stores serve as the major source of the retinoid that is utilized by all tissues throughout the body; retinyl ester reserves are hydrolyzed by hydrolytic enzymes to yield retinol when its plasma concentration declines. Additionally, a minor part of postprandial retinyl ester is taken up by extrahepatic tissues, and stored in extrahepatic stellate cells that were identified in lung, kidney, and intestine (Nagy et al. 1997). Despite intensive investigations the exact mechanisms underlying retinol uptake by organs and tissues are still unknown (Vogel et al. 1999).

Vitamin A is absorbed in the small intestine, where it is incorporated into chylomicrons as retinyl esters for release into the lymph, and further distributed via blood to the liver for storage (Fig. 8). Chylomicron remnants containing retinyl esters are internalized exclusively by hepatocytes, and no other type of liver cells. Very shortly after receptor-mediated endocytosis, retinyl esters present in chylomicron remnants are hydrolyzed in early endosomes to retinol (Matsuura et al. 1993). Depending on the vitamin A nutritional status of the animal, retinol combines in the endoplasmic reticulum with retinol-binding protein, RBP, and is either secreted from the hepatocytes, oxidized to retinoic acid or stored as retinyl esters (Fig. 8). Retinol binds intracellularly to specific cellular retinal-binding proteins, CRBP-I and CRBP-II, that also play an important role in retinyl ester hydrolysis and formation, and oxidation of retinal in retinoic acid (Vogel et al. 1999). Although hepatocytes can contain 5%–30% of the total retinol (retinol + retinyl ester) present in the liver of normal rats, most of

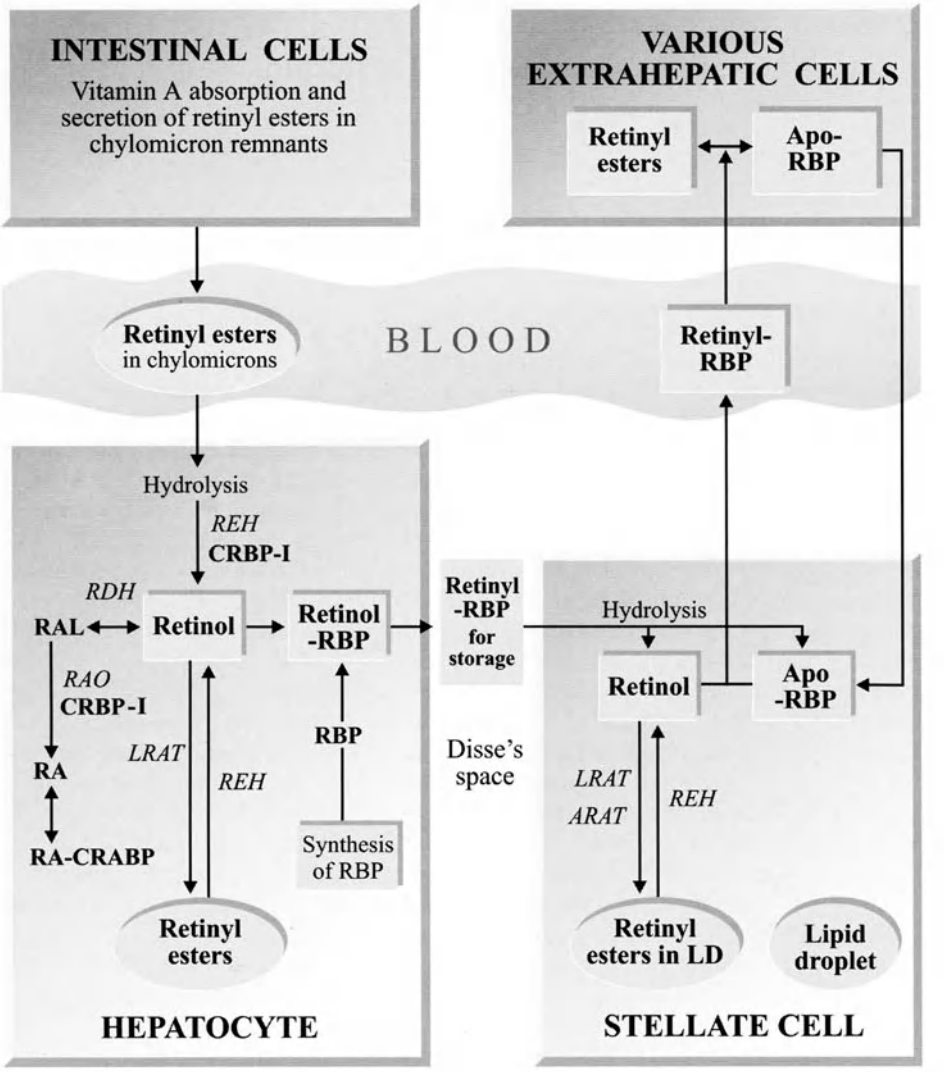

Fig. 8. Main pathways of retinol metabolism in the liver (description in text). *RBP*, retinol-binding protein; *CRBP*, cellular retinol-binding protein; *LD*, lipid droplets in stellate cells; *ARAT*, acyl-CoA:retinal acyltransferase; *LRAT*, lecithin:retinol acyltransferase; *RA*, retinoic acid; *CRABP*, cellular retinoic acid binding protein; *RAL*, retinal; *RDH*, retinol dehydrogenase (catalyzes reversible reaction); *REH*, retinyl ester hydrolase; *RAO*, retinal oxidase (catalyzes irreversible reaction)

it (70%–95%) is stored in stellate cells (Hendriks et al. 1985) after uptake of RBP-bound retinol and its re-esterification to retinyl esters (Vogel et al. 1999).

Although the interactions between hepatocytes and stellate cells need to be precisely regulated for the control of retinoid homeostasis, little is known about the ways in which these types of liver cells communicate. It had been assumed that RBP-retinol

was secreted by hepatocytes into extracellular space and taken up by stellate cells (Blomhoff et al. 1988). However, it has also been shown that the uptake of retinol into stellate cells may not be dependent on RBP (Matsuura et al. 1993). The liver is the major site of RBP synthesis in the body, and this synthesis takes place only in hepatocytes and not nonparenchymal liver cells (Vogel et al. 1999). However, stellate cells represent the main cellular storage site of vitamin A in the liver as they were shown to contain 300 times the amount of retinyl esters present in hepatocytes on a cell protein basis (Hendriks et al. 1985). Hepatic stellate cells are highly enriched in cellular retinol-binding protein type I (CRBP-I), and in enzymes active in retinal metabolism as compared with hepatocytes (Vogel et al. 1999). CRBP-I plays an essential role for vitamin A homeostasis, since this protein was shown to be indispensable for efficient synthesis and storage of retinyl ester in mice with inactivated gene encoding CRBP-I (Ghyselinck et al. 1999). CRBP-I directs retinol for esterification with long-chain fatty acids by lecithin:retinol acyltransferase (LRAT), an enzyme whose specific activity was circa tenfold greater in nonparenchymal liver cells than in the hepatocyte fraction of vitamin A-sufficient rats (Matsuura et al. 1997). LRAT may additionally contribute to the process of retinol uptake by HSC and to the maintenance of nontoxic retinol levels in plasma (Matsuura et al. 1999). Stellate cells also contain an intracellular binding protein for retinoic acid, cellular retinoic acid-binding protein type I (CRABP-I; Vogel et al. 1999).

In the rat, the amount of retinoids present in stellate cells and the relative distribution of the hepatic retinoid stores between the parenchymal and stellate cells is dependent on the body's vitamin A status, and on the cell-to-cell interactions between hepatocytes and HSC (Vogel et al. 1999). At times of low utilization of retinol in the peripheral tissues, when the levels of retinol-RBP are high within the liver, the stellate cells actively take up retinol for storage as retinyl ester. At times of high retinol utilization by the extrahepatic tissues, retinol-RBP, newly secreted by hepatocytes, enters the circulation to deliver retinol to peripheral tissues. The apo-RBP formed upon uptake of retinol in the periphery recycles to the liver, where additional retinol is taken up from the storage depots in the stellate cells for delivery to peripheral tissues (Fig. 8). In this way hepatic stellate cells play a primary role in providing tissues and cells with optimal amounts of vitamin A despite huge fluctuations in daily vitamin A intake (Vogel et al. 1999). However, when liver retinoid reserves are low, most of the liver retinoid is found in parenchymal cells (Batres and Olson 1987).

10 Cooperation of Liver Cells in the Regulation of Glucose Metabolism

The liver plays a central role in the regulation of glucose homeostasis in mammals through the ability of hepatocytes to store glycogen during the resorptive phase (glucogenesis), and to release glucose in the postprandial period as a result of glycogenolysis and de novo glucose synthesis from noncarbohydrate substrates, such as lactate, glycerol, or amino acids, i.e., gluconeogenesis. Hepatic glucose production, necessary for survival in the fasting state, plays a key role in the maintenance of glycemia. The glucoregulatory function of hepatocytes is controlled mainly by insulin, glucagon, catecholamines, and glucocorticoids, whereas many other hormones, cytokines, and various mediators play an accessory role in the fine-tuning of glucose homeostasis to the requirements of specific physiological and pathophysiological situations (Cherrington 1999). In acute systemic inflammation many mediators released both extra- and intrahepatically are of great importance for the adaptation of the liver to the energetic needs of the whole body.

10.1 Effects of Prostaglandins

It has been shown that many mediators that reach the liver during inflammatory processes, such as endotoxins, immune-complexes, anaphylatoxins, and PAF, increase glucose output in the perfused liver, but fail to do so in isolated hepatocytes (reviewed by Wake et al. 1989). The release of glucose under these circumstances was inhibited by cyclooxygenase inhibitors (Casteleijn et al. 1988), which suggested that the aforementioned activators acted indirectly on hepatocytes via prostanoids synthesized in nonparenchymal liver cells (mainly Kupffer and endothelial cells). Indeed, it has been demonstrated that exogenous prostaglandins may stimulate glucose production in the perfused liver and isolated hepatocytes through the direct activation of glycogen phosphorylase *a* via a phospholipase C-linked signal transduction pathway (Casteleijn et al. 1988). In another experimental approach inactivation of Kupffer cells by gadolinium chloride almost completely abolished adenosine or ATP-stimulated glycogenolysis in the perfused rat liver (Nukina and Thurman 1994).

Anaphylatoxin C5a, the plasma-born small inflammatory protein generated as *N*-terminal cleavage product during the activation of complement factor C5, was found to enhance glucose output from hepatocytes through the prostanoid release from nonparenchymal liver cells, mainly Kupffer cells (Hespeling et al. 1995b) and stellate cells (Schieferdecker et al. 1998). The hypothesis of the indirect action of C5a was further supported by the results of quantitative RT-PCR, immunocytochemical

Table 15. Substances stimulating hepatic glycogenolysis via prostaglandin-mediated intercellular communication between nonparenchymal liver cells and hepatocytes

Factor [a]	Prostanoid	Liver cell(s) releasing PGs[a]	References
Anaphylatoxin C5a	Prostanoids	KC, HSC	Hespeling et al. 1995b
Adenosine	PGD_2	nd	vom Dahl et al. 1990
ATPb	nd	KC	Nukina and Thurman 1994
ATP	$PGF_{2\alpha}$, PGD_2	HSC	Athari et al. 1994
Endotoxin (LPS)	PGD_2, $PGF_{2\alpha}$, PGE_2	KC	Casteleijn et al. 1988
Endothelin-1	nd	SEC, HSC	Gandhi et al. 1992
Immune complexes	nd	nd	Buxton et al. 1987
Nitric oxide	nd	nd	Borgs et al. 1996
Noradrenaline	$PGF_{2\alpha}$, PGD_2	HSC	Athari et al. 1994
PAF	PGD_2	KC	Kuiper et al. 1988

nd, not determined.

a Various factors may be generated either locally in the liver or systematically.

b ATP was released from hepatocytes stimulated with adenosine.

and functional studies, which showed C5a receptor (C5aR) mRNA and C5aR protein expression mainly on Kupffer cells, strongly on stellate cells, but much weaker on sinusoidal endothelial cells (Schlaf et al. 1999). Although under normal conditions rat hepatocytes did not show C5aR mRNA or protein (Schlaf et al. 1999), functional C5a receptors have been induced in liver parenchymal cells both on the mRNA and protein level by the in vivo treatment of rats with IL-6, a major proinflammatory cytokine (Schieferdecker et al. 2000). These findings indicate that hepatocyte-specific defense reactions might be differentially regulated under normal and inflammatory conditions. In the later phase of liver inflammation, which is mediated by intra- or extrahepatically released cytokines and other substances, the products of complement activation may directly stimulate functions of liver parenchymal cells. Interestingly, endotoxin, a major activator of liver macrophages, did not stimulate glucose output from the perfused liver (Pestel et al. 2000), although endotoxin is known to release large amounts of prostaglandins from Kupffer cells (Sect. 7.1).

Many substances (Table 15) synthesized within the liver during a local inflammatory process or generated systematically, e.g., in the course of sepsis, may indirectly increase hepatic glycogenolysis through the prostaglandin-mediated intercellular communication between nonparenchymal liver cells (mainly Kupffer cells) and hepatocytes (Fig. 9). The release of glucose from glycogen supports the increased demand for energetic fuel by the inflammatory cells such as leukocytes, and additionally

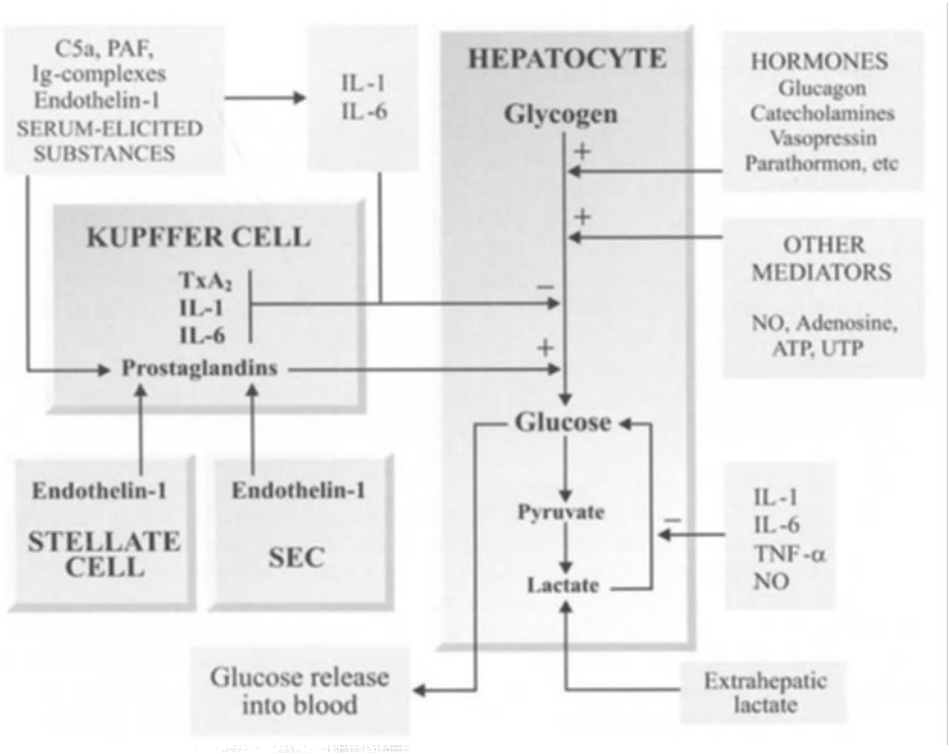

Fig. 9. Substances released from nonparenchymal liver cells or derived from extrahepatic sources that stimulate glycogenolysis or inhibit gluconeogenesis (from lactate) in hepatocytes directly or through cell-to-cell communication. *Symbols:* +, stimulation; –, inhibition of the process

enables enhanced glucose turnover in sinusoidal endothelial cells and Kupffer cells which is necessary for effective defense of these cells against invading microorganisms and oxidative stress in the liver (Sect. 13.5.2).

Glucagon-stimulated hepatic gluconeogenesis has been shown to be antagonized by prostaglandin E_2 through the inhibition of cAMP formation, which suppressed glycogen phosphorylase activity and glucose output from hepatocytes (Puschel and Christ 1994). Moreover, in isolated hepatocytes PGE_2 accelerated the decay of phosphoenolpyruvate carboxykinase mRNA and reduced activity of its key gluconeogenic enzyme, which was previously induced by glucagons (Puschel and Christ 1994), probably via a G_i-linked signal transduction (cf. Table 13). These data imply that during inflammation PGE_2, derived mainly from activated Kupffer cells, may reduce liver gluconeogenic capacity as a part of strategy to spare liver resources (amino acids) for the acute-phase response (see Sect. 13.5.1).

10.2
Effects of Cytokines and Nitric Oxide

Proinflammatory mediators, such as interleukin-1β, interleukin-6, TNF-α, and nitric oxide, which may be elicited systematically or released from nonparenchymal liver cells, have been shown to modulate the regulation of hepatic glucose metabolism.

It has been found that IL-1β, IL-6 (Kanemaki et al. 1998), and nitric oxide (Sprangers et al. 1998) markedly diminish insulin-stimulated deposition of glycogen in primary rat hepatocyte cultures through the inhibition of glycogen synthase activity and stimulation of glycogen phosphorylase activity. IL-6 inhibited also insulin-stimulated increase of glucokinase activity, an important glycolytic enzyme (Christ et al. 1994). These actions of cytokines and NO may be interpreted as directed towards providing more glucose for the immediate use by hepatocytes, or for its release by the liver.

Gluconeogenesis, a process that supplies the body with glucose after exhaustion of hepatic glycogen stores, was shown to be inhibited in cultured hepatocytes activated by endotoxin (LPS) and a combination of cytokines (Ceppi et al. 1996; Stadler et al. 1995). This effect was mediated by nitric oxide through the inhibition of key gluconeogenic enzyme activities, pyruvate carboxylase, and phosphoenolpyruvate carboxykinase (Horton et al. 1994). It was also shown that IL-6 (Christ et al. 1994), or IL-1β and TNF-α (Christ and Nath 1996) impaired glucagon-dependent induction of phosphoenolpyruvate carboxinase, in part by the inhibition of cyclic AMP formation (Christ et al. 1997), and also through the increased degradation of the enzyme's mRNA (Christ and Nath 1996). After exhaustion of hepatic glycogen stores these effects may impair glucagon-stimulated gluconeogenesis, and decrease glucose release from the liver.

It seems plausible that the modulation of the hepatic effects of main glucoregulatory hormones, such as insulin and glucagon, by proinflammatory mediators elicited systematically or released intrahepatically from nonparenchymal (or parenchymal) liver cells may underlie the early hyperglycemic and the late hypoglycemic phase of endotoxic shock (Sect. 13.5.2). In the normal liver IL-1, IL-6, or TNF-α may directly affect hepatic glucose metabolism, or interfere with hormonal control of glucose homeostasis, and thus participate in the regulation of glucose production and supply to other tissues.

11 Cooperation of Liver Cells in the Synthesis of Growth Factors and Respective Binding Proteins

Growth factors represent a heterogeneous group of peptides that express various effects on almost each cell type; however, the stimulation of cell proliferation represents one of their main characteristics. Both parenchymal and nonparenchymal liver cells are not only targets but also sources of some important growth factors, and of their respective binding proteins. Many growth factors produced by liver cells act not only on extrahepatic cells, but influence also functions of neighboring cells in the hepatic lobule. Some of these cell-to-cell interactions mediated by growth factors will be shortly described.

11.1 Insulin-Like Growth Factors and IGF-Binding Proteins

Insulin-like growth factors I and II (IGF-I and IGF-II) are single-chain peptides with structure similar to proinsulin, which are involved in the regulation of cell growth, differentiation, and metabolism. The production and release of IGFs are primarily regulated by growth hormone (GH) and nutritional status. Although most cells throughout the body can synthesize IGFs (LeRoith 1997), circulating IGF-I, which exhibits particular metabolic responsiveness, originates predominantly in the liver (Sjogren et al. 1999). Interestingly, IGF-I synthesis was demonstrated not only in hepatocytes (Scott and Baxter 1986), but also in cultured stellate cells (Pinzani et al. 1990), and IGF-I mRNA was localized in Kupffer and sinusoidal endothelial cells (Zimmermann et al. 2000). These findings indicate that IGF-I secreted from nonparenchymal liver cells may contribute to the circulating pool of IGF-I derived from liver, albeit at lower levels than IGF-I derived from hepatocytes.

IGFs bind specifically to cell membrane receptors, and also to high-affinity binding proteins present in serum and tissue fluids, termed IGF-binding proteins (IGFBP) -1 to -6, of which IGFBP-3 is regarded as the major carrier protein. The IGFBPs are expressed in a tissue-specific manner, and have different affinities for the IGFs. Liver is the primary source of two major circulating forms of IGFBPs, IGFBP-1 and IGFBP-3 (LeRoith 1997). IGFBP-3 binds 80%–95% of the circulating IGF-I and IGF-II in a stable ternary complex that includes also an additional 85-kDa protein termed acid-labile subunit (ALS). This complex prolongs the plasma half-life of IGF-I and limits the amounts of free, biologically active IGFs in the circulation, determining their bioavailability to tissues. In contrast to IGFBP-3, other IGFBPs form binary complexes with IGF-I (LeRoith 1997).

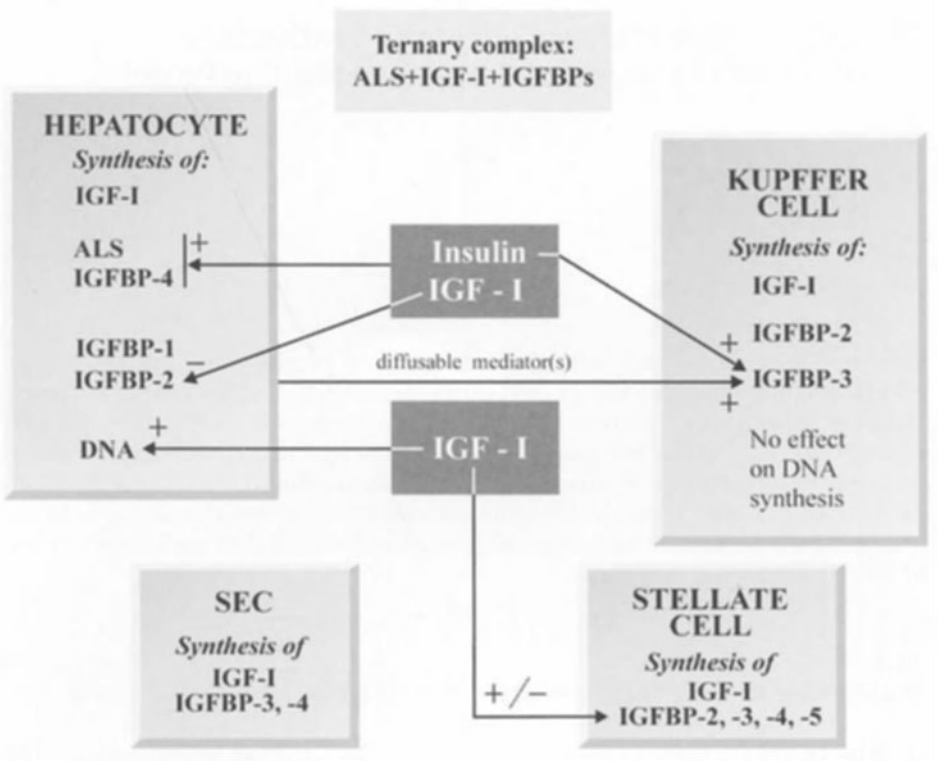

Fig. 10. Cooperation of liver cells in the synthesis of IGF-binding proteins and HGF (see text). *Symbols*: +, stimulation; –, inhibition of synthesis; +/–, differential effects on specific IGFBPs

Growth hormone is the primary inducing factor of IGF-I, IGFBP-3, and ALS synthesis, acting indirectly through insulin and IGF-I, but its effects may vary as individual components of the ternary complex are synthesized in different cell populations within rat liver (Fig. 10). Whereas ALS and IGFBP-1 expression was found only in hepatocytes (Saile et al. 1995; Scharf et al. 1996; Villafuerte et al. 1994; Zimmermann et al. 2000), IGFBP-3 mRNA and protein were expressed in Kupffer and SEC (Scharf et al. 1996; Villafuerte et al. 1994; Zimmerman et al. 2000). IGFBP-2 was synthesized in hepatocytes and Kupffer cells (Scharf et al. 1996), and IGFBP-4, which is the second most abundant serum IGFBP in adult rat and other species, was expressed at high levels in hepatocytes, and at low levels in nonparenchymal liver cells (Gentilini et al. 1998; Zimmermann et al. 2000). Cultured HSC secreted IGFBP-2, -3, -4 and -5 into medium (Gentilini et al. 1998; Scharf et al. 1998). Thus, liver nonparenchymal cells may constitute important sites of synthesis and cell-specific regulation of IGFBPs.

The cell-specific expression of distinct IGFBPs in the liver provides potential for specific regulation of hepatic IGF-I action (Fig. 10). In cultured hepatocytes growth hormone did not affect biosynthesis of IGFBPs (Villafuerte et al. 1994); however, GH, insulin, and IGF-I enhanced ALS expression (Scharf et al. 1996). In cultured hepato-

cytes insulin and IGF-I decreased the synthesis of IGFBP-1 and -2, while it increased that of IGFBP-4 (Scharf et al. 1996; Zimmermann et al. 2000). Kupffer cells were shown to synthesize IGFBP-2 and IGFBP-3 independently of insulin and IGF-I (Scharf et al. 1996); however, the stimulation of IGFBP-3 synthesis by insulin and IGF-I in these cells required a diffusable mediator derived from cocultured hepatocytes (Villafuerte et al. 1994). In hepatic stellate cells IGF-I induced IGFBP-3 and -5 proteins in a time-dependent manner without an increase in the corresponding mRNAs; however, IGFBP-4 protein levels decreased in response to IGF-I (Gentilini et al. 1998). These data clearly show that in the adult rat liver the biosynthesis of individual IGFBPs and ALS is compartmentalized in different cell populations, and is differentially regulated by insulin, IGF-I, and growth hormone (Fig. 10). The IGF-stimulated proliferation of cultured stellate cells was shown to be profoundly enhanced in the presence of conditioned media of cultured hepatocytes, suggestive of paracrine regulation of stellate cell growth and function by hepatocyte-derived factors (Gressner et al. 1993a).

Recent studies have suggested that hepatic production of IGFs and IGFBPs may be regulated not only by hormones and nutritional factors, but also by some growth factors and cytokines acting via paracrine or autocrine interactions. For example, IGF-I production by adult rat hepatocytes was stimulated by TGF-α and TGF-β1 (Voci et al. 1999), cytokines synthesized in the liver by hepatic stellate cells (Table 10) and Kupffer cells (Table 7). TGF-β stimulated IGFBP-3, and decreased IGFBP-5 mRNA and protein synthesis in cultured human stellate cells (Gentilini et al. 1998). IL-1α and TNF-α, cytokines secreted by activated Kupffer cells, increased circulating levels of IGFBP-1 through direct effects on the IGFBP-1 mRNA abundance in the liver (Benbassat et al. 1999). It is possible that stimulation of hepatic IGFBP production by cytokines and growth factors may contribute to the alterations in IGF bioactivity and impaired anabolism in clinical conditions when cytokine production is high. For instance, it was shown that in liver biopsies obtained from patients with active chronic viral-induced hepatitis that were characterized by high TGF-β expression, IGFBP-3 mRNA was increased in the majority of samples compared with normal controls (Gentilini et al. 1998).

11.2 Role of Hepatocyte Growth Factor in the Communication Between Hepatocytes and Stellate Cells

Hepatocyte growth factor (HGF) is a polypeptide growth factor that exhibits mitogenic, motogenic, and morphogenic activities for a wide variety of cells. Moreover, HGF plays an essential part in the development and regeneration of the liver, being the most potent known stimulator of hepatocyte proliferation (Matsumoto and Nakamura 1996). In the liver synthesis of HGF has been localized to nonparenchymal cells (Maher 1993), mainly to hepatic stellate cells that were shown to synthesize a large part of the HGF released from normal liver (Maher 1993; Ramadori et al. 1992b).

It is plausible that stellate cells may influence the turnover of hepatocytes in a paracrine way via the secretion of HGF that is directed by hepatocyte synthesis of insulin-like growth factor(s). IGF-I, synthesized in the liver both by parenchymal and nonparenchymal cells, was shown to increase HGF synthesis in the early cultures of stellate cells (Skrtic et al. 1997, 1999). Although in the normal liver hepatocytes pro-

duce large quantities of IGF-I (Scott and Baxter 1986), in vitro hepatocytes do not proliferate in response to IGF-I (Gohda et al. 1990), probably because they display only a few IGF-I receptors (Caro et al. 1988). [However, in some strictly defined culture conditions a quick appearance of IGF-induced hepatocyte proliferation has been observed (Kimura and Ogihara 1998)]. Interestingly, in the close vicinity of hepatocytes, a vast amount of IGF-I receptors was found on HSC (Brenzel and Gressner 1996; Scharf et al. 1998), and a smaller number on SEC (Zimmermann et al. 2000). Thus, stellate cells may represent local target cells for IGF-I action in the liver, and, through the secretion of HGF, may affect proliferation of hepatocytes (Skrtic et al. 1997). Moreover, it was shown that conditioned media of cultured hepatocytes contained some factor(s) that stimulated HGF secretion by stellate cells independently of the IGF-I presence (Skrtic et al. 1999).

Thus IGF-I, and other hepatocyte-derived factors, may indirectly affect hepatocytes via paracrine loops involving the release of HGF from hepatic stellate cells. The existence of such communication between hepatocytes and stellate cells may play a role in vivo in situations that lead to liver regeneration (Gohda et al. 1990). Secretion of HGF from HSC may also be beneficiary in liver inflammation as it was shown that HGF protected hepatocytes (Kosai et al. 1999) and sinusoidal endothelial cells (Seto et al. 1998) in experimental endotoxic liver injury. On the other hand, the synthesis by stellate cells of TGF-β, one of the most potent growth inhibitors for hepatocytes (Strain et al. 1987), may provide negative signals for hepatocyte proliferation both in resting and regenerating liver (Bissell et al. 1995).

11.3 Cross-Talk of Liver Cells in the Synthesis of TGF-β and TGF-β-Binding Proteins

Transforming growth factor-β (TGF-β) superfamily comprises a group of structurally related growth factors that function as potent inducers of cell differentiation and proliferation, modulation of extracellular matrix secretion, as well as wound healing (Grande 1997). TGF-β potently suppresses the proliferation of hepatocytes (Strain et al. 1987), stimulates production of ECM components in stellate cells (see Chap. 14), and can mediate hepatocyte apoptosis (Oberhammer et al. 1991). Injury to the liver caused by various factors results in rapid induction of TGF-β predominantly in hepatic stellate cells (Bauer et al. 1998a), consistent with a ubiquitous role for TGF-β in wound healing. Because of the pleiotropic effects of TGF-β, and its role in the development of liver fibrosis (Chap. 14), the expression and activation of this cytokine in normal and diseased liver became the subject of intensive investigations.

Three subtypes of TGF-β (TGF-β1, TGF-β2, and TGF-β3), which possess nearly identical biological properties, are found in mammals (Grande 1997). TGF-βs are synthesized and secreted in a biologically latent large homodimer form that must be proteolytically activated before it can bind to receptors and perform biological activities. Large latent TGF-β (235–280 kDa) consists of the mature 25-kDa TGF-β protein, the N-terminal part of its precursor (latency-associated peptide, LAP, 75 kDa), and the latent TGFβ-binding protein (LTBP, 120–160 kDa). Latent TGF-β molecules are localized to the cell surface and extracellular matrix via binding to type II IGF/mannose-6-phosphate receptor, through mannose-6-phosphate-containing carbohydrates in the

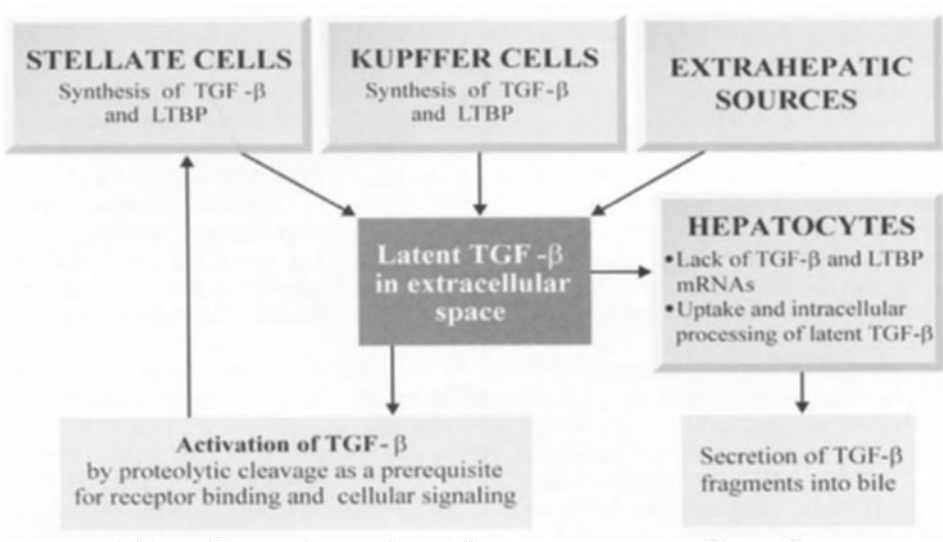

Fig. 11. Cooperation of hepatocytes and nonparenchymal liver cells in the synthesis of IGF and TGF-β and their binding proteins

LAP, and through the association of LTBP with fibrillar extracellular matrix molecules. The proteolytic cleavage of matrix-bound LTBP (possibly mediated in the liver by plasmin and other proteases), and the release of the small latent TGF-β complex (TGFβ-LAP) is regarded as the initial step in TGF-β activation. Binding of the mature TGF-β (25-kDa homodimer) to its type II receptor recruits and phosphorylates TGF-β type I receptor, which in turn phosphorylates its intracellular targets, such as Smad proteins that propagate the TGF-β signal from cytoplasm into the nucleus to activate gene expression (Breitkopf et al. 2001; Hu et al. 1998).

TGF-β1 is the most abundant TGF-β isoform in both the normal and fibrotic liver (De Bleser et al. 1997a); however, in normal rat or human liver TGF-β expression has been found exclusively in Kupffer and stellate cells (Bauer et al. 1998a), with much stronger signals present in KC (De Bleser et al. 1997a). Moreover, the presence of LTBP (LTBP-1, -2, -3), and TGF-β isoform transcripts (TGF-β-1, -2, -3), and LTBP-1, LTBP-2, LAP and TGF-β proteins, as well as the secretion of large latent TGF-β complexes were found in normal rat liver in Kupffer (Roth et al. 1998a) and stellate cells (Breitkopf et al. 2001). In contrast to the normal situation, stellate cells constitute the major TGF-β-expressing and -secreting cell type in the rat (Bachem et al. 1992) and human (Bauer et al. 1998a; Casini et al. 1993) fibrotic liver. The expression of all three components of the large latent TGF-β complex and three TGF-β and LTBP isoforms, detected in HSC by RT-PCR, and confirmed by sequence analysis (Breitkopf et al. 2001; Gong et al. 1998), indicates that stellate cells may constitute the major source of all latent TGF-β complex components both in normal and fibrotic liver.

Although in situ also parenchymal cells were recognized to contain the large latent TGF-β complex (Roth et al. 1998b), no transcripts of any of the TGF-β and LTBP

isoforms were found in liver sections (Breitkopf et al. 2001) or in hepatocytes freshly isolated from normal rat liver (Breitkopf et al. 2001; De Bleser et al. 1997a; Roth-Eichhorn et al. 1998), which strongly suggested that hepatocytes did not synthesize either LTBP or TGF-β. However, detection of TGF-β, LAP, and LTBP in cultured hepatocytes (Roth-Eichhorn et al. 1998) and the presence of TGF-β fragments in bile (Coffey et al. 1987) suggests that latent TGF-β is taken up, stored, and metabolized in parenchymal liver cells (Roth-Eichhorn et al. 1998). The latent form of TGF-β can be released from hepatocytes or intracellularly activated, and therefore may modulate TGF-β-sensitive pathways extra- and intracellularly (Roth et al. 1998b). The emerging concept of the cooperation of nonparenchymal and parenchymal liver cells in the synthesis and release of latent and active forms of TGF-β and their binding proteins is shown in Fig. 11.

Latent TGF-β is extracellularly activated by the action of proteolytic enzymes such as plasmin (Grande 1997). Nonparenchymal liver cells release factors that regulate the activity of the plasminogen/plasmin system and may modulate TGF-β bioactivity. In normal liver, stellate cells (Leyland et al. 1996) and sinusoidal endothelial cells (Kuiper et al. 1989; Rieder et al. 1993) are important sources of plasminogen activator inhibitor 1 (PAI-1), the most efficient inhibitor of plasminogen activators present in plasma. PAI-1 synthesis and release from these cells may represent an important control site for the regulation of TGF-β bioavailability inside and outside the liver. The observation that in experimental liver fibrosis only stellate cells expressed surface type II IGF/mannose-6-phosphate receptor, known to facilitate activation of TGF-β (De Bleser et al. 1995), is in line with the suggested key role of TGF-β in the development of hepatic fibrosis (Chap. 14).

12 Cooperation of Liver Cells in the Regulation of Sinusoidal Contractility

The regulation of sinusoidal blood flow and intrahepatic vascular resistance in normal and damaged liver has been the subject of intensive studies aimed at the elucidation of the pathogenesis of portal hypertension, a life-threatening complication of liver fibrosis and cirrhosis. Apart from regulatory mechanisms operating at the level of pre- and postcapillary vessels, it was shown in vivo that sinusoids constrict in a graded and reversible manner in response to specific mediators acting on hepatic stellate cells both under normal and pathological conditions (Clemens and Zhang 1999). Hepatic stellate cells have a strategic position in the perisinusoidal space and their long cytoplasmic processes, containing vast numbers of microfilaments, embrace endothelial cells (Fig. 3). Moreover, processes of stellate cells were found to be in the vicinity of nerve endings (Bioulac-Sage et al. 1990; Tiniakos et al. 1996; Ueno and Tanikawa 1997). In vitro nonactivated quiescent HSC do not contract (Kawada et al. 1992); however, contraction of activated stellate cells in culture mediated by increase in intracellular calcium concentration (Oide et al. 1999) can be directly quantified (Thimgan and Yee 1999).

Activated stellate cells express receptors for vasoactive agents (Table 4) and exhibit contractile or relaxing, although variable, responses to these factors (Table 16). Endothelins are the most important modulators of hepatic microcirculation (Clemens and Zhang 1999). Through the use of intravital microscopic imaging techniques it was shown that endothelin-1 induced contractile response of hepatic sinusoids that was correlated with decreased oxygen saturation of sinusoidal blood (Okumura et al. 1994; Zhang et al. 1994b). In normal liver preproET-1 mRNA was expressed in endothelial cells of the interlobular veins and arteries (Fukushiga et al. 2000), and in nonparenchymal liver cells, predominantly in sinusoidal endothelial cells (Eakes et al. 1997). ET-1 release was shown also from other nonparenchymal liver cells, mostly stellate cells, in response to such factors as TGF-β (Rieder et al. 1991) or endotoxin (Eakes et al. 1997). It has been demonstrated that exogenous ET-1 increased portal vascular resistance through the constriction of sinusoids (Bauer et al 1994) and preterminal portal venules (Kaneda et al. 1998).

Hepatic effects of endothelins are mediated by two receptor subtypes, ET_A and ET_B (Zhang et al. 1997), which are heterogeneously expressed on all major hepatic cell types, with the highest density on stellate cells (Housset et al. 1993). Stellate cells and hepatocytes express both ET_A and ET_B receptors (Fukushiga et al. 2000; Housset et al. 1993) with the predominance of the ET_B subtype in rat HSC (Gabriel et al. 1999). Some authors found only ET_B receptors on Kupffer cells and SEC (Housset et al. 1993), while others detected both types of ET receptors on sinusoidal endothelial cells (Diamantis et al. 1998).

Table 16. Substances contracting and relaxing stellate cells

	Secreting cell(s)[a]	References
Vasoconstrictors		
Endothelin-1	SEC, HSC	Kawada et al. 1993; Sakamoto et al. 1993
Angiotensin-II	Serum	Pinzani et al. 1992c
Arginine vasopressin	Hypothalamus	Bataller et al. 1997
ATP and ADP	HC, KC	Takemura et al. 1994
Leukotriene D_4	HC	Titos et al. 2000
$PGF_{2\alpha}$	KC, SEC	Kawada et al. 1992
Substance P	Neurons	Sakamoto et al. 1993
Thromboxane A_2	KC, SEC	Kawada et al. 1992
Thrombin	Serum	Pinzani et al. 1992a
Vasodilators		
NO	HC, KC, SEC	Kawada et al. 1993; Rockey and Chung 1995
CO	KC	Suematsu et al. 1995
PGE_2	KC, SEC	Kawada et al. 1992, 1993
PGI_2	KC, SEC	Kawada et al. 1992, 1993
Atrial natriuretic peptide (ANP)	Heart	Gorbig et al. 1999
Adrenomedullin	KC, SEC	Kawada and Inoue 1994
IL-1	KC	Sakamoto et al. 1997

a Mainly liver cells; however, many of the listed substances may be released into blood by extrahepatic cells.

In liver disease or injury, other types of nonparenchymal liver cells, such as the sinusoidal endothelial cells and/or Kupffer cells, become important contributors to the contractile response. Endothelin levels are elevated in chronic liver disease (Alam et al. 2000; Pinzani et al. 1996a), and the source of excess endothelin is probably the liver itself (Clemens and Zhang 1999). A paracrine signalling mechanism was postulated to operate between endotoxin-activated Kupffer cells that released TGF-β, and sinusoidal endothelial cells, in which TGF-β stimulated ET-1 synthesis and secretion (Eakes et al. 1997); this intercellular signaling relationship may represent an important component in the hepatic responses to endotoxin. However, since TGF-β is also secreted by activated stellate cells, it may increase ET-1 synthesis via an autocrine loop and act in a paracrine way on SEC (Fig. 12). The observation that endotoxin (Gandhi

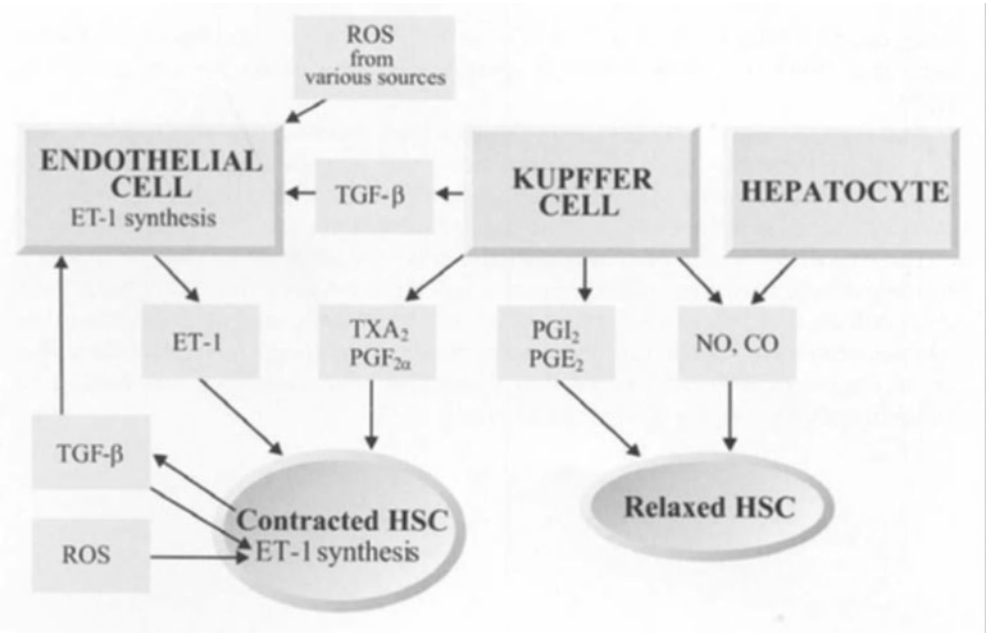

Fig. 12. Main factors released from cells of the liver lobule that affect the contractility of hepatic stellate cells

et al. 2000) or reactive oxygen species (Gabriel et al. 1998) increase ET-1 release from cultured stellate cells may be relevant for the increased portal resistance in endotoxemia and liver cirrhosis (Clemens and Zhang 1999). The increased sensitivity of endothelin receptors found in activated stellate cells (Reinehr et al. 1998; Gandhi et al. 2000) may contribute to the altered contractility of HSC in pathological conditions.

In contractile cells of the vessel wall the effects of vasoconstrictor agonists can be attenuated by vasodilators (Table 16; Fig. 12). NO, an important vasodilatory agent, has been shown to modulate liver blood flow mainly via its relaxing effects on stellate cells (Clemens 1999). In activated HSC exogenous NO was capable of preventing ET-induced contraction as well as causing precontracted cells to relax (Rockey and Chung 1995). Although all types of liver cells can produce NO (Chap. 8), in the normal liver the constitutive isoform of NO synthase (eNOS) was exclusively found in endothelial cells; it was activated by shear stress and sinusoidal blood flow (Shah et al. 1997). However, under pathological conditions, Kupffer cells and hepatocytes challenged with endotoxin, administered either in vivo or in vitro, released vast amounts of NO (Curran et al. 1990), an effect that was found to improve oxygen transport from sinusoids to hepatocytes (Huang et al. 1997).

Carbon monoxide, a by-product of the heme oxygenase (HO) activity, constitutes another endogenous factor that was shown to reduce sinusoidal tone, and was necessary for maintaining liver perfusion (Suematsu et al. 1995). In normal liver the action of CO, which is produced predominantly by hepatocytes that constitutively express HO-2 activity (Goda et al. 1998), is probably involved in the relaxation of hepatic stellate cells (Clemens and Zhang 1999). Under pathological conditions carbon mon-

oxide, produced also by the inducible HO-1 activity of Kupffer cells (Bauer et al. 1998b; Goda et al. 1998), was found to protect hepatic microcirculation (Clemens and Zhang 1999).

Although NO and CO are key molecules that relax stellate cells acting via paracrine and autocrine mechanisms, also prostaglandins that increase cyclic AMP concentration (PGE_2 and PGI_2) and may be released locally from Kupffer or sinusoidal endothelial cells, may relax stellate cells (Clemens and Zhang 1999).

The elucidation of the mechanisms that control the interplay of vasoconstrictory and vasodilatory substances and gaseous molecules released from sinusoidal liver cells and other types of liver cells, which act on hepatic stellate cells and other components of the hepatic vascular system, may help in designing new therapies for the treatment of sinusoidal blood flow disturbances in portal hypertension, or in inflammatory liver injury (Clemens and Zhang 1999).

13 Cross-Talk of Liver Cells in Response to Endotoxin

The gastrointestinal tract is a reservoir of viable bacterial organisms and biologically active microbiological products, such as endotoxin. Endotoxin is the general term for a class of lipopolysaccharide (LPS) molecules located in the outer cell membrane of gram-negative bacteria. Endotoxin constitutes physiological constituent of portal-venous blood (Jacob et al. 1977), and under normal conditions, in contrast to extrahepatic tissues, it does not cause hepatic inflammation. However, an abnormally high release of endotoxin from gram-negative bacteria results in sepsis (defined as the systemic response to bacteremia) and its complications: systematic inflammatory response syndrome (SIRS) and septic shock characterized by hypotension, vascular injury, disseminated intravascular coagulopathy, multiple organ failure, and ultimately death (Parillo 1993). Many mediators, such as cytokines, eicosanoids, PAF, activators of complement cascade, reactive oxygen species, nitric oxide, kinins, thrombin, procalcitonin, and endothelin-1, participate in the pathogenesis of sepsis (Parillo 1993). Due to its integral role in metabolism and host defense mechanisms, the liver is a major organ responsible for initiation of multiple organ failure during sepsis. It is also one of the major organs damaged during endotoxic shock. Endotoxin-triggered inflammatory response in the liver is associated with many structural and functional changes such as early depression of hepatocellular function, activation of parenchymal and nonparenchymal cells, platelet aggregation, intravenous thrombosis, and infiltration of polymorphonuclear leukocytes, mainly neutrophils (Hewett and Roth 1993).

13.1 Endotoxin Uptake by the Liver

The liver plays a major role in clearing circulating LPS from the blood (Freudenberg et al. 1982; Ruiter et al. 1981) mainly by Kupffer cells (Van Bossuyt et al. 1988; Catala et al. 1999), and to a lesser extent by sinusoidal endothelial cells (Catala et al. 1999; Ruiter et al. 1981) and hepatocytes (Mimura et al. 1995). Upon binding of LPS to cells, it is internalized, processed, and released (Fox et al. 1988). On both SEC and Kupffer cells, LPS binds mainly to a special class of scavenger receptors (van Oosten et al. 1998) and to a minor extent to CD14 receptor protein and macrosialin (CD68) present on Kupffer cells (van Oosten et al. 1998). The uptake of LPS by Kupffer cells leads to the

release of many mediators that exert profound effects on liver and body functions (Table 17).

In the circulation, LPS is complexed to several compounds, including its specific carrier, the LPS-binding protein (LBP), which is an acute-phase protein produced by hepatocytes. The LPS/LBP complex binds to the cell surface receptor CD14, a glycosylphosphatidylinositol protein anchored on the cell surface of myeloid lineage cells, including Kupffer cells (Ulevitch and Tobias 1995). Attachment of the LPS/LBP complex to the membrane-bound CD14 (mCD14) and endocytosis of this complex (Ulevitch and Tobias 1995) result in cell activation, nuclear translocation of the transcription factor NF-κB, and synthesis of proinflammatory cytokines, prostanoids, and other soluble mediators (Bauerle and Henkel 1994). However, the effects of LBP on Kupffer cell activation by LPS were shown to be dependent on functional Toll-like receptor 4 (Tlr 4), suggesting that LBP/CD14 pathway may play a minor role in KC activation by LPS (Su et al. 2000). Low levels of CD14 mRNA and protein, observed in normal rat (Liu et al. 1998b) and human hepatocytes (Su et al. 1999), and rat Kupffer cells (Lichtman et al. 1998), were found to be rapidly increased in Kupffer cells after LPS administration (Lichtman et al. 1998; Su et al. 2000).

The early response of hepatocytes to radiolabelled LPS injected into portal vein takes place by the direct endotoxin uptake, only a few minutes after intraportal LPS administration, and leads to the excretion of endotoxin into the bile (Mimura et al. 1995; Van Bossuyt et al. 1988). However, an indirect endotoxin effect on hepatocytes could proceed via induction of hepatocyte LBP and CD14 gene expression by cytokines released from Kupffer cells (Liu et al. 1998b; Wan et al. 1995), parallel to the activation of other acute-phase proteins (see further). Thus endotoxin upregulates in hepatocytes the synthesis of proteins involved in its own uptake by the liver and other organs.

13.2 Activation of Nonparenchymal Liver Cells as the Early Response to Endotoxin

It was demonstrated that direct LPS action on hepatocytes (Milosevic et al. 1999) or Kupffer cells (Peters et al. 1990) made no damage to the cells. However, acute endotoxemia induced by the treatment of rats or mice with bacterially derived LPS results in inflammatory response characterized in the early phase by the accumulation of neutrophils and macrophages in the liver (Hewett and Roth 1993; Jaeschke and Smith 1997), microcirculatory dysfunction (McCuskey et al. 1999), depression of SEC function (Deaciuc et al. 1994b), and, after 1–2 days, injury of hepatocytes and endothelial cells accompanied by their reactive proliferation (Helyar et al. 1994; Hewett and Roth 1993; Laskin et al. 1995). LPS-activated liver cells release a variety of cytotoxic and inflammatory mediators (Table 17) that participate in the propagation of the inflammatory response and endotoxin-induced cell and tissue damage both at the local and systemic levels.

It is believed that the LPS-induced activation of Kupffer cells constitutes the key primary event in the pathogenesis of LPS-induced liver injury through the release of a variety of substances that have been implicated as cytotoxic or modulatory mediators of hepatic inflammation (Table 17). This hypothesis has been supported by experi-

Table 17. Factors released from liver cells after challenge with endotoxin (LPS)

Cell type	References
Hepatocytes	
IL-6	Saad et al. 1995
TNF-α	Saad et al. 1995
NO (induction by cytokines)	Curran et al. 1990; Saad et al. 1995
Macrophage inflammatory protein-2	Dong et al. 1998
Chemokines (KC/gro, CINC)	Maher 1995; Mawet et al. 1996; Shiratori et al. 1994
Kupffer cells	
Reactive oxygen species	Arthur et al. 1988
NO	Curran et al. 1990; Gaillard et al. 1991
Cytokines	
CINCa	Deutschman et al. 1996
CC-chemokines (MIP-1α, MCP-1)	Bukara and Bautista 2000
IFN-α, IFN-β	Decker 1990
IL-1α and -1β	Aono et al. 1997; Chensue et al. 1991
IL-6	Busam et al. 1990; Callery et al. 1990; Knolle et al. 1997
IL-10	Knolle et al. 1995
IL-12	Takahashi et al. 1996; Tsutsui et al. 1997
IL-18	Tsutsui et al. 1997
TNF-α	Callery et al. 1990; Chensue et al. 1991; Karck et al. 1988
Eicosanoids	
Prostaglandins	Kawada et al. 1992; Kmiec et al. 1993; Peters et al. 1990
Thromboxane A_2	Decker 1990
Leukotrienes	Spitzer et al. 1993
Lysosomal enzymes	Decker 1990
Sinusoidal endothelial cells	
Endothelin-1	Eakes et al. 1997
IL-1	Feder et al. 1993
IL-6	Feder et al. 1993; Knolle et al. 1997
PAF	Mizoguchi et al. 1991
Reactive oxygen species	McCloskey et al. 1992
Prostanoids	Eyhorn et al. 1988

Table 17 *(continued)*. Factors released from liver cells after challenge with endotoxin (LPS)

Cell type	References
Hepatic stellate cells[b]	
Endothelin-1 and NO	Gandhi et al. 2000
NO synthesis induced by TNF-α, IFN-γ or LPS	Helyar et al. 1994a; Rockey and Chung 1995
MCP-1	Sprenger et al. 1999; Xu et al. 1996

a Observed in acute endotoxemia (after treatment of animals with LPS).

b Only transformed (activated) HSC responded to LPS (Sprenger et al. 1997).

ments demonstrating that inactivation or elimination of Kupffer cells by chemical agents such as gadolinium chloride, administered before (Iimuro et al. 1994; Sarphie et al. 1996) induction of sepsis, prevented or attenuated many of the LPS effects on the liver. The products of activated liver macrophages may directly and indirectly affect surrounding parenchymal and sinusoidal endothelial cells as well as infiltrating leukocytes and platelets, with the end result of inflammatory response and tissue injury. Moreover, Kupffer cells receive from other liver cells paracrine signals that modulate the extent of hepatic inflammation. However, it has been shown that endotoxin can also directly induce IL-6, TNF-α, NO, and acute-phase protein synthesis in cultured rat hepatocytes without the involvement of factors released from Kupffer cells (Saad et al. 1995). Some of the complex relationships existing between nonparenchymal and parenchymal liver cells under the conditions of endotoxic injury will be presented in the following sections.

13.2.1 TNF-α as a Paracrine Mediator in Liver Responses to Endotoxin

Many data suggest that TNF-α, a multifunctional cytokine produced mainly by monocytes and tissue macrophages, is the principal mediator of the pathophysiological effects of inflammation, cachexia, and endotoxic shock (Beutler and Cerami 1989). Administration of TNF-α has been found to reproduce many of the effects of endotoxin-induced acute inflammation, including hepatocellular necrosis and changes in lipid metabolism (Wang et al. 1995a). In rat, intraperitoneal injection of LPS or its infusion into the portal vein increased TNF-α levels in arterial serum, indicating that the liver is an important site of TNF-α production in the body (Asari et al. 1996). Inhibition of Kupffer cell function with dietary glycine (Ikejima et al. 1996) or destruction of Kupffer cells with liposome-encapsulated dichloromethylene diphosphonate (Bautista et al. 1994) largely reduced endotoxin-induced TNF-α formation and blocked mortality in rats treated with a lethal dose of LPS, implying a role of Kupffer cells as the major source of TNF-α in the liver (Luster et al. 1994). In vitro

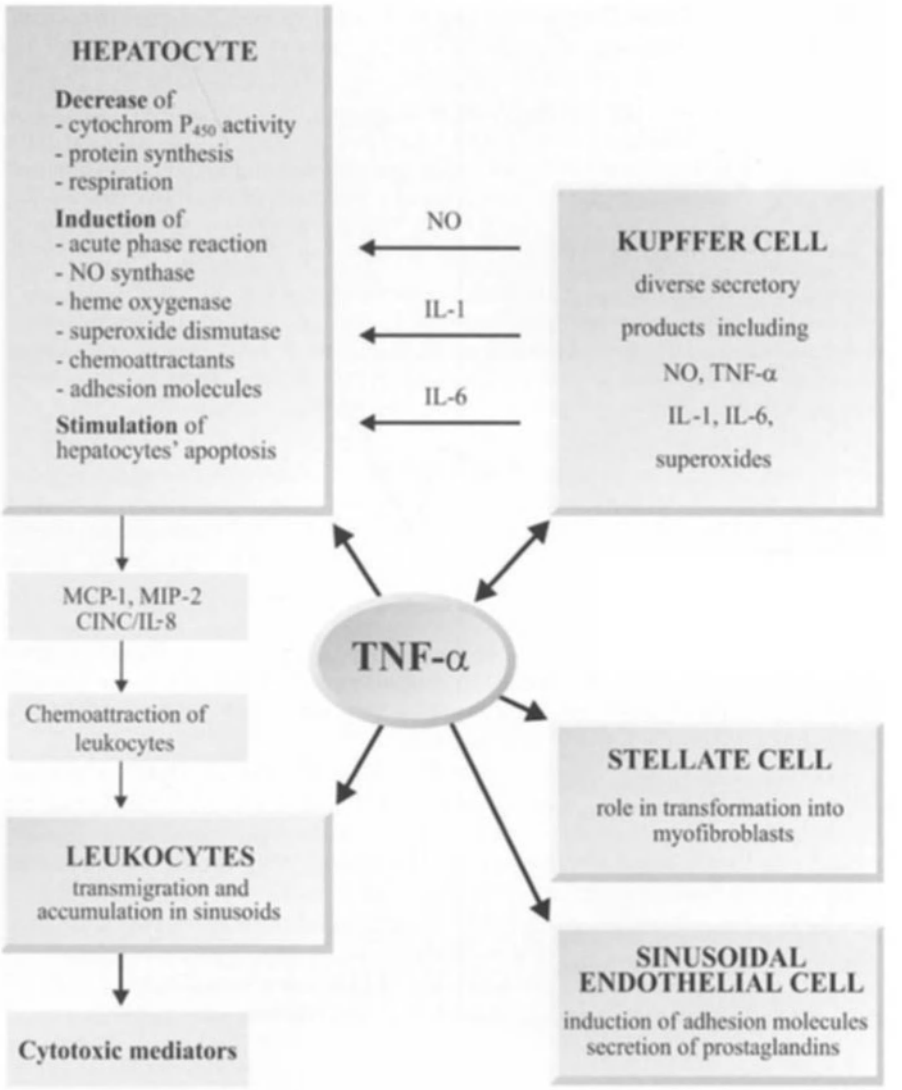

Fig. 13. Some of the TNF-α effects in the liver (description in text)

studies showed that TNF-α was the major mediator elicited in Kupffer cells by endotoxin (Decker 1990).

The local production of TNF-α by LPS-elicited liver macrophages plays an important role in endotoxin-induced liver damage by the way of intercellular communication within the liver, and by the systemic effects of TNF-α, which has been shown to stimulate other cells to the release of terminal mediators of shock such as PAF, kinins, leukotrienes, reactive oxygen species, proteases, or nitric oxide (Parillo 1993). Patho-

physiological responses mediated by TNF-α in the liver include inflammatory cell infiltration, hyperlipidemia, fibrogenesis, and cholestasis (Simpson et al. 1997). The effects of TNF-α on liver cells are mediated through its binding to the type I 55-kDa membrane receptor of target cells (Zhang et al. 1994a), and signal transduction via the activation of transcription factor NF-κB (Tran-Thi et al. 1995). TNFα-induced activation of NF-κB in endothelial cells and hepatocytes during endotoxemia (Essani et al. 1996) leads to the transcriptional activation of a number of proinflammatory genes, e.g., of chemokine MIP-2 (Dong et al. 1998), nitric oxide synthase (Shiratori et al. 1998), and heme oxygenase 1 (Downard et al. 1997). TNFα-mediated upregulation of the expression of adhesion molecules has been involved in the neutrophil influx observed in endotoxemic liver (Jaescke 1997). TNF-α was also shown to induce oxidative stress in isolated mouse hepatocytes (Adamson et al. 1993), and suppress mitochondrial respiration in cultured rat hepatocytes (Stadler et al. 1992), an effect probably mediated by the upregulation of nitric oxide synthesis in hepatocytes (Shiratori et al. 1998; Stadler et al. 1992). Other hepatic actions of TNF-α during endotoxemia involve suppressed induction of cytochrome P450 isoforms (Monshouwer et al. 1996), and elimination of damaged hepatocytes via the activation of apoptosis (Hamada et al. 1999; Jaeschke et al. 1998; Leist et al. 1995; Tsustsui et al. 1997), although anti-apoptotic effects of this cytokine have also been reported (Nagaki et al. 2000). TNF-α, acting together with IL-1 and IL-6, further products of activated Kupffer cells (Table 17), was shown to induce acute-phase proteins in hepatocytes (Sect. 13.5.1), and to upregulate the regenerative response to liver injury through the induction of hepatocyte proliferation (Beyer and Theologides 1993). Figure 13 presents some of the complex interactions between the main types of liver cells (and leukocytes) that are involved in TNF-α effects in endotoxemic liver.

Although TNF-α was shown to stimulate the activation of NF-κB in primary cultures of rat Kupffer cells, this cytokine alone did not induce its own synthesis (Tran-Thi et al. 1995), but rather inhibited it in an autoregulatory negative feedback through the simultaneous increase of PGE_2 (Karck et al. 1988) and IL-10 (Knolle et al. 1995) secretion from Kupffer cells. The suppression of TNF-α synthesis in Kupffer cells by Kupffer cell-derived PGE_2 can be interpreted as the way to provide counterregulation of the developing inflammatory cascade. The synthesis of TNF-α in Kupffer cells was shown to be inhibited also by glucocorticoids (Decker 1990), epinephrine (Liao et al. 1995), and all-*trans* retinoic acid (Motomura et al. 1997).

13.2.2 The Role of Nitric Oxide in the Response of Liver Cells to Endotoxin

Nitric oxide, released during endotoxemia, has been implicated as the important mediator of hypotension, peripheral vasodilation, and tissue damage during endotoxic shock (Clemens 1999). A few hours after endotoxin administration increased expression of iNOS messenger RNA was found both in nonparenchymal and parenchymal liver cells (Kawada et al. 1998; Laskin et al. 1995). Although various liver cell types may produce NO, it has been assumed that in endotoxin-treated animals hepatocytes and Kupffer cells, due to their number and size, are the major hepatic source of NO during the early stage of endotoxemia. The induction of NOS in hepatocytes of endotoxin-treated animals occurs through the action of cytokines, such as

IL-1, IL-6, and TNF-α, released from activated Kupffer cells, and probably sinusoidal endothelial cells (Sect. 8.1.1) or from extrahepatic sources.

The role of NO in the pathogenesis of endotoxin-induced liver injury is controversial. In vitro observations suggested damaging effects of NO on hepatocytes. For example, NO synthesized by endotoxin-activated Kupffer cells induced prominent reduction of total protein synthesis (Curran et al. 1990), depression of mitochondrial respiration (Kurose et al. 1996; Stadler et al. 1992), and inhibition of cytochrome-P450 activity (Monshouwer et al. 1996; Stadler et al. 1994) in cultured hepatocytes.

However, in vivo experiments that demonstrated the exacerbation of endotoxin-induced liver damage by nonspecific inhibitors of nitric oxide synthase (Harbrecht et al. 1992; Ou et al. 1997) suggested that putative hepatoprotective effects of NO during endotoxemia may be mediated partially by the stabilization of hepatic microcirculation (Nishida et al. 1994), increased oxygen delivery to hepatocytes (Huang et al. 1997), suppression of prostanoid and IL-6 synthesis in Kupffer cells (Stadler et al. 1993), and inhibition of endotoxin-induced activation of neutrophils (Liu et al. 1998a). Moreover, the observation that in vivo inhibition of NO formation enhanced superoxide release by the perfused liver (Bautista and Spitzer 1994) suggested that nitric oxide may protect hepatocytes against reactive oxygen species released from neutrophils and Kupffer cells.

The data describing the involvement of nitric oxide in the process of hepatocyte apoptosis are unequivocal. It has been shown that NO suppressed apoptosis, induced by TNF-α and actinomycin D in primary cultures of rat hepatocytes, through the proteolytic processing and activation of multiple pro-caspases, including caspase-3 and caspase-8 (Li et al. 1999). However, in the LPS/galactosamine model of hepatic injury in mice, characterized by prominent apoptosis of hepatocytes (Leist et al. 1995), activation of iNOS did not prevent liver failure, whereas anti-TNFα neutralizing antibody inhibited liver damage and iNOS expression (Morikawa et al. 1999). In contrast, it has also been demonstrated that NO may be involved in hepatic injury through the induction of hepatocyte apoptosis (Wang et al. 1998c).

Although beneficial effects of increased NO synthesis or delivery were shown in some models of liver injury, NO was found to have detrimental effects in hemorrhagic shock, and in ischemia-reperfusion model of liver injury (Clemens 1999). Thus, the protective or toxic role of nitric oxide in hepatic injury may depend on the particular model of liver damage, cellular source (nonparenchymal and parenchymal liver cells, infiltrating leukocytes) and amount of NO released, presence of oxidative stress, and time phase in the development of liver damage (Clemens 1999). It is, however, clear that paracrine interactions between activated Kupffer cells, acting as resident macrophages, and hepatocytes, major effector cells responsible for liver function, as well as hepatic stellate cells involved in the regulation of sinusoidal blood flow (Chap. 12), are of major importance for the NO actions in the injured liver.

13.3 The Interactions of Neutrophils with Liver Cells in Endotoxemia

The initial manifestation of the LPS-induced liver injury represents a hepatic microvascular inflammatory response that includes activation of endothelium, increased

leukocyte-endothelial interactions, impaired sinusoidal pressure, and activation of Kupffer cells (McCuskey 1999).

Ultrastructural studies showed decrease in the number of fenestrations in the cytoplasm of SEC (Sarphie et al. 1996) already after 3 h, and pronounced swelling of endothelial cells 24 h after LPS administration (Ito et al. 2000). The activation of SEC shifts these cells from their normal anticoagulant state to a procoagulant state with increased adhesiveness for leukocytes and platelets. Neutrophils (polymorphonuclear granulocytes, PMN) accumulate in the liver prior to the onset of its injury (Jaeschke 1997). Considerable body of evidence indicates that neutrophil accumulation in the liver after inflammatory or oxidative stress contributes to hepatocyte injury. Subsequent tissue damage results from the action of various mediators released from activated sequestered leukocytes and Kupffer cells (Fig. 14).

The recruitment of neutrophils into the liver during endotoxin-induced hepatitis involves release of chemotactic activity from Kupffer cells (CINC, Deutschman et al. 1996; MCP-1, Marra et al. 1998b), hepatocytes (CINC, Dong et al. 1998; Mawet et al. 1996; Shiratori et al. 1994; MCP-1 and MIP-2, Dong et al. 1998), and stellate cells (MCP-1, Marra et al. 1998b; Sprenger et al. 1997). The transmigration of neutrophils through the sinusoidal wall into the liver parenchyma has been shown to be primarily dependent on the upregulation of the β_2 integrin Mac-1 (CD11b/CD18) on neutrophils by TNF-α and complement, and its counter-receptor on endothelial cells, intercellular adhesion molecule (ICAM-1, CD54; Jaeschke 1997). The induction of vascular adhesion molecule-1 (VCAM-1, CD106) expression on Kupffer cells and sinusoidal endothelial cells (but not on hepatocytes) by endotoxin, TNF-α, and IL-1 has also been found to participate in the transendothelial migration of neutrophils (Essani et al. 1997a). Induction of E-selectin expression, absent on SEC in normal liver, has been shown to be important for the full activation of neutrophils during the transmigration process (Lawson et al. 2000).

Parenchymal cell injury represents an important stimulus for the sequestration of leukocytes in sinusoids and their transmigration through the release of chemotactic activity (Fig. 13). In the endotoxin/galactosamine model of liver injury, the accumulation of activated neutrophils in sinusoids occurred before TNFα-mediated hepatocyte apoptosis (Leist et al. 1995). However, hepatocyte apoptosis has been shown to be a critical trigger for transendothelial migration of neutrophils and the subsequent aggravation of liver injury in this murine model of sepsis (Lawson et al. 1998).

The adherence of neutrophils to hepatocytes, clearly documented by electron microscopy (Ohira et al. 1995), which is dependent on β_2 integrins and ICAM-1 interactions (Nagendra et al. 1997), represents the next step, after neutrophil transendothelial migration, in the development of neutrophil-induced liver damage. Adherence-dependent release of cytotoxic mediators from neutrophils (and Kupffer cells), such as proteases and reactive oxygen species, results in hepatocellular necrosis (Ho et al. 1996). Although Kupffer cells play a major role in the initiation of endotoxin-induced liver damage, they are also active during the later stages of hepatic inflammation through the intensive phagocytosis of apoptotic neutrophils, which accumulate in the sinusoidal lumen several hours after endotoxin administration (Shi et al. 1996).

Thus, the involvement of neutrophils in the development of hepatocellular injury is the consequence of their interactions with the resident cells of liver lobule, especially endothelial cells, Kupffer cells, and hepatocytes. However, neutrophil-induced injury represents only one of several pathomechanisms that can be involved in the patho-

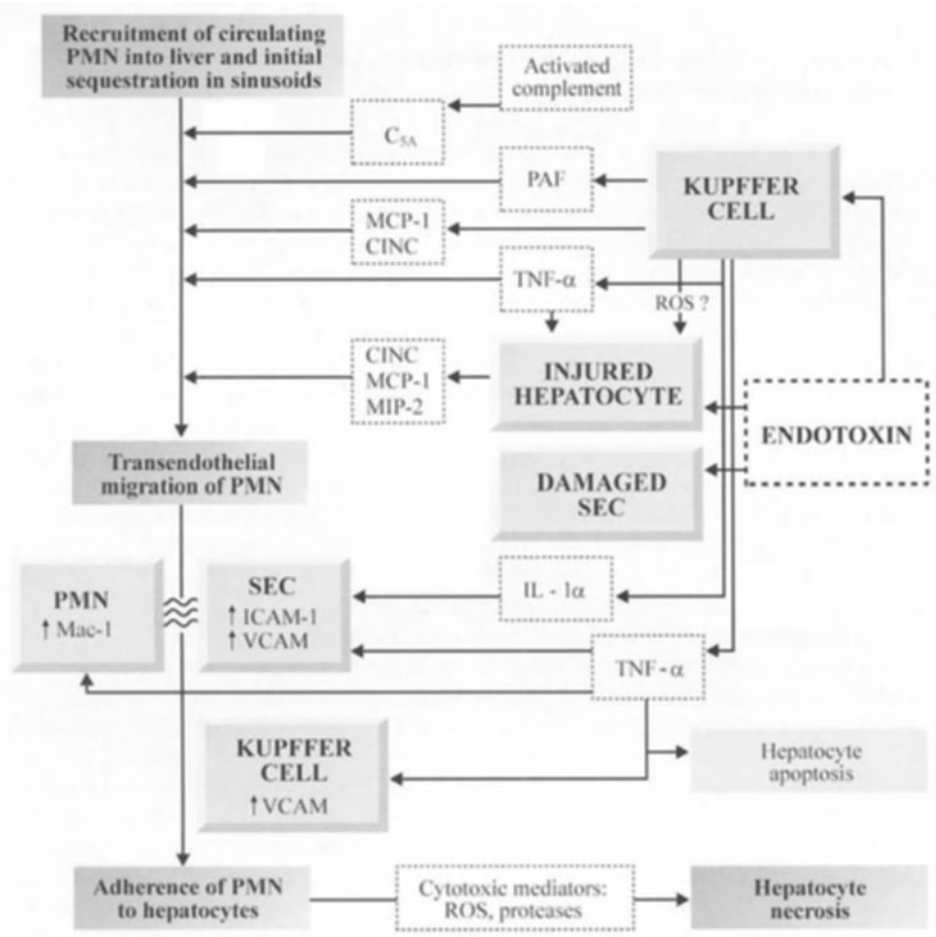

Fig. 14. Schematic representation of neutrophil-induced hepatocyte injury (see text). ↑ denotes upregulation of the adhesion molecule by cytokine(s). *PMN*, polymorphonuclear granulocytes (neutrophils); other abbreviations have been explained in text

physiology of inflammatory liver injury. Other important contributing factors include the platelet aggregation, microcirculatory disturbances, or accumulation of cytotoxic lymphocytes; however, the discussion of these mechanisms, as well as the role of neutrophils in other forms of liver injury (Jaeschke and Smith 1997), are beyond the scope of this mongraph.

13.4
Interactions of Liver Cells in the Production and Removal of Reactive Oxygen Species

In vivo or in vitro endotoxin administration results in the hepatic release (Bautista et al. 1990) of vast amounts of superoxide anion (O_2^-), a principal form of oxygen radical. Although superoxide anion and its dismutation product, hydrogen peroxide, are the primary radical oxygen species (ROS) formed by many cells (Fig. 15A), the release of myeloperoxidase from activated neutrophils leads to the formation of hypochlorous acid (HOCl) as the major oxidant. ROS are thought to be responsible in a great part for the liver damage in such models of experimental injury as ischemia-reperfusion, chemical-induced hepatotoxicity, or endotoxemia (Jaeschke et al. 1999; Jaeschke 2000). ROS have been shown to inactivate plasma antiproteases (Weiss 1989), and in this supportive role may enable protease-dependent hepatocyte damage. ROS are involved in the activation of transcription factors such as nuclear factor-κB (NF-κB) and activator protein 1 (AP-1) that provide signals for the activation of proinflammatory genes (Bauerle and Henkel 1994). Antioxidants were found to inhibit NF-κB activation, cytokine formation, and ICAM-1 upregulation in the endotoxin-damaged liver (Essani et al. 1997b). Thus, the positive feedback by ROS may play an important role in amplifying the excessive inflammatory responses.

The intercellular communication may be important for protecting the liver from the endotoxin-induced enhanced superoxide anion production. It has been demonstrated that in vivo LPS challenge upregulated, in a coordinated way, glucose transport and glucose-6-phospate dehydrogenase (G6PDH) activity in sinusoidal endothelial cells, as well as superoxide dismutase and glutathione peroxidase gene expression, which resulted in augmented ROS detoxifying activity (Spolarics 1998). In LPS-activated Kupffer cells the elevated expression of G6PDH, the key enzyme of the NADPH-providing pentose cycle, correlated well with the increased synthesis of superoxide anion, hydrogen peroxide, and nitric oxide (Spolarics and Navarro 1994). However, no changes in the activities of superoxide dismutase and glutathione peroxidase, the main anti-oxidant enzymes, were observed either in Kupffer cells or hepatocytes after LPS administration (Spolarics 1996). These data indicate that Kupffer cells (and infiltrating neutrophils) show a dominance of pro-oxidant pathways, whereas endothelial cells are dominantly antioxidant.

It has been proposed that an intercellular oxidant balance (Fig. 15B) exists in the hepatic microenvironment (Spolarics 1998). Endotoxemia sets this intercellular (and intracellular) balance at a new level by stimulating the pentose cycle and dependent pathways in a cell-specific way: the endothelial cell response may buffer the harmful effects of activated phagocytes and neutrophils, and protect the underlying parenchymal cells (Spolarics 1998). This mechanism may be operative also in normal liver as small amounts of endotoxin are constantly found in the portal vein (Jacob et al. 1977). Additionally, the production of NO by LPS-elicited Kupffer cells and hepatocytes may represent another protection mechanism against ROS-mediated liver damage since nitric oxide was shown to operate as a scavenger of superoxide anion (Casini et al. 1997).

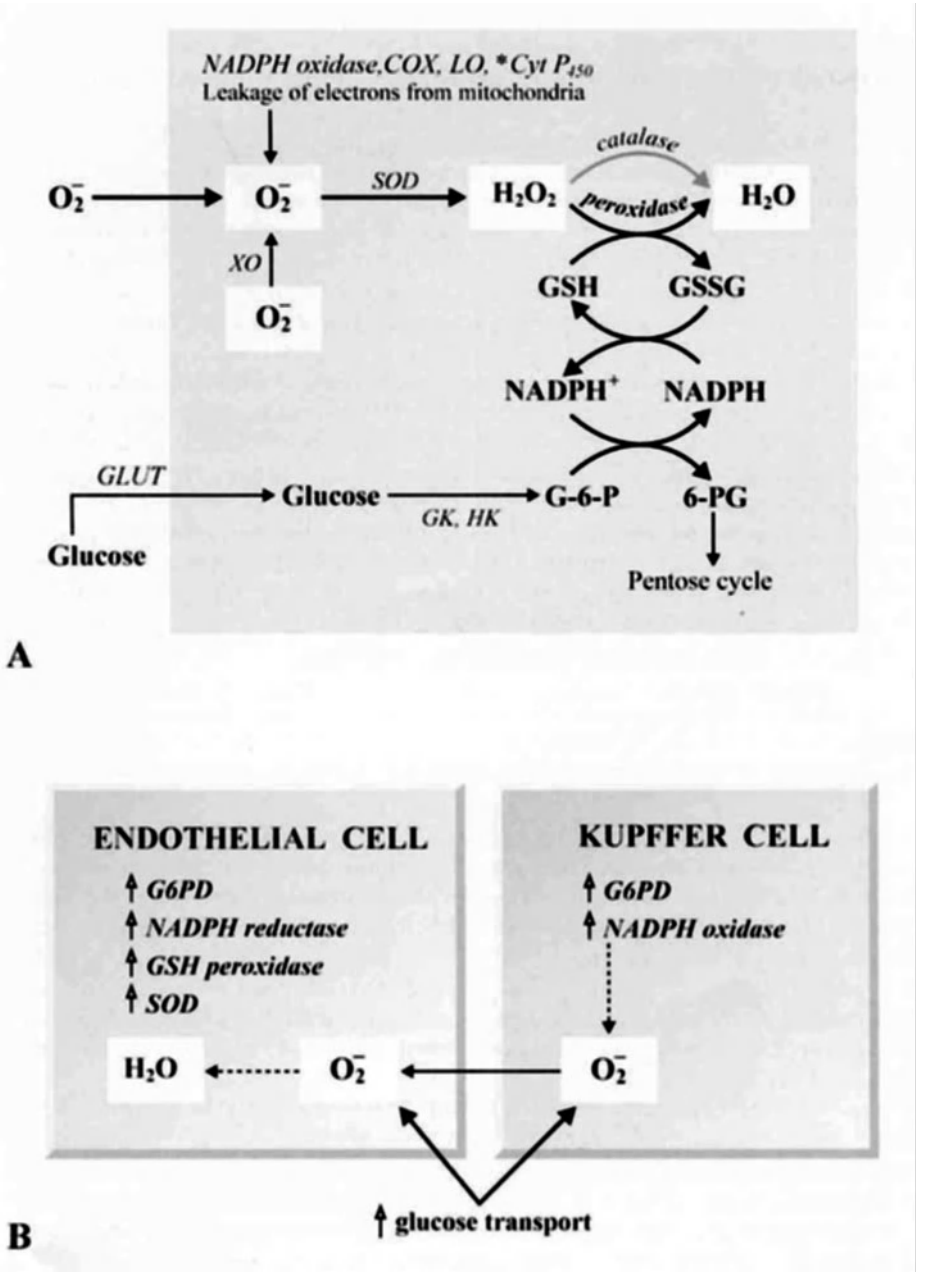

Fig. 15. A Main pathways of reactive oxygen radical metabolism. ROS such as H_2O_2, O_2^-, and OH are generated intracellularly or derive from cellular environment. *COX*, cyclooxygenase; *GK*, glucokinase; *GLUT*, glucose transporter(s); *G-6P*, glucose-6-phosphate; *G6PD*, G-6-P dehydrogenase; *6-PG*, 6-phospho-gluconolactone; *GSH*, reduced glutathione; *GSSG*, oxidized glutathione; *HK*, hexokinase; *LO*, lipooxygenase; O_2^-, superoxide anion; *SOD*, superoxide dismutase; *XO*, xanthine oxidase; *asterisk* denotes NADPH:cytochrome P_{450} reductase. B Intercellular oxidant balance during endotoxemia between pro-oxidant Kupffer cells and anti-oxidant sinusoidal endothelial cells, *arrows* indicate increased cellular activity or content as described in text

13.5
Cross-Talk of Liver Cells in Endotoxin-Induced Metabolic Alterations

In mammals, disturbances of homeostasis due to infection, tissue injury, neoplastic growth, or immunological disorders induce profound changes in host metabolism reflected by increases in energy expenditure and enhanced glucose, protein, and lipid turnover, referred to collectively as the acute-phase response (Gabay and Kushner 1999). The hypercatabolic state elicited in endotoxemia, which may be regarded as directed to the creation of sufficient response by the immune system, is caused by classical hormones such as adrenalin, cortisol, and glucagon, and by many cytokines such as IL-1, TNF-α, IL-6, IGF-I, EGF, and TGF-β (Filkins 1985). Increased protein breakdown in muscles provides amino acids as substrates for the synthesis of acute-phase proteins in the liver, for the proliferation of inflammatory cells, and synthesis of immunoglobulins (Hasselgren 1995). Altered metabolism of plasma lipids and lipoproteins, leading to a hyperlilidemic state, delivers important energetic fuels, and may be important for the inactivation of endotoxin (Hardardotirr et al. 1994). The initial increase of glucose release from hepatocytes provides energetic substrates for the ischemic tissues; however, hypoglycemia develops in the later phase of septicemia (Filkins 1985). Many of these changes, which are caused by altered metabolism of hepatocytes, are induced by mediators released both from extrahepatic sources, as well as from hepatocytes and nonparenchymal liver cells.

13.5.1
Interactions of Liver Cells During Synthesis of Acute-Phase Proteins

In the course of acute-phase response a family of gene products known as acute-phase proteins (APPs) is expressed in the liver. APPs act as inhibitors of plasma proteinases that play an important role in the activation of complement, kallikrein-kinin, thrombosis, and fibrinolytic systems; moreover, APPs may also function as scavengers, metal chelators, or coagulants (Koj 1996). During acute-phase reaction hepatocyte synthesis of so-called positive APPs, such as α_2-macroglobulin, C-reactive protein, α_1-acidic glycoprotein, or α_1-antitrypsin is upregulated, whereas the synthesis of "negative" proteins, such as albumin or phosphoenolpyruvate kinase, is decreased (Koj 1996; Wang et al. 1995b).

The increased expression of APPs by the liver in response to inflammation at extrahepatic sites is mediated primarily by the concerted action of IL-1- and IL-6-type cytokines (such as IL-11, leukemia inhibitory factor, and oncostatin M), TNF-α, IFN-γ, and glucocorticoids (Gabay and Kushner 1999). It was found that the in vivo endotoxin administration resulted already after 1 h in the prominent increases of IL-1α, IL-6, and TNF-α levels in the rat liver (Luster et al. 1994), due to the activation of NF-κB observed in Kupffer cells, hepatocytes, and SEC (Essani et al. 1996; Saad et al. 1995). It was shown that IL-1, IL-6, and TNF-α released from Kupffer cells inhibited albumin synthesis in hepatocytes co-cultured with Kupffer cells, with IL-6 having the greatest effect (Itoh et al. 1994; Kowalski et al. 1992). Proinflammatory cytokines released from Kupffer cells may also impair hepatic drug metabolism through the suppression of cytochrome P450- and UDP glucuronosyl transferase-dependent activities in hepatocytes (Monshouwer et al 1996; Morgan et al. 1994). Moreover, IL-6

secreted by cultured rat hepatocytes challenged in vitro with endotoxin has been shown to modulate synthesis of acute-phase proteins in an autocrine way (Saad et al. 1995). Thus, both endotoxin-activated Kupffer cells and hepatocytes, acting in the paracrine and autocrine way, play an important role in the induction of acute-phase reaction in the liver via the release of proinflammatory cytokines.

13.5.2 Cooperation of Liver Cells in the Regulation of Glucose Metabolism in Endotoxemia

Sepsis and endotoxemia lead to the profound changes of the whole body carbohydrate metabolism that are caused by cytokine- and hormone-mediated mechanisms. Administration of a moderately low dose of endotoxin to the intact animal has been found to evoke an initial hyperglycemia with increased glucose turnover and elevated lactate levels, followed by hypoglycemia (Filkins 1985). These alterations are thought to be caused by the elevated glucose output from hepatocytes through the stimulation of glycogenolysis by prostanoids, IL-1β, IL-6, or NO released from Kupffer cells (Chap. 10), which is followed by decreased gluconeogenesis and glycogen synthesis (Horton et al. 1994), concurrent with increased glycolysis (Wang et al. 1995b). The inhibition of gluconeogenesis has resulted partially from the suppression of phosphoenolpyruvate carboxinase gene transcription in hepatocytes, which was caused by IL-6 and TNF-α (Christ et al. 1997; Hill and McCallum 1992), cytokines released in the endotoxin-challenged liver mainly from Kupffer cells. Moreover, the expression of another key gluconeogenic enzyme, glucose-6-phosphatase, was strongly downregulated in the septic rat (Deutschman et al. 1997), and the enzyme transcription rate was strongly decreased in mice treated with TNF-α (Metzger et al. 1997). A few hours after in vivo endotoxin challenge, nitric oxide was found to be partially responsible for the inhibition of the gluconeogenic flux in the liver (Horton et al. 1994; Stadler et al. 1995), and for the decreased activity of the GLUT-2 glucose transporter (Casada et al. 1996). These data strongly suggest that a general impairment of hepatic gluconeogenesis in endotoxemia may be caused by the action of proinflammatory cytokines and nitric oxide generated locally in activated Kupffer, or systematically in extrahepatic cells.

The impaired hepatic glucose production in endotoxemia is accompanied by increased glucose use by peripheral tissues, including the liver itself (Meszaros et al. 1987). Endotoxin (LPS), cytokine, or phagocytic challenge augmented glucose use two- to threefold in the whole liver due to the increase in glucose use by nonparenchymal liver cells (Kupffer cells and SEC), and infiltrating hepatic neutrophils (Meszaros et al. 1991). The increased glucose uptake by the LPS-activated Kupffer and endothelial cells resulted from the upregulation of gene expression and increased membrane translocation of GLUT1 glucose transporter (Spolarics 1998). Although most of the glucose was used for glycolysis, LPS-primed Kupffer cells and sinusoidal endothelial cells, but not hepatocytes, showed increased gene expression and activity of glucose-6-phosphate dehydrogenase, a rate-limiting enzyme of the pentose cycle that generates NADPH, the obligatory reduced equivalent for all major ROS producing and detoxifying pathways and fatty acid synthesis (Spolarics and Navarro 1994; Spolarics and Wu 1997). The increase of glucose flux through the pentose cycle observed in Kupffer cells and SEC after LPS or TNF-α administration was highly correlated with

elevated superoxide anion production (Spolarics et al. 1993; Spolarics and Wu 1997). Thus, during endotoxemia elevated glucose use by the liver serves not only the increased energy need of extrahepatic cells and organs, but also supports, in a cell-specific way, glucose-dependent ROS metabolism in Kupffer and endothelial cells (Fig. 15).

Although Kupffer cells play an important physiological role in the clearance of bacteria and/or endotoxins from the circulation, abnormally high exposure of liver macrophages to bacterial products may lead to the elevated production of inflammatory mediators, and ultimately to liver injury. The data reported in this chapter suggest that activated Kupffer cells play the key role in the pathogenesis of LPS-induced liver injury by the virtue of (1) the ability to capture endotoxins from the circulation, (2) the capacity to respond to LPS by secreting an array of mediators (see Table 17), and (3) the capacity of these mediators to elicit various responses both at the organ and systemic levels. Sinusoidal endothelial cells enable the transmigration of neutrophils, which constitute important effector of hepatotoxic cells, into the direct vicinity of parenchymal liver cells. However, hepatocytes seem to represent not only a "passive" object of inflammatory attack, since these cells react to the damage by secretion of substances such as nitric oxide and acute-phase proteins, which may function in the cell-cytotoxic or cell-protective ways.

Besides the above-described involvement of cytokines, nitric oxide, and reactive oxygen species in the endotoxin-induced hepatic injury, other potent intercellular messengers, such as endothelin, cysteinyl leukotrienes, and platelet-activating factor (Bautista and Spitzer 1992; Denzlinger 1996; Eakes et al. 1997), have been found to play an important role in the pathogenesis of septic liver injury. Moreover, intercellular communication was found to be similarly important in the pathogenesis of other forms of liver damage such as ischemia-reperfusion (Jaeschke and Smith 1997), hepatotoxic (Laskin 1990), alcoholic (Casini 2000), or preservation (Bilzer and Gerbes 2000) liver injury. Several possibilities can be proposed for the cross-talk of different mediators culminating in the liver damage. First, several of these substances may act simultaneously so that pathophysiological changes in the liver would result from their synergistic action. Secondly, a single mediator acting in an autocrine manner may stimulate the production of several other mediators that contribute to the damage of liver tissue. Thirdly, substances released from neighboring cells may act in a paracrine way to augment or decrease the synthesis of inflammatory mediators or expression of adhesion molecules involved in the interactions of infiltrating mononuclear cells with nonparenchymal and parenchymal liver cells. Increased understanding of the basic mechanisms of communication between nonparenchymal and parenchymal liver cells, and between resident liver cells and infiltrating leukocytes (neutrophils, lymphocytes, and monocytes) in endotoxemic shock, will undoubtedly help in the development of new diagnostic and therapeutic measures.

14 Cooperation of Liver Cells in the Process of Liver Fibrosis

Fibrosis is the common response to chronic liver injury from various origins including metabolic diseases, viral infections, alcohol abuse, and various chemicals. Liver fibrosis is characterized by both quantitative and qualitative changes in the composition and distribution of extracellular matrix (ECM) that are reflected by a three- to fivefold net increase of ECM and replacement of low-density basement membrane-like material by interstitial type matrix abundant in fibril-forming collagens (Friedman 1993; Gressner 1998). This gross remodeling of ECM in the fibrotic liver represents an imbalance between the deposition and degradation of ECM molecules. Hepatic stellate cells, which are involved in the regulation of ECM production and degradation (Table 10), have been found to play a pivotal role in the initiation and progression of hepatic fibrosis (Friedman 1993, 2000; Gressner 1998). Following acute or chronic liver injury, hepatic stellate cells transdifferentiate: they proliferate, lose lipid droplets, change morphology from the star-shaped cells to that of myofibroblasts with the expression of smooth muscle α-actin (reviewed by Gressner 1998), and migrate to sites of tissue damage (Ikeda et al. 1999; Marra et al. 1998b). Activation of stellate cells that initiates the development of the inflammatory process results from multiple interactions between many cell types (injured hepatocytes, Kupffer cells, endothelial cells, platelets, infiltrating inflammatory cells) mediated by cytokines and reactive oxygen species, and from the changes in the composition of the perisinusoidal matrix (Arthur 2000; Gressner 1998). In the case of chronic liver damage, HSC activation persists during the "perpetuation phase" (Friedman 1993; Gressner 1998), and progressive accumulation of ECM leads to liver fibrosis, and finally to cirrhosis. The key role of hepatic stellate cells in the development of liver fibrosis may be deduced from the correlation between the number of HSC and the extent of liver fibrosis observed both in experimental liver injury (Friedman 1993) or in patients with chronic hepatitis C treated with interferon (Sakaida et al. 1999).

14.1 Factors Involved in the Activation of Hepatic Stellate Cells

Fibrogenesis is regarded as a dynamic process related to the extent and duration of parenchymal cell injury. The cascade of events that leads in vivo to the development of liver fibrosis is initiated by noxious agents that may be different in various kinds of liver damage. It is, however, widely believed that injury to hepatocytes and/or Kupffer and endothelial cells results in the release of many substances that cause transformation of quiescent stellate cells into myofibroblast-like cells. The activation of HSC may

result from both direct action of toxic molecules such as reactive oxygen species or paracrine and autocrine effects of many mediators (Friedman 2000).

It has been assumed that the in vivo activation of hepatic stellate cells, which leads to the increase of their number and secretory activity, may be recapitulated in vitro as a process of transformation of quiescent HSC in myofibroblast-like cells (see Sect. 5.4). However, by the use of the proteomics technique it was demonstrated that several proteins found to be prominently upregulated in cultured activated HSC were unchanged during physiological activation in vivo, implicating that culture-induced activation does not always correspond to the in vivo situation (Bach Kristensen et al. 2000). Moreover, some effects of cytokines on HSC suggest that "activation" of cultured stellate cells (assessed, e.g., by the expression of smooth muscle α-actin and TGF-β/activin type I receptor) is not always followed by their increased proliferation (Knittel et al. 1997b).

Under in vitro conditions platelet-derived growth factor (PDGF), released in the liver by platelets and Kupffer cells, was shown to be the most potent mitogen for rat (Friedman and Arthur 1989) and human (Friedman and Rockey 1992) stellate cells. Overexpression of PDGF receptors on HSC (absent on normal, quiescent cells) has been found both in human liver disease and experimental models of liver injury (Pinzani et al. 1996b; Wong et al. 1994). In cultured rat HSC the cell-cycle transition from G1 to S phase was closely related to the upregulation of cyclin D1, D2, and E expression, with a concomitant expression of PDGF-β receptor (Kawada et al. 1999). PDGF-regulated proliferation of hepatic stellate cells was shown to be transduced by the activation of two different pathways: the PI-3 kinase/MAP kinase pathway (Kawada et al. 1999), and the Na+/H+ exchange (Di Sario et al. 1999).

Apart from the mitogenic effect, PDGF (Carloni et al. 1997; Raghow et al. 1994) and basic FGF (Fibbi et al. 1999) increase motility of hepatic stellate cells through chemotactic activity of both cytokines, which may be promoted by the urokinase-type plasminogen activator (uPA), synthesized by HSC, which was shown to activate latent forms of both PDGF and basic FGF (Fibbi et al. 1999). Moreover, monocyte chemotactic peptide-1 released by activated stellate cells (Marra et al. 1993, 1998b) induced not only the migration of monocytes and lymphocytes, but also HSC (Marra et al. 1999). In the presence of chronic liver damage this action of MCP-1 may represent an additional stimulus for the recruitment of stellate cells into damaged regions of the liver and thus promote the development of hepatic fibrosis and scarring (Fig. 16).

The most important fibrogenic mediator in the liver is transforming growth factor-β, and excessive TGF-β activity of fibroblast-like cell types has been implicated in the pathogenesis of tissue fibrosis (Border and Noble 1994). A causative role for TGF-β1 in hepatic fibrosis has been supported by studies in transgenic mice in which the chronic production of mature TGF-β1 resulted in hepatic (and renal) fibrosis with increased collagen deposition and hepatocyte apoptosis (Sanderson et al. 1995). A prolonged increase in TGF-β expression at the organ level and in hepatic stellate cells has been demonstrated during human (Castilla et al. 1991) and experimental (Bissel et al. 1995) liver fibrosis.

TGF-β1 actively contributes to the shift of the balance between the deposition and degradation of extracellular matrix through multiple actions. It stimulates the transformation of quiescent HSC into myofibroblast-like cells (Bachem et al. 1993). In activated cells TGF-β augments synthesis of ECM proteins and proteoglycans, decreases production of ECM-degrading enzymes (collagenases and other metallopro-

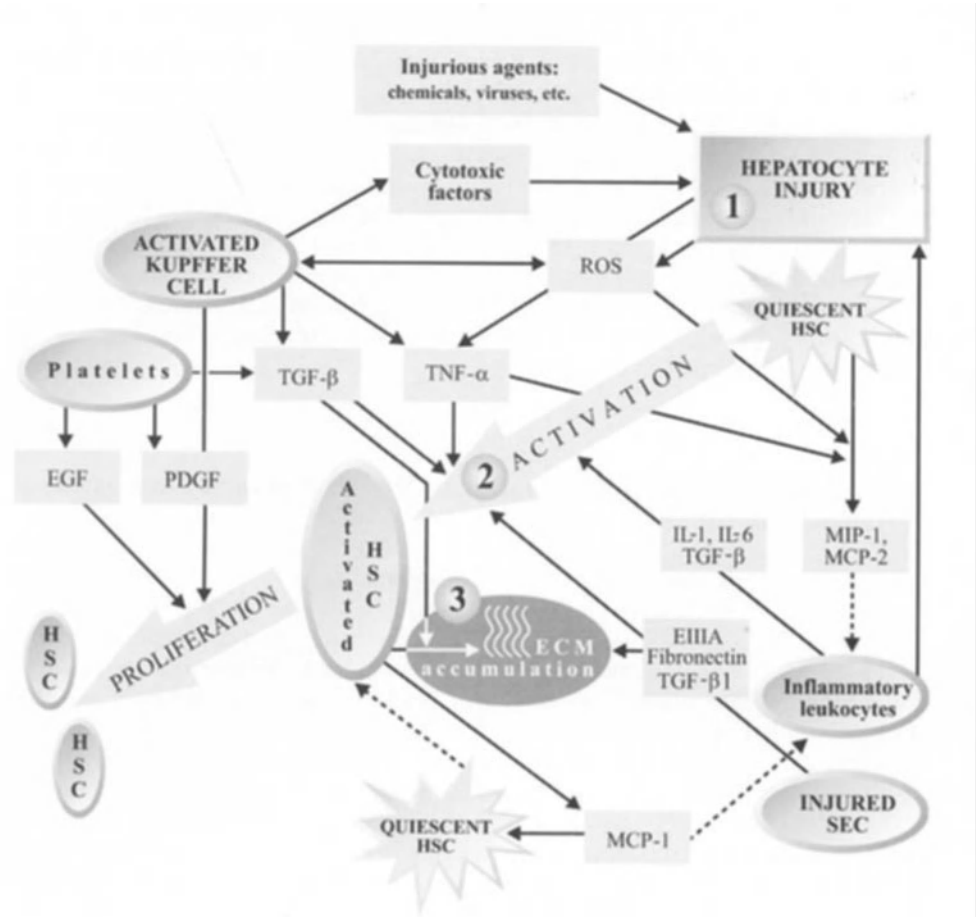

Fig. 16. Hypothetical model of mechanisms involved in hepatic stellate cell activation. Preinflammatory step (*1*) is initiated by the damage of hepatocytes that release substances stimulating transformation of stellate cells into myofibroblast-like cells (activated HSC). The HSC activation (*2*) is enhanced during the inflammatory step due to the action of mediators released from various cell types, some mediators are also potent inducers of HSC proliferation. Activated Kupffer cells and inflammatory leukocytes may additionally damage hepatocytes via the release of ROS and proteases. In the postinflammatory, or perpetuation step, the fully activated stellate cells release various cytokines and other substances (Table 10), whose activities result (*3*) in the net increase of the ECM deposition (see description in text). Only some major mediators involved in the intercellular crosstalks have been shown. Modified from Friedman (1993) and Gressner (1998). *Dotted lines* denote chemoattraction

teinases listed in Table 10), and increases synthesis of the inhibitors of metalloproteinases (Arthur 2000). TGF-β may indirectly enhance matrix degradation by inducing synthesis of plasminogen activator inhibitor (PAI-1) in sinusoidal endothelial cells (Rieder et al. 1993): decreased plasminogen activation would result in reduced activation of latent metalloproteinases by plasmin, and thus would favor accumulation of ECM. Although TGF-β alone did not show mitogenic or morphogenic effects on

quiescent stellate cells (Bachem et al. 1989b), it was indirectly involved in HSC proliferation via the induction of PDGF receptor expression, thereby increasing the mitogenic action of PDGF (Pinzani et al. 1995). Although TGF-β mRNA and immunoreactivity have been found in all resident types of nonparenchymal liver cells (De Bleser et al. 1997a; Bissel et al. 1995), HSC have been identified as the major TGFβ-expressing and -secreting cell types in normal rat (Bachem et al. 1992) and human (Casini et al. 1993) fibrotic liver. Studies of the TGF-β1 knockout mouse have revealed markedly reduced collagen accumulation in response to liver injury; however, increased expression of smooth muscle α-actin, a marker of stellate cell activation, which was observed in the liver, suggested a role for TGF-β in perpetuation rather than initiation of stellate cell activation (Hellerbrand et al. 1999).

14.2 Cellular Cross-Talk During Activation of Stellate Cells and Progression of Hepatic Fibrogenesis

Several phenotypic characteristics of activated stellate cells are important for the perpetuation of the initial profibrogenic response in the damaged liver. These changes, which include retinoid loss, increased proliferation, chemotaxis, fibrogenesis, and contractility, decreased matrix degradation, and chemoattraction of white blood cells (Friedman 2000), are driven by cytokines and other factors (Table 18) released locally both from activated stellate cells and other types of liver cells. The importance of autocrine loops for the sustained activation of stellate cells was shown for the action of PDGF (Mallat et al. 1998), FGF (Fibbi et al. 1999; Rosenbaum and Blazejewski 1995), TGF-β (Gressner 1998), and endothelin-1 (Pinzani et al. 1996a). However, the initial activation of HSC is thought to be mainly influenced by products released locally from damaged hepatocytes and activated Kupffer cells, sinusoidal endothelial cells, inflammatory cells infiltrating the liver, and platelets (Fig. 16).

The greatest stimulation of extracellular matrix synthesis by HSC was observed when their culture medium was simultaneously enriched in the conditioned media (CM) of cultured hepatocytes and Kupffer cells (Gressner et al. 1993b). When added separately, CM of Kupffer cells enhanced proliferation of stellate cells, promoted transition of the quiescent into the myofibroblast-like phenotype, and increased ECM production. Also CM of cultured hepatocytes activated quiescent stellate cells (Gressner et al. 1993b). In similar experiments the chemical nature of mediators that stimulate the activity of stellate cells has been partially elucidated (Table 11). For example, release of TGF-β from Kupffer cells was shown to stimulate matrix synthesis and stellate cell activation (Friedman and Arthur 1989; Matsuoka and Tsukamoto 1990). In some models of liver injury reactive oxygen species released from damaged hepatocytes may increase profibrogenic activity of hepatic stellate cells through their increased proliferation and enhanced collagen type I synthesis (Casini et al. 1997; Lee et al. 1995), or by the induction in quiescent stellate cells of a potent mononuclear cell chemoattractant, MCP-1 (Xu et al. 1996). Fully activated stellate cells were shown to respond in vitro directly to bacterial endotoxin (LPS) by enhanced expression of MCP-1 (Sprenger et al. 1999). For the in vivo situation this suggests that the more stellate cells have been transformed to the activated phenotype, the more the liver becomes responsive to LPS, which may lead to a vicious circle of enhanced chemotac-

Table 18. Substances affecting fibrogenesis and proliferation of hepatic stellate cellsa

	References
Factors stimulating fibrogenesis by activated HSC	
TGF-β1	Bachem et al. 1993
Reactive oxygen species (ROS)	Casini et al. 1997; Lee et al. 1995
Acetaldehyde	Moshage et al. 1990
9-*cis*-retinoic acidb	Okuno et al. 1997
TNF-αc	Bachem et al. 1993; Knittel et al. 1997b
IGF-I	Svegliati-Baroni et al. 1999
IL-1β	Armendariz-Borunda et al. 1992
IL-6	Greenwel et al. 1995
Oncostatin M	Levy et al. 2000
Factors promoting proliferation of HSC	
PDGFβ	Friedman and Arthur 1989; Mallat et al. 1998
TGF-α	Bachem et al. 1989b; Reeves et al. 2000
TGF-β1d	Pinzani et al. 1995
EGF	Bachem et al. 1989b
b-FGFe	Rosenbaum and Blazejweski 1995
HGF	Maher 1993
IGF-I	Gentilini et al. 1998; Pinzani et al. 1990; Skrtic et al. 1997
VEGF	Ankoma-Sey et al. 1998
Phosphatidic acid	Reeves et al. 2000
9-*cis*-retinoic acid	Hellemans et al. 1999
Sphingosine 1-phosphate	Ikeda et al. 2000
Vasoactive agents	
Thrombin	Mallat et al. 1998; Marra et al. 1995
Arginin vasopressin	Bataller et al. 1997
Atrial natriuretic peptide	Gorbig et al. 1999
Factors inhibiting fibrogenesis by activated HSC	
HGF	Matsuda et al. 1997
Interferon-γ and -α	Mallat et al. 1995
TNF-α	Armendariz-Borunda et al. 1992; Irabaru et al. 2000
Endothelin-1 (effect in activated HSC)	Mallat et al. 1996

Table 18 *(Continued).* Substances affecting fibrogenesis and proliferation of hepatic stellate cellsa

	References
All-*trans*-retinoic acidf	Hellemans et al. 1999; Wang et al. 2000
NO donors	Casini et al. 1997
α_2-Macroglobulin	Schuftan and Bachem 1999
Factors inhibiting proliferation of HSCa	
Interferon-γ and -α	Mallat et al. 1995; Rockey et al. 1992
TNF-α	Knittel et al. 1997b; Saile et al. 1999
TGF-βg	Bachem et al. 1989b; Dooley et al. 2000; Saile et al. 1999
LPS	Kawada et al. 1998
Thrombin	Mallat et al. 1998
Endothelin-1	Mallat et al. 1996
Retinoids	Pinzani et al. 1992b
Retinoic acid	Davis et al. 1990
Ligands of PPARγ	Galli et al. 2000
Nitric oxide donorsh	Failli et al. 2000
Rapamycini	Zhu et al. 1999
Inhibitors of GTPase RhoA	Iwamoto et al. 2000

a Many liver and extrahepatic cells secrete growth factors promoting proliferation of stellate cells; they include hepatocytes, Kupffer cells, infiltrating macrophages and platelets, and activated stellate cells.
b Effect mediated by inducing the activation of latent TGF-β in HSC.
c TNF-α stimulated fibronectin and tenascin synthesis (Knittel et al. 1997b); however, it decreased total and type I collagen synthesis (Armendariz et al. 1992; Hernandez-Munoz et al. 1997) in cultured stellate cells.
d Indirect effect via the transmodulation of PDGF receptors.
e Indirect effect.
f Retinoic acid decreased the activation of the α_2(I) collagen promoter but had no effect on the activation of the α_1(I) collagen promoter in cultured stellate cells.
g Different activities dependent on the activation status of HSC have been reported.
h Indirect effect by inhibiting the mitogenic effect of PDGF mediated partially by the increased cAMP synthesis resulting from PGE_2 synthesis.
i Indirect effect by inhibiting the mitogenic effect of PDGF.

tic mediator production. One of many autocrine loops that are involved in progression of the fibrogenic activity of HSC may be based on the stimulation by TGF-β, secreted by activated stellate cells, of MCP-1 and matrix production, and the induced changes in the matrix composition may further stimulate chemokine secretion (Marra et al. 1998b). Another autoregulatory feedback loop involves, related to the

stellate cell activation, stabilization of the endothelin-converting enzyme mRNA (ECE-1, required for the proteolysis of prepro-endothelin into mature endothelin), which results in the enhanced ET-1 synthesis (Shao et al. 1999). Because TGF-β, a major product of activated HSC, stabilizes ECE-1 mRNA, endothelin-1 may activate stellate cells in an autocrine fashion by a mechanism that involves TGF-β (Friedman et al. 2000).

It is widely believed that activation of Kupffer cells represents a critical component in the cascade of liver fibrosis. Rapid accumulation of Kupffer cells often precedes the onset of stellate cell activation in vivo (Johnson et al. 1992). Kupffer cells and activated stellate cells seem to be the most important sources of TGF-β in the liver (De Bleser et al. 1997a; Matsuoka and Tsukamoto 1990; Meyer et al. 1990a). However, beside TGF-β, Kupffer cells release a spectrum of other cytokines, including PDGF, TNF-α, IL-lα, IL-6, and TGF-α, which affect proliferation and modulate phenotype of stellate cells (Table 15; Gressner 1998). It was found that TNF-α promoted transdifferentiation of quiescent stellate cells into myofibroblasts (Bachem et al. 1993), and showed differentiated action on the secretory activity of HSC: it increased synthesis of some ECM proteins, particularly of fibronectin and tenascin, but decreased collagen type III (Knittel et al. 1997b) and collagen type I (α_1) expression (Hernandez-Munoz et al. 1997). It has been suggested that the initial matrix breakdown following liver injury might be enhanced by TNF-α, which has been found to stimulate both matrix metalloproteinases and TIMP expression of HSC, while diminished matrix degradation during chronic tissue injury might be due to the action of TGF-β1 through TIMP induction (Arthur 2000). However, TNF-α may directly antagonize some fibrogenic actions of TGF-β1 through the induction of proteinase expression (Armendariz-Borunda et al. 1992) and down-regulation of the α(I) collagen gene expression in rat hepatic stellate cells (Irabaru et al. 2000).

Proinflammatory cytokines, IL-1, TNF-α, and interferon-γ, secreted by activated Kupffer cells, are very potent stimuli for MCP-1 gene and protein expression (Marra et al. 1993; Sprenger et al. 1999; Xu et al. 1996), which may be important for the chemoattraction of not only inflammatory cells, but also quiescent stellate cells (Fig. 16). TNF-α has been found to induce both in quiescent and activated stellate cells synthesis of a neutrophil-specific chemokine, MIP-2 (Sprenger et al. 1997). Thus, products of activated Kupffer cells are important for the generation of chemotactic gradients for neutrophils, monocytes, and lymphocytes recruited into the damaged liver. Infiltrating inflammatory cells may accelerate fibrogenic activity through the secretion of cytokines that activate HSC such as IL-1, IL-6, and TGF-β. Interleukin-6 produced during the acute-phase response, both in Kupffer cells and outside the liver, may play a role in hepatic fibrogenesis via induction of collagen, fibronectin, and TGF-β mRNA expression in HSC (Greenwel et al. 1995). Moreover, neutrophils can activate stellate cells by an oxidant-dependent mechanism (Casini et al. 1997).

Sinusoidal endothelial cells, following early liver injury, produce a fetal isoform of fibronectin, a splice variant (containing in the rat the EIIIA region) that was shown to stimulate transformation of resting stellate cells to myofibroblast-like cells (Jarnagin et al. 1994). Because EIIIA fibronectin expression in cultured SEC was shown to be stimulated by TGF-β1 (George et al. 2000), this effect may be involved in the initiation of liver fibrogenesis by TGF-β. Moreover, in experimental liver fibrosis TGF-β1 expression was increased not only in stellate cells, but also in sinusoidal endothelial cells, which may suggest a role for SEC in hepatic fibrogenesis (De Bleser et al. 1997a).

During liver fibrogenesis injured hepatocytes are not only a source of oxygen radicals, chemokines, and substances that increase proliferation of stellate cells (Gressner et al. 1993b), but release also a soluble factor that can activate a latent form of ECM-remodelling enzyme, matrix metalloproteinase-2, which is secreted into the medium by activated stellate cells (Theret et al. 1997).

Besides hepatocytes and nonparenchymal liver cells, another important source of fibrogenic cytokines constitute platelets, present within injured liver, that have been shown to be a potent source of PDGF, TGF-β1, and EGF (Bachem et al. 1989a,b). Moreover, sphingosine 1-phosphate (S1P), a lipid mediator that is stored in platelets and released on their activation, has been found to enhance proliferation of culture-activated HSC, and to increase contraction of collagen lattices containing HSC (Ikeda et al. 2000). Because platelet consumption occurs in acute liver injury, S1P may play a role in the initiation of the wound healing process in the liver.

It has been recently increasingly recognized that not only cellular cross-talk, but also the status of the extracellular matrix plays an important role in the progression of tissue fibrosis. It has been shown that almost each ECM component has the ability to modulate cell growth, migration, and gene expression directly through the interactions with cell adhesion molecules, and indirectly by trapping mitogenic growth factors in their active or inactive forms (Raghow 1994).

Apart from paracrine interactions the activation of hepatic stellate cells has been found to be markedly augmented via autocrine action of above-mentioned cytokines and other factors, such as HGF, PAF, MCP-1, and endothelin-1 (Table 10). Because many mediators released from liver cells not only recruit and activate quiescent stellate cells in a paracrine way, but also induce vicious circles of progressing stellate cell activation, a mechanism for self-perpetuation in liver fibrosis may exist (Bachem et al. 1993; Bissell et al. 1995; Friedman 1993).

14.3 Factors Influencing the Resolution of Initial Fibrotic Changes of the Liver

Fibrosis of the liver represents a dynamic process characterized by the imbalance between increased matrix secretion and decreased matrix degradation. However, early in fibrosis the potential to remodel the fibrillar matrix exists, and hepatotoxin withdrawal may be associated with a return to normal liver histology. For example, it was shown that successful treatment with interferon of patients with hepatitis C-associated fibrosis, which led to the irradication of the virus, was correlated with significant improvement of the liver architecture (Sakaida et al. 1999).

The development of experimental liver fibrosis, and finally, cirrhosis, requires prolonged administration of the inducing agent such as, e.g., CCl_4 or alcohol. It has been demonstrated that with the removal of the fibrogenic stimulus, the activated stellate cells may be eliminated by their reversion to the quiescent phenotype (Iredale et al. 1998) or their death by apoptosis.

Apoptosis of stellate cells observed during the recovery phase from acute liver damage (Iredale et al. 1998; Saile et al. 1997) was shown to proceed through a Fas-mediated pathway (Saile et al. 1997, 1999). Moreover, HSC apoptosis was associated with the decreased expression of tissue inhibitors of metalloproteinases (TIMPs), which

may lead to a net increase in collagenase activity and account for matrix resorption associated with the liver's return to (almost) normal histology after self-limited injury (Iredale et al. 1998). Thus, apoptosis may represent an important mechanism terminating the proliferation of activated hepatic stellate cells. Whereas TGF-β either had no direct effect (Dooley et al. 2000) or inhibited (Saile et al. 1999) the proliferation of activated hepatic stellate cells, TNF-α inhibited their proliferation (Saile et al. 1999). However, both cytokines were also shown to decrease spontaneous apoptosis of cultured stellate cells, and thus to sustain their activation and increase production of ECM components (Saile et al. 1999). The antiapoptotic and antiproliferatory actions of TNF-α and TGF-β may thus represent the mechanism responsible for prolonged survival of activated stellate cells in chronic liver damage in vivo.

Anti-inflammatory cytokines produced by nonparenchymal liver cells have also been identified as possible factors that may contribute to the reversal of hepatic fibrosis. IL-10 has been shown to downregulate inflammation by inhibiting macrophage production of TNF-α (Knolle et al. 1997). Induction of IL-10 has been observed during the activation of stellate cells (Thompson et al. 1998; Wang et al. 1998b) or Kupffer cells (Knolle et al. 1995); however, in progressive human fibrosis due to hepatitis C virus the levels of IL-10 were reduced (Napoli et al. 1996). Down-regulation by IL-10 of TNF-α production in Kupffer cells could hamper the development of liver fibrosis, since TNF-α was shown to stimulate the synthesis of extracellular matrix proteins in stellate cells (Knittel et al. 1997b). Interferon-γ, a product of activated monocytes and lymphocytes, inhibited both proliferation and activation of cultured rat stellate cells, and reduced the expression of extracellular matrix proteins, fibronectin, tenascin, and collagen type III (Knittel et al. 1997b). These observations suggest that IL-10 and IFN-γ may play an important role in liver repair, and thus may represent a potential therapy for progressive liver disease. Indeed, the first pilot trial of IL-10 in patients with chronic hepatitis C, interferon nonresponders, showed a decrease of liver fibrosis after 3 months of treatment, despite lack of apparent antiviral activity (Nelson et al. 2000).

The biotechnologically-engineered, soluble TGF-β type II receptor fused to human immunoglobulin G was shown to effectively decrease experimental liver fibrosis through the in vivo inhibition of stellate cell activation (George et al. 1999). A local infusion of adenoviral vectors expressing a soluble TGF-β receptor was shown to suppress the development of experimental liver fibrosis (Ueno et al. 2000). It is hoped that anti-TGF-β intervention, e.g., through the blockade of TGF-β signaling (Qi et al. 1999), may open new avenues for the effective therapy of human liver fibrosis. Interestingly, another potent cytokine, hepatocyte growth factor, a major hepatocyte mitogen, was shown to suppress experimental liver fibrosis (Matsuda et al. 1997).

Antifibrogenic action of some NO donors may affect direct inhibition of PDGF-induced proliferation, motility, and contractility of HSC (Failli et al. 2000), in addition to a reduction of fibrillar extracellular matrix accumulation (Casini et al. 1997). Some other antifibrogenic substances, which have been shown to inhibit the proliferation of stellate cells and/or production of ECM components, have been listed in Table 18.

15 Perspectives

The methodological advances in the isolation and culturing of different types of liver cells have shown that the reciprocal interactions between hepatocytes and nonparenchymal liver cells, and between different types of nonparenchymal cells, play an important role in liver functions under both normal and pathological situations. The expression of many intercellular mediators has been shown to be regulated by other molecules released by various types or the same type of liver cell, thus providing a basis for the existence of positive or negative feedback loops. From the teleological point of view, the intercellular signaling networks within the liver act to provide effective support of parenchymal liver cell function. Thus, sinusoidal endothelial cells and Kupffer cells protect hepatocytes from huge amounts of noxious materials arriving in the blood, due to their huge capacity for endocytosis and phagocytosis, and the ability to induce in the liver rather the state of peripheral tolerance than induction of immunity (Knolle and Gerken 2000). Stellate cells in normal liver, apart from their role in the metabolism of retinoids and the regulation of sinusoidal blood flow, control the production and degradation of extracellular matrix components that build the basal framework of the organ structure.

The interplay of intercellular mediators released from both parenchymal and nonparenchymal liver cells is responsible for many pathological effects observed in the course of liver inflammation resulting from its injury. The outcome of liver injury, its resolution or protraction into a chronic process that involves fibrotic response results from multiple interactions between activated resident cells (hepatocytes, Kupffer, stellate and endothelial cells), platelets, and infiltrating inflammatory cells; however, the exact roles played by the individual factors released from various cell types have to be further delineated. The intensive research aimed at the dissection of intricate networks of intercellular communication during liver inflammation is essential for the introduction of novel therapeutic strategies to treat such grave conditions as endotoxic shock or liver fibrosis with its complications: portal hypertension and cirrhosis.

The liver has a dual role in the immunological system through the elimination of antigens and pathogens, and the avoidance of immune response against these antigens, i.e., immunological tolerance. The investigations of local liver immune responses may greatly expand the knowledge about the mechanisms of immune-mediated liver damage (Knolle and Gerken 2000), as well as explain the phenomena of transplantation tolerance that may lead to improved rates of successful allogenic liver transplantation. The elucidation of the cooperation between resident liver cells (KC, SEC, and IHL) and infiltrating tumor cells will greatly improve the knowledge about the role played by the liver in the antitumor defenses (Vidal-Vanaclocha et al. 2000).

Activation of hepatic NK T or pit cell function by the administration of IL-2, IL-12, or IL-18 may be used as a way to decrease hepatic metastasis formations in patients operated on because of tumors of other organs, e.g., colon cancer (Okuno et al. 2000; Tsutsui et al. 1996). Since the adult mammalian liver contains hematopoietic pluripotent stem cells, which give rise to multiple cell lineages, including thymic and extrathymic T cells, granulocytes, and erythroid lineage cells (Crispe et al. 1996), further studies on the role played by the cross-talks of parenchymal and nonparenchymal liver cells with intrahepatic lymphocytes for the regulation of liver homeopoiesis, as well as other immunological functions of the liver, may be expected.

The clinical use of isolated liver cells may be available in a relatively short perspective. Recent progress in the field of hepatocyte culture methods has led to the expression of differentiated liver functions in primary cultures of human hepatocytes maintained for several weeks in serum-free medium, which allows for performing metabolic, pharmacologic-toxicologic studies, and investigations on human pathogens under defined chemical conditions (Runge et al. 2000). The experimentation of hepatocyte-based bioartificial liver systems (BAL) is being used to support acute liver failure, a chronically diseased liver, and to correct genetic disorders resulting in metabolically deficient states (Puviani et al. 1998). The next logical step in the construction of more effective BALs should lead to the enrichment of these systems in nonparenchymal liver cells (Bhatia et al. 1999). Indeed, such an approach allowed to obtain three-dimensional acinar or cyst-like structures (Mitaki et al. 1999), or acinar-like plates of hepatocytes that exhibited canaliculus-like channels between hepatocytes after 2–3 weeks of culturing hepatocytes in the medium enriched in nonparenchymal cells (Michalopoulos et al. 1999; Mitaka et al. 1999). The supportive role of the co-proliferating nonparenchymal cells, principally stellate cells, for the functions of hepatocytes may be in the synthesis of endogenous matrix, which is difficult to be replaced by bio-engineered matrices, and in the development of multiple hepatocyte-nonparenchymal cell interactions, which are important also for growth and regeneration of the liver. The potential of nonparenchymal liver cells for supporting the efficient repopulation of damaged liver by purified hematopoietic stem cells that differentiate into hepatocytes and/or cholangiocytes (e.g., Lagasse et al. 2000; Theisse et al. 2000) will soon become the subject of investigations.

Site-specific delivery of drugs such as chemotherapeutics or antisense prodrugs potentially effective in modulating the expression of adhesion molecules or transcription factors (e.g., NF-κB) implicated in the development and progression of inflammatory processes in the liver may be approached by the use of endocytic and phagocytic properties of sinusoidal endothelial cells and Kupffer cells (Terpstra et al. 2000).

Besides a major role in the pathogenesis of liver-associated dysfunction, Kupffer cell activation was shown to play an important role in the systemic inflammatory response to acute pancreatitis (Folch et al. 2000) or in the endotoxin-related lung injury (Wheeler et al. 2000a). It may be envisaged that the use of novel approaches such as the use of recombinant adenoviral vectors to target and inactivate Kupffer cells (Wheeler et al. 2000b) may lead to the therapeutically beneficial suppression of the activation of liver macrophages which is involved in the diseases of liver and other organs.

16 Summary

The liver lobule is formed by parenchymal cells, i.e., hepatocytes and nonparenchymal cells. In contrast to hepatocytes that occupy almost 80% of the total liver volume and perform the majority of numerous liver functions, nonparenchymal liver cells, which contribute only 6.5% to the liver volume, but 40% to the total number of liver cells, are localized in the sinusoidal compartment of the tissue. The walls of hepatic sinusoid are lined by three different cell types: sinusoidal endothelial cells (SEC), Kupffer cells (KC), and hepatic stellate cells (HSC, formerly known as fat-storing cells, Ito cells, lipocytes, perisinusoidal cells, or vitamin A-rich cells). Additionally, intrahepatic lymphocytes (IHL), including pit cells, i.e., liver-specific natural killer cells, are often present in the sinusoidal lumen. It has been increasingly recognized that both under normal and pathological conditions, many hepatocyte functions are regulated by substances released from neighboring nonparenchymal cells.

Liver sinusoidal endothelial cells constitute the lining or wall of the hepatic sinusoid. They perform important filtration function due to the presence of small fenestrations that allow free diffusion of many substances, but not of particles of the size of chylomicrons, between the blood and the hepatocyte surface. SEC show huge endocytic capacity for many ligands including glycoproteins, components of the extracellular matrix (ECM; such as hyaluronate, collagen fragments, fibronectin, or chondroitin sulphate proteoglycan), immune complexes, transferrin and ceruloplasmin. SEC may function as antigen-presenting cells (APC) in the context of both MHC-I and MHC-II restriction with the resulting development of antigen-specific T-cell tolerance. They are also active in the secretion of cytokines, eicosanoids (i.e., prostanoids and leukotrienes), endothelin-1, nitric oxide, and some ECM components.

Kupffer cells are intrasinusoidally located tissue macrophages with a pronounced endocytic and phagocytic capacity. They are in constant contact with gut-derived particulate materials and soluble bacterial products so that a subthreshold level of their activation in the normal liver may be anticipated. Hepatic macrophages secrete potent mediators of the inflammatory response (reactive oxygen species, eicosanoids, nitric oxide, carbon monoxide, TNF-α, and other cytokines), and thus control the early phase of liver inflammation, playing an important part in innate immune defense. High exposure of Kupffer cells to bacterial products, especially endotoxin (lipopolysaccharide, LPS), can lead to the intensive production of inflammatory mediators, and ultimately to liver injury. Besides typical macrophage activities, Kupffer cells play an important role in the clearance of senescent and damaged erythrocytes. Liver macrophages modulate immune responses via antigen presentation, suppression of T-cell activation by antigen-presenting sinusoidal endothelial cells via parac-

rine actions of IL-10, prostanoids, and TNF-α, and participation in the development of oral tolerance to bacterial superantigens. Moreover, during liver injury and inflammation, Kupffer cells secrete enzymes and cytokines that may damage hepatocytes, and are active in the remodeling of extracellular matrix.

Hepatic stellate cells are present in the perisinusoidal space. They are characterized by abundance of intracytoplasmic fat droplets and the presence of well-branched cytoplasmic processes, which embrace endothelial cells and provide focally a double lining for sinusoid. In the normal liver HSC store vitamin A, control turnover of extracellular matrix, and regulate the contractility of sinusoids. Acute damage to hepatocytes activates transformation of quiescent stellate cells into myofibroblast-like cells that play a key role in the development of inflammatory fibrotic response.

Pit cells represent a liver-associated population of large granular lymphocytes, i.e., natural killer (NK) cells. They spontaneously kill a variety of tumor cells in an MHC-unrestricted way, and this antitumor activity may be enhanced by the secretion of interferon-γ. Besides pit cells, the adult liver contains other subpopulations of lymphocytes such as γδT cells, and both "conventional" and "unconventional" αβ T cells, the latter containing liver-specific NK T cells.

The development of methods for the isolation and culture of main liver cell types allowed to demonstrate that both nonparenchymal and parenchymal cells secrete tens of mediators that exert multiple paracrine and autocrine actions. Co-culture experiments and analyses of the effects of conditioned media on cultures of another liver cell type have enabled the identification of many substances released from nonparenchymal liver cells that evidently regulate some important functions of neighboring hepatocytes and non-hepatocytes. To the key mediators involved in the intercellular communication in the liver belong prostanoids, nitric oxide, endothelin-1, TNF-α, interleukins, and chemokines, many growth factors (TGF-β, PDGF, IGF-I, HGF), and reactive oxygen species (ROS). Paradoxically, the cooperation of liver cells is better understood under some pathological conditions (i.e., in experimental models of liver injury) than in normal liver due to the possibility of comparing cellular phenotype under in vivo and in vitro conditions with the functions of the injured organ.

The regulation of vitamin A metabolism provides an example of the physiological role for cellular cross-talk in the normal liver. The majority (up to 80%) of the total body vitamin A is stored in the liver as long-chain fatty acid esters of retinal, serving as the main source of retinoids that are utilized by all tissues throughout the body. Hepatocytes are directly involved in the uptake from blood of chylomicron remnants, and the synthesis of retinol-binding protein that transfers retinol to other tissues. However, more than 80% of the liver retinoids are stored in lipid droplets of hepatic stellate cells. HSC are capable of both uptake and release of retinol depending on the body's retinol status. The activity of some major enzymes of vitamin A metabolism have been found to be many times higher per protein basis in stellate cells than in hepatocytes. Despite progress in the understanding of the roles played by these two cell types in hepatic retinoid metabolism, the way in which retinoids move between the parenchymal cells, stellate cells, and blood plasma has not been fully elucidated.

Sinusoidal blood flow is, to a great extent, regulated by hepatic stellate cells that can contract due to the presence of smooth muscle α-actin. The main vasoactive substances that affect constriction or relaxation of HSC derive both from distant sources and from neighboring hepatocytes (carbon monoxide, leukotrienes), endothelial cells (endothelin, nitric oxide, prostaglandins), Kupffer cells (prostaglandins, NO), and

stellate cells themselves (endothelin, NO). The cellular cross-talk reflected by the fine-tuned modulation of sinusoidal contraction becomes disturbed under pathological conditions, such as endotoxemia or liver fibrosis, through the excess synthesis of vasoregulatory compounds and the involvement of additional mediators acting in a paracrine way.

The liver is an important source of some growth factors and growth factor-binding proteins. Although hepatocytes synthesize the bulk of insulin-like growth factor I (IGF-I), also other types of nonparenchymal liver cells may produce this peptide. Cell-specific expression of distinct IGF-binding proteins observed in the rat and human liver provides the potential for specific regulation of hepatic IGF-I synthesis not only by growth hormone, insulin, and IGF-I, but also by cytokines released from activated Kupffer (IL-1, TNF-α, TGF-β) or stellate cells (TGF-α, TGF-β). Hepatic stellate cells may affect turnover of hepatocytes through the synthesis of potent positive as well as negative signals such as, respectively, hepatocyte-growth-factor or TGF-β. Although hepatocytes seem not to produce TGF-β, a pleiotropic cytokine synthesized and secreted in the latent form by Kupffer and stellate cells, they may contribute to its actions in the liver by the intracellular activation of latent TGF-β, and secretion of the biologically active isoform.

Many mediators that reach the liver during inflammatory processes, such as endotoxins, immune-complexes, anaphylatoxins, and PAF, increase glucose output in the perfused liver, but fail to do so in isolated hepatocytes, acting indirectly via prostaglandins released from Kupffer cells. In the liver, prostaglandins synthesized from arachidonic acid mainly in Kupffer cells in a response to various inflammatory stimuli, modulate hepatic glucose metabolism by increasing glycogenolysis in adjacent hepatocytes. The release of glucose from glycogen supports the increased demand for energetic fuel by the inflammatory cells such as leukocytes, and additionally enables enhanced glucose turnover in sinusoidal endothelial cells and Kupffer cells which is necessary for effective defense of these cells against invading microorganisms and oxidative stress in the liver. Leukotrienes, another oxidation product of arachidonic acid, have vasoconstrictive, cholestatic, and metabolic effects in the liver. A transcellular synthesis of cysteinyl leukotrienes (LTC_4, LTD_4, and LTE_4) functions in the liver: LTA_4, an important intermediate, is synthesized in Kupffer cells, taken up by hepatocytes, converted into the potent LTC_4, and then released into extracellular space, acting in a paracrine way on Kupffer and sinusoidal endothelial cells. Thus, hepatocytes are target cells for the action of eicosanoids and the site of their transformation and degradation, but can not directly oxidate arachidonic acid to eicosanoids.

The exposure to high doses of endotoxin (LPS) results in sepsis and its complications, which may ultimately lead to death. Due to its integral role in metabolism and host defense mechanisms, the liver is a key organ responsible for the initiation of multiple organ failure during sepsis. Binding of endotoxins, contained in the portal blood, by Kupffer cells and hepatocytes results in the activation of Kupffer cells, and damage to hepatocytes and sinusoidal endothelial cells. Endotoxin-related injury of hepatocytes and SEC is not caused by direct toxic effect of LPS, but by proinflammatory mediators released from activated Kupffer cells and recruited inflammatory cells. Local and systemic effects of TNF-α released by LPS-elicited liver macrophages play an important role in endotoxin-induced liver damage. In contrast to the TNF-α action, in vivo models of sepsis-related liver injury suggested protective action of nitric oxide, another major intercellular mediator released from Kupffer cells, on liver function.

Because in vitro effects of nitric oxide on hepatocytes were cytotoxic, it is thought that the detrimental or beneficial NO action in the liver depends on its cellular concentration, presence of oxygen radicals, and different pathomechanisms of particular models of liver injury. TNF-α, interleukin-1, and interleukin-6, cytokines released from activated Kupffer cells, have been shown to induce synthesis of acute-phase proteins in hepatocytes, a part of nonspecific body's response to inflammation.

Accumulation of polymorphonuclear leukocytes (neutrophils) in the liver contributes prominently to the endotoxin-induced hepatocellular injury. Subsequent tissue damage results from the action of various mediators released from activated neutrophils and Kupffer cells. All major steps in the neutrophil-related liver injury (the initial sequestration of neutrophils in hepatic vasculature, transendothelial migration, and adherence-dependent cytotoxicity against hepatocytes) are dependent on intercellular cross-talk between Kupffer cells and damaged hepatocytes on one hand, and neutrophils and SEC on the other. The available data show that activated Kupffer cells play a key role in the pathogenesis of LPS-induced liver injury due to their (1) ability to capture endotoxins from the circulation, (2) capacity to respond to LPS through the secretion of many mediators, and (3) capacity of these mediators to elicit various responses both at the organ and systemic levels. In this complicated network of inflammatory events Kupffer cells may be, at the same time, targets and effectors of different, self-perpetuating processes that involve in the liver also hepatocytes, sinusoidal endothelial cells, stellate cells, and infiltrating leukocytes.

Fibrosis is the common response to chronic liver injury from various origins including metabolic diseases, viral infections, alcohol abuse, and various chemicals. Liver fibrosis is characterized by both quantitative and qualitative changes in the composition of extracellular matrix and its spatial distribution, which result from the imbalance between the deposition and degradation of ECM components. Hepatic stellate cells play a pivotal role in the initiation and progression of hepatic fibrosis. Fibrogenesis in the liver is initiated by hepatocyte damage that leads to the recruitment of inflammatory blood cells, platelets, and activation of Kupffer and endothelial cells with subsequent release of cytokines and reactive oxygen species. Various mediators, released mainly from neighboring cells, alter the phenotype of stellate cells from "quiescent" into "activated" one. Upon activation stellate cells proliferate, transform into myofibroblast-like cells (change shape from star- into spindle-like, loose lipid droplets, express smooth muscle α-actin and many other proteins not present in "quiescent" cells), migrate at the periphery of the liver lobule, and synthesize large amounts of connective tissue components. Sustained activation of stellate cells in chronic liver injury disturbs the balance between extracellular matrix synthesis and degradation, and a net increase of liver connective tissue occurs, leading to liver fibrosis, and finally to cirrhosis. The process of stellate cell activation is dependent on multiple interactions between many cell types present in the injured liver, and on alterations in the composition of extracellular matrix. Among the growth factors examined, PDGF and TGF-β are the most potent cytokines for HSC proliferation and fibrogenesis, respectively. The development of experimental liver fibrosis requires prolonged administration of the inducing agent; however, with the early removal of the fibrogenic stimulus, activated stellate cells may be eliminated by their reversion to the quiescent phenotype or their death by apoptosis. Antifibrotic substances may act at the various stages of the profibrogenic processes. Some of them may suppress the progression of the vicious circles that result in liver fibrosis. For example, IL-10, the

powerful inhibitor of macrophage activation, was found to decrease liver fibrosis in hepatitis C patients, whereas molecules that block signal transduction of TGF-β showed antifibrotic effectiveness in experimental liver fibrosis.

The extraordinary progress made during the last decade in the elucidation of the functions of nonparenchymal liver cells and their cooperation in normal and diseased liver suggests that further studies of the interactions between cells of the liver lobule will help in the implementation of novel diagnostic and therapeutic regimens in such areas as the control of the immune responses in viral liver diseases and liver transplantation, construction of bioartificial liver systems that may sustain organ functions before liver transplantation can take place, and pathomechanism-based antifibrotic therapy.

References

Adamson GM, Billings RE (1993) Cytokine toxicity and induction of NO synthase activity in cultured mouse hepatocytes. Toxicol Appl Pharmacol 119:100–107

Alam I, Bass NM, Bacchetti P, Gee L, Rockey DC (2000) Hepatic tissue endothelin-1 levels in chronic liver disease correlate with disease severity and ascites. Am J Gastroenterol 95:199–203

Anasagasti MJ, Alvarez A, Avivi C, Vidal-Vanaclocha F (1996) Interleukin-1-mediated H2O2 production by hepatic sinusoidal endothelium in response to B16 melanoma cell adhesion. J Cell Physiol 167:314–323

Andus T, Ramadori G, Heinrich PC, Knittel T, Meyer-zum-Buschenfelde KH (1992) Cultured Ito cells of rat liver express the α2-macroglobulin gene. Eur J Biochem 168:641–646

Ankoma-Sey V, Matli M, Chang KB, Lalazar A, Donner DB, Wong L, Warren RS, Friedman SL (1998) Coordinated induction of VEGF receptors in mesenchymal cell types during rat hepatic wound healing. Oncogene 17:115–121

Aono K, Isobe K, Kiuchi K, Fan-ZH, Ito M, Takeuchi A, Miyachi M, Nakashima I, Nimura Y (1997) In vitro and in vivo expression of inducible nitric oxide synthase during experimental endotoxemia: involvement of other cytokines. J Cell Biochem 65:349–358

Arany E, Afford S, Strain AJ, Winwood PJ, Arthur MJ, Hill DJ (1994) Differential cellular synthesis of insulin-like growth factor binding protein-1 (IGFBP-1) and IGFBP-3 within human liver. J Clin Endocrinol Metab 79:1871–1876

Armbrust T, Schmitt E, Ramadori G (1995) Viable rat Kupffer cells synthesize but do not secrete interleukin-1: indications for necrosis-induced maturation of interleukin-1α, but not of interleukin-1β. Biochem Biophys Res Commun 207:637–645

Armbrust T, Nordmann B, Kreissig M, Ramadori G (1997) C1q synthesis by tisue mononuclear phagocytes from normal and damaged rat liver: up-regulation by dexamethason, down-regulation by interferon γ, and lipopolysaccharide. Hepatology 26:98–106

Armendariz-Borunda J, Katayama K, Seyer JM (1992) Transcriptional mechanisms of type I collagen gene expression are differentially regulated by interleukin-1β, tumor necrosis factor-α, and transforming growth factor-β in Ito cells. J Biol Chem 267:14316–14321

Armendariz-Borunda J, Simkevitch CP Roy N, Raghow R, Kang AH, Seyer JM (1994) Activation of Ito cells involves regulation of AP-1 binding proteins and induction of type 1 collagen gene expression. Biochem J 304:817–824

Arthur MJ (2000) Fibrogenesis II. Metalloproteinases and their inhibitors in liver fibrosis. Am J Physiol Gastrointest Liver Physiol 279:G245–G249

Arthur MJP, Kowalski Saunders P, Wright R (1988) Effect of endotoxin on release of reactive oxygen intermediates by rat hepatic macrophages. Gastroenterology 95:1588–1594

Arthur MJP, Stanley A, Iredale JP Rafferty JA, Alembry RM, Friedman SL (1992) Secretion of 72 kDa type IV collagenase/gelatinase by cultured human lipocytes: analysis of gene expression, protein synthesis and proteinase actitivity. Biochem J 287:701–707

Asari Y, Majima M, Sugimoto K, Katori M, Ohwada T (1996) Release site of TNF-α after intravenous and intraperitoneal injection of LPS from Escherichia coli in rats. Shock 5:208–212

Athari A, Hänecke K, Jungermann K (1994) Prostaglandin F2α and D2 release from primary Ito cell cultures after stimulation with noradrenaline and ATP but not adenosine. Hepatology 20:142–149

Bach Kristensen D, Kawada N, Imamura K, Miyamoto Y, Tateno C, Seki S, Kuroki T, Yoshizato K (2000) Proteome analysis of rat hepatic stellate cells. Hepatology 32:268–277

Bachem MG, Melchior R, Gressner AM (1989a) The role of thrombocytes in liver fibrogenesis: effects of platelet-lysate and thrombocyte-derived growth factors on the mitogenic activity and glycosaminoglycan synthesis of cultured rat liver fat storing cells. J Clin Chem Clin Biochem 27:555–565

Bachem MG, Riess U, Gressner AM (1989b) Liver fat storing cell proliferation is stimulated by epidermal growth factor/transforming growth factor α and inhibited by transforming growth factor β. Biochem Biophys Res Commun 162:708–714

Bachem MG, Meyer D, Melchior R, Sell KM, Gressner AM (1992) Activation of rat liver perisinusoidal lipocytes by transforming growth factors derived from myofibroblastlike cells. A potential mechanism of self perpetuation in liver fibrogenesis. J Clin Invest 89:19–27

Bachem MG, Sell KM, Melchior R, Kropf J, Eller T, Gressner AM (1993) Tumor necrosis factor α (TNFα) and transforming growth factor-β1 (TGFβ1) stimulate fibronectin synthesis and the transdifferentiation of fat-storing cells in the rat liver into myofibroblasts. Virchows Arch B 63:123–130

Bachmann S (1997) Erythropoietin gene (EPO) expression and EPO synthesis. Kidney Int 51:479–482

Bailey SM, Cunningham CC (1998) Acute and chronic ethanol increases reactive oxygen species generation and decreases viability in fresh, isolated rat hepatocytes. Hepatology 28: 1318–1326

Bakkeren HF, Kuipers F, Vonk RJ, Van Berkel TJC (1990) Evidence for reverse cholesterol transport in vivo from liver endothelial cells to parenchymal cells and bile by high density lipoprotein. Biochem J 268:685–691

Ballardini G, Groff P, Badiali DGL, Schuppan D, Bianchi FB (1994) Ito cell heterogeneity: desmin-negative Ito cells in normal rat liver. Hepatology 19:440–446

Bardadin KA, Scheuer PJ, Peczek A, Wejman J (1991) Immunocytochemical observations on macrophage populations in normal fetal and adult human liver. J Pathol 164:253–259

Bataller R, Nicolas JM, Gines P, Esteve A, Nieves Gorbig M, Garcia Ramallo E, Pinzani M, Ros J, Jimenez W, Thomas AP, Arroyo V, Rodes J (1997) Arginine vasopressin induces contraction and stimulates growth of cultured human hepatic stellate cells. Gastroenterology 113:615–624

Batres RO, Olson JA (1987) A marginal vitamin A status alters the distribution of vitamin A among parenchymal and stellate cells in rat liver. J Nutr 117:874–879

Bauer M, Zhang JX, Bauer I, Clemens MG (1994) ET-1 induced alterations of hepatic microcirculation: sinusoidal and extrasinusoidal sites of action. Am J Physiol 267:G143–G149

Bauer HU, Friess H, Abou-Shady M, Berberat P, Zimmermann A, Gold LI, Korc M, Buchler MW (1998a) Transforming growth factor betas and their receptors in human liver cirrhosis. Eur J Gastroenterol Hepatol 10:1031–1039

Bauer I, Wanner GA, Rensing H, Alte C, Miescher EA, Wolf B, Pannen BH, Clemens MG, Bauer M (1998b) Expression pattern of heme oxygenase isoenzymes 1 and 2 in normal and stress-exposed rat liver. Hepatology 27:829–838

Bauerle AP, Henkel T (1994) Function and activation of NF-κB in the immune system. Annu Rev Immunol 12:141–179

Bautista AP, Spitzer JJ (1992) Platelet activating factor stimulates and primes the liver, Kupffer cells and neutrophils to release superoxide anion. Free Radic Res Commun 17:195–209

Bautista AP, Spitzer JJ (1994) Inhibition of nitric oxide formation in vivo enhances superoxide release by the perfused liver. Am J Physiol 266:G783–G788

Bautista AP, Meszaros K, Bojta J, Spitzer JJ (1990) Superoxide anion generation in the liver during the early stage of endotoxemia in rats. J Leukoc Biol 48:123–128

Bautista AP, Skrepnik N, Niesman MR, BagbyGJ (1994) Elimination of macrophages by liposome-encapsulated dichloromethylene-diphosphonate suppresses the endotoxin-induced priming of Kupffer cells. J Leukoc Biol 55:321–327

Beckh K, Kneip S, Arnold R (1994) Direct regulation of bile secretion by prostaglandins in perfused rat liver. Hepatology 19:1208–1213

Bedossa P, Peltier E, Terris B, Franco D, Poynard T (1995) Transforming growth factor-β1 (TGF-βl) and TGF-β1 receptors in normal, cirrhotic, and neoplastic human livers. Hepatology 21:760–766

Benbassat CA, Lazarus DD, Cichy SB, Evans TM, Moldawer LL, Lowry SF, Unterman TG (1999) Interleukin-1α (IL-1α) and tumor necrosis factor α (TNFα) regulate insulin-like growth factor binding protein-1 (IGFBP-1) levels and mRNA abundance in vivo and in vitro. Horm Metab Res 31:209–215

Bendelac A, Rivera MN, Park SH, Roark JH (1997) Mouse CD1-specific NK1 T cells: development, specificity, and function. Annu Rev Immunol 15:535–562
Benyon RC, Hovell CJ, Da Gaca M, Jones EH, Iredale JP, Arthur MJ (1999) Progelatinase A is produced and activated by rat hepatic stellate cells and promotes their proliferation. Hepatology 30:977–986
Berry MN, Friend DS. (1969) A high yield preparation of isolated, rat liver parenchymal cells. J Cell Biol 43:506–520
Beutler B, Cerami A (1989) The biology of cachectin/TNF – a primary mediator of the host response. Annu Rev Immunol 7:625–655
Beyer HS, Theologides A (1993) Tumor necrosis factor-α is a direct hepatocyte mitogen in the rat. Biochem Mol Biol Int 29:1–4
Bhatia SN, Balis UJ, Yarmush ML, Toner M (1999) Effect of cell-cell interactions in preservation of cellular phenotype: cocultivation of hepatocytes and nonparenchymal cells. FASEB J 13:1883–1900
Billiar TR, Curran RD, Ferrari FK, Williams DL, Simmons RL (1990a) Kupffer cell:hepatocyte cocultures release nitric oxide in response to bacterial endotoxin. J Surg Res 48:349–353
Billiar TR, Lysz TW, Curran RD, Bentz BG, Machiedo GW, Simmons RL (1990b) Hepatocyte modulation of Kupffer cell prostaglandin E_2 production in vitro. J Leukoc Biol 47:305–311
Bilzer M, Gerbes AL (2000) Preservation injury of the liver: mechanisms and novel therapeutic strategies. J Hepatol 32:508–515
Bilzer M, Jaeschke H, Vollmar AM, Paumgartner G, Gerbes AL (1999) Prevention of Kupffer cell-induced oxidant injury in rat liver by atrial natriuretic peptide. Am J Physiol 276:G1137–G1144
Bioulac-Sage P, Lafon ME, Saric J, Balabaud C (1990) Nerves and perisinusoidal cells in human liver. J Hepatol 10:105–112
Bioulac-Sage P, Kuiper J, van Berkel TJC, Saric J, Balabaud C (1996) Lymphocyte and macrophage populations in the liver. Hepato-Gastroenterology 43:4–14
Bissel DM, Hammaker L, Schmid R (1972) Hemoglobin and erythrocyte catabolism in rat liver:the separate roles of parenchymal and sinusoidal cells. Blood 40:812–822
Bissell DM, Wang SS, Jarnagin WR, Roll FJ (1995) Cell-specific expression of transforming growth factor-β in rat liver. Evidence for autocrine regulation of hepatocyte proliferation. J Clin Invest 96:447–455
Blom WM, De Bont HJ, Meijerman I, Kuppen PJ, Mulder GJ, Nagelkerke JF (1999) Interleukin-2-activated natural killer cells can induce both apoptosis and necrosis in rat hepatocytes. Hepatology 29:785–792
Blomhoff R, Berg T, Norum KR (1988) Transfer of retinol from parenchymal to stellate cells in liver is mediated by retinol-binding protein. Proc Natl Acad Sci USA 85:3455–3458
Blouin A, Bolender RP, Weibel ER (1977) Distribution of organelles and membranes between hepatocytes and nonhepatocytes in the rat liver parenchyma. J Cell Biol 72:441–455
Bode JG, Peters Regehr T, Gressner AM, Haussinger D (1998) De novo expression of glutamine synthetase during transformation of hepatic stellate cells into myofibroblast-like cells. Biochem J 335:697–700
Bogers WM, Stad RK, Van-Es LA, Daha MR (1992) Both Kupffer cells and liver endothelial cells play an important role in the clearance of IgA and IgG immune complexes. Res Immunol 143:219–224
Border WA, Noble NA (1994) Transforming growth factor β in tissue fibrosis. N Engl J Med 331:1286–1292
Borgs M, Bollen M, Keppens S, Yap SH, Stalmans W, Vanstapel F (1996) Modulation of basal hepatic glycogenolysis by nitric oxide. Hepatology 23:1564–1571
Bouwens L, Baekeland M, De Zartger R, Wisse E (1986) Quantitation, tissue distribution and proliferation kinetics of Kupffer cells in normal rat liver. Hepatology 6:718–722
Bouwens L, Remels L, Baekeland M, Van Bossuyt H, Wisse E (1987) Large granular lymphocytes or "pit cells" from rat liver: isolation, ultrastructural characterization and natural killer activity. Eur J Immunol 17:37–42
Bouwens L, Marinelli A, Kuppen PJ, Eggermont AM, van de Velde CJ, Wisse E (1990) Electron microscopic observations on the accumulation of large granular lymphocytes (pit cells) and Kupffer cells in the liver of rats treated with continuous infusion of interleukin-2. Hepatology 12:1365–1370
Bouwens L, De Bleser P, Vanderkerken K, Geerts B, Wisse E (1992) Liver cell heterogeneity: functions of non-parenchymal cells. Enzyme 46:155–168

Breitkopf K, Lahme B, Tag CG, Gressner AM (2001) Expression and matrix deposition of latent transforming growth factor β binding proteins in normal and fibrotic rat liver and transdifferentiating hepatic stellate cells in culture. Hepatology 33:387–396
Brenzel A, Gressner AM (1996) Characterization of insulin-like growth factor (IGF)-I-receptor binding sites during in vitro transformation of rat hepatic stellate cells to myofibroblasts. Eur J Clin Chem Clin Biochem 34: 401–409
Brix K, Wirtz R, Herzog V (1997) Paracrine interaction between hepatocytes and macrophages after extrathyroidal proteolysis of thyroglobulin. Hepatology 26:1232–1240
Bukara M, Bautista AP (2000) Acute alcohol intoxication and gadolinium chloride attenuate endotoxin-induced release of CC chemokines in the rat. Alcohol 20:193–203
Buniatan G, Hamprecht B, Gebhardt R (1996) Glial fibryllary acidic protein as a marker of perisinusoidal stellate cells that can distinguish between the normal and myofibroblast-like phenotypes. Biol Cell 87:65–73
Busam KJ, Bauer TM, Bauer J, Gerok W, Decker K (1990) Interleukin-6 release by rat liver macrophages. J Hepatol 11:367–373
Buxton DB, Fisher RA, Briseno DL, Hanahan DJ, Olson MS (1987) Glycogenolytic and haemodynamic responses to heat-aggregated immunoglobulin G and prostaglandin E2 in the perfused rat liver. Biochem J 243:493–498
Callery MP, Mangino MJ. Kamei T, Flye MW (1990) Interleukin-6 production by endotoxin-stimulated Kupffer cells is regulated by prostaglandin E2. J Surg Res 48:523–527
Cardarelli PM, Blumenstock FA, McKeown-Longo PJ, Saba TM, Mazurkiewicz JE (1990) A high afinnity binding of fibronectin to cultured Kupffer cells. J Leukoc Biol 48:426–437
Cardier JE, Dempsey J (1998) Thrombopoietin and its receptor, c-mpl, are constitutively expressed by mouse liver endothelial cells: evidence of thrombopoietin as a growth factor for liver endothelial cells. Blood 91:923–929
Carloni V, Romanelli RG, Pinzani M, Laffi G, Gentilini P (1997) Focal adhesion kinase and phospholipase Cγ involvement in adhesion and migration of human hepatic stellate cells. Gastroenterology 112:522–531
Caro JF, Poulos J, Ittoop O, Pories WJ, Flickinger EG, Sinha MK (1988) Insulin-like growth factor I binding in hepatocytes from human liver, human hepatoma, and normal, regenerating, and fetal rat liver. J Clin Invest 81:976–981
Casada M, Diaz Guerra MJ, Bosca L, Martin-Sanz P (1996) Characterization of nitric oxide dependent changes in carbohydrate hepatic metabolism during septic shock. Life Sci 58:561–572
Casini A, Pinzani M, Milani S, Grappone C, Galli G, Jezequel AM, Schuppan D, Rotella CM, Surrenti C (1993) Regulation of extracellular matrix synthesis by transforming growth factor β1 in human fat-storing cells. Gastroenterology 105:245–253
Casini A, Ceni E, Salzano R, Biondi P, Parola M, Galli A, Foschi M, Caligiuri A, Pinzani M, Surrenti C (1997) Neutrophil-derived superoxide anion induces lipid peroxidation and stimulates collagen synthesis in human hepatic stellate cells: role of nitric oxide. Hepatology 25:361–367
Casini A (2000) Alcohol-induced fatty liver and inflammation: where do Kupffer cells act? Editorial. J Hepatol 32:1026–1030
Cassiman D, van Pelt J, De Vos R, Van Lommel F, Desmet V, Yap S-H, Roskams T (1999) Synaptophysin: a novel marker for human and rat hepatic stellate cells. Am J Pathol 155:1831–1839
Cassiman D, Denef C, Desmet VJ, Roskams T (2001) Human and rat hepatic stellate cells express neurotrophins and neurotrophin receptors. Hepatology 33:148–158
Casteleijn E, Kuiper J, van Rooij HC, Kamps JAAM, Koster JF, van Berkel TJC (1988) Hormonal control of glycogenolysis in parenchymal liver cells by Kupffer and endothelial liver cells. J Biol Chem 263:2699–2703
Castilla A, Prieto J, Fausto N (1991) Transforming growth factors -β1 and -α in chronic liver disease: effects of interferon therapy. N Engl J Med. 324:933–940
Catalá M, Antón A, Portoles MT (1999) Characterization of the simultaneous binding of Escherichia coli endotoxin to Kupffer and endothelial liver cells by flow cytometry. Cytometry 36:123–130
Ceppi ED, Smith FS, Titheradge MA (1996) Effect of multiple cytokines plus bacterial endotoxin on glucose and nitric oxide production by cultured hepatocytes. Biochem J 317:503–507
Chao W, Liu H, DeBuysere M, Hanahan DJ, Olson MS (1989) Identification of receptors for platelet activating factor in rat Kupffer cells. J Biol Chem 264:13591–13598

Chensue SW, Terebuh PD, Remick DG, Scales WE, Kunkel SL (1991) In vivo biologic and immunohistochemical analysis of interleukin-1α, -β and tumor necrosis factor during experimental endotoxemia: kinetics, Kupffer cell expression, and glucocorticoid effects. Am J Pathol 1338:395–402

Cherrington AD (1999) Control of glucose uptake and release by the liver in vivo. Diabetes 48:1198–1214

Christ B, Nath A (1996) Impairment by interleukin 1β and tumour necrosis factor-α of the glucagon-induced increase in phosphoenolpyruvate carboxykinase gene expression and gluconeogenesis in cultured rat hepatocytes Biochem J 320:161–166

Christ B, Nath A, Heinrich PC, Jungermann K (1994) Inhibition by recombinant human interleukin-6 of the glucagon-dependent induction of phosphoenolpyruvate carboxykinase and of the insulin-dependent induction of glucokinase gene expression in cultured rat hepatocytes: regulation of gene transcription and messenger RNA degradation. Hepatology 20:1577–1583

Christ B, Nath A, Jungermann K (1997) Mechanism of the impairment of the glucagon-stimulated phosphoenolpyruvate carboxykinase gene expression by interleukin-6 in rat hepatocytes: inhibition of the increase in cyclic 3',5' adenosine monophosphate and the downstream cyclic 3',5' adenosine monophosphate action. Hepatology 26:73–80

Chroneos ZC, Baynes JW, Thorpe SR (1995) Identification of liver endothelial cells as the primary site of IgM catabolism in the rat. Arch Biochem Biophys 319:63–73

Cincu RN, Rodriguez Ortigosa CM, Vesperinas I, Quiroga J, Prieto J (1997) S-adenosyl-L-methionine protects the liver against the cholestatic, cytotoxic, and vasoactive effects of leukotriene D4: a study with isolated and perfused rat liver. Hepatology 26:330–335

Clemens MG (1999) Nitric oxide in liver injury. Hepatology 30:1–5

Clemens MG, Zhang JX (1999) Regulation of sinusoidal perfusion: in vivo methodology and control by endothelins. Semin Liver Dis 19:383–396

Coffey RJ Jr, Kost LJ, Lyons RM, Moses HL, LaRusso NF (1987) Hepatic processing of transforming growth factor-β in the rat. Uptake, metabolism, and biliary excretion. J Clin Invest 80:750–777

Coleman RA, Smith WL, Narumiya S (1994) International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. Pharmacol Rev 46:205–229

Crispe IN, Mehal WZ (1996) Strange brew: T cells in the liver. Immunol Today 17:522–525

Crosbie OM, Reynolds M, McEntee G, Traynor O, Hegarty JE, O'Farrelly LN (1999) In vitro evidence for the presence of hematopoietic stem cells in the adult human liver. Hepatology 29:1193–1198

Curran RD, Billiar TR, Stuehr DJ, Hofmann K, Simmons RL (1989) Hepatocytes produce nitrogen oxide from L-arginine in response to inflammatory products of Kupffer cells. J Exp Med 170:1769–1774

Curran RD, Billiar TR, Stuehr DJ, Ochoa JB. Harbrecht BG. Flint SG, Simmons RL (1990) Multiple cytokines are required to induce hepatocyte nitric oxide production and inhibit total protein synthesis. Ann Surg 1212:462–469

Davis BH, Kramer RT, Davidson NO (1990) Retinoic acid modulates rat Ito cell proliferation, collagen and transforming growth factor-β production. J Clin Invest 86:2062–2070

Dawson PA, Lukaszewski LM, Ells PF, Malbon CC, Williams DL (1989) Quantification and regulation of apolipoprotein E expression in rat Kupffer cells. J Lipid Res 30:403–413

Deaciuc IV, Alappat JM, D'Souza NB (1994a) Effect of acute and chronic alcohol administration to rats on the expression of interleukin-6 cell-surface receptors of hepatic parenchymal and non-parenchymal cells. Alcohol Clin Exp Res 18:1207–1214

Deaciuc IV, Bagby GJ, Niesman MR, Skrepnik N, Spitzer JJ (1994b) Modulation of hepatic sinusoidal endothelial cell function by Kupffer cells: an example of intercellular communication in the liver. Hepatology 19:464–470

Deaciuc IV, D'Souza NB, Spitzer JJ (1995) Tumor necrosis factor-α cell-surface receptors of liver parenchymal and nonparenchymal cells during acute and chronic alcohol administration to rats. Alcohol Clin Exp Res 19:332–338

De Bleser PJ, Jannes P, Buul-Offers SC van, Hoogerhrugge C.M, Schravendijk CFH van, Niki T, Rogiers V, von den Brande JL, Wisse E, Geerts A (1995) Insulin-like growth factor-II/mannose 6-phosphate receptor is expressed on CCl4-exposed rat fat-storing cells and facilitates activation of latent

transforming growth factor-β in cocultures with sinusoidal endothelial cells. Hepatology 21:1429–1437
De Bleser PJ, Niki T, Rogiers V,Geerts A (1997a) Transforming growth factor β gene expression in normal and fibrotic rat liver. J Hepatol 26:886–893
De Bleser PJ, Niki T, Xu G, Rogiers V, Geerts A (1997b) Localization and cellular sources of activins in normal and fibrotic rat liver. Hepatology 26:905–912
Decker K (1990) Biologically active products of stimulated liver macrophages (Kupffer cells). Eur J Biochem 192:245–261
Decker K, Zhang F, Grewe M, Hoffman R (1995) The regulation of cytokine receptor expression in rat Kupffer cells. In: Wisse E, Knook DL, Wake K (eds) Cells of the Hepatic Sinusoid. Vol 5, Leiden, Kupffer Cell Foundation, pp 318–323
De Leeuw AM, McCarthy SP, Geerts A, Knook DL (1984) Purified rat liver fat-storing cells in culture divide and contain collagen. Hepatology 4:392–403
Denzlinger C (1996) Biology and pathophysiology of leukotrienes. Crit Rev Oncol Hematol 23:167–223
Deutschman CS, Haber BA, Andrejko K, Cressman DE, Harrison R, Elenko E, Taub R (1996) Increased expression of cytokine-induced neutrophil chemoattractant in septic rat liver. Am J Physiol 271:R593–600.
Deutschman CS, Andrejko KM, Haber BA, Bellin L, Elenko E, Harrison R, Taub R (1997) Sepsis-induced depression of rat glucose-6-phosphatase gene expression and activity. Am J Physiol 273:R1709–1718
De Vera ME, Taylor BS, Wang Q, Shapiro RA, Billiar TR, Geller DA (1997) Dexamethasone suppresses iNOS gene expression by upregulating I-kappa B and inhibiting NF-kappa B. Am J Physiol 273:G1290–1296
Diamantis I, Van de Castelle M, Hurni B, Reichen J (1998) Detection of endothelin-1 and its receptors in rat liver endothelial cells by in situ reverse trascriptase-polymerase chain reaction. J Hepatol 28:157–160
Dieter P, Ambs P, Fitzke E, Hidaka H, Hoffmann R, Schwende H (1995) Comparative studies of cytotoxicity and the release of TNF-α, nitric oxide, and eicosanoids of liver macrophages treated with lipopolysaccharide and liposome-encapsulated MTP-PE. J Immunol 155:2595–604
Dini L, Carla EC (1998) Hepatic sinusoidal endothelium heterogeneity with respect to the recognition of apoptotic cells. Exp Cell Res 240:388–393
Di Sario A, Bendia E, Svegliati-Baroni G, Ridolfi F, Bolognini L, Feliciangeli G; Jezequel AM, Orlandi F, Benedetti A (1999) Intracellular pathways mediating Na+/H+ exchange activation by platelet-derived growth factor in rat hepatic stellate cells. Gastroenterology 116:1155–1166
Dobashi H, Seki S, Habu Y, Ohkawa T, Takeshita S, Hiraide H, Sekine I (1999) Activation of mouse liver natural killer and NK1.1+ T cells by bacterial superantigen-primed Kupffer cells. Hepatology 30:430–436
Doherty DG, Norris S, Madrigal-Estebas L, McEntee G, Traynor O, Hegarty JE, O'Farelly C (1999) The human liver contains multiple populations of NK cells, T cells, and CD3+ CD56+ natural T cells with distinct cytotoxic activities and Th1, Th2 and Th0 cytokine secretion patterns. J Immunol 163:2314–2321
Dong W, Simeonova PP, Gallucci R, Matheson J, Fannin R, Montuschi P, Flood L, Luster MJ (1998) Cytokine expression in hepatocytes: role of oxidant stress. J Interferon Cytok Res 18:629–638
Dooley S, Delvoux B, Lahme B, Mangasser-Stephan K, Gressner AM (2000) Modulation of transforming growth factor-β response and signaling during transdifferentiation of rat hepatic stellate cells to myofibroblasts. Hepatology 31:1094–1106
Downard PJ, Wilson MA, Spain DA, Matheson PJ, Siow Y, Garrison RN (1997) Heme oxygenase dependent carbon monoxide production is a hepatic adaptive response to sepsis. J Surg Res 71:7–12
Drakes ML, Zahorchak AF, Takayama T, Lu L, Thomson AW (2000) Chemokine and chemokine receptor expression by liver-derived den-dritic cells: MIP-1α production is induced by bacterial lipopolysaccharide and interaction with allogeneic T cells. Transpl Immunol 8:17–29
Dupont G, Tordjmann T, Clair C, Swillens S, Claret M, Combettes L (2000) Mechanism of receptor-oriented intercellular calcium wave propagation in hepatocytes. FASEB J 14:279–289
Eakes AT, Howard KM, Miller JE, Olson MS (1997) Endothelin-1 production by hepatic endothelial cells: characterization and augmentation by endotoxin exposure. Am J Physiol 272:G605–G611

Eakes AT, Olson MS (1998) Regulation of endothelin synthesis in hepatic endothelial cells. Am J Physiol 274:G1068–G1076
Eckardt KU, Pugh CW, Meier M, Tan CC, Ratcliffe PJ, Kurtz A (1994) Production of erythropoietin by liver cells in vivo and in vitro. Ann NY Acad Sci 718:50–60
Elsharkaw AM, Wright MC, Hay RT, Arthur MJP, Hughes T, Matthias J. Bahr MJ, Degitz K, MannDA (1999) Persistent activation of nuclear factor-κB in cultured rat hepatic stellate cells involves the induction of potentially novel Rel-like factors and prolonged changes in the expression of NFκB family proteins. Hepatology 30:761–769
Emeis JJ, Lindeman J, Nieuwenhuizen W (1981) Immunoenzyme histochemical localization of fibrin degradation products in tissues. Am J Pathol 103:337–344
Essani NA, McGuire GM, Manning AM, Jaeschke H (1995) Differential induction of mRNA for ICAM-1 and selectins in hepatocytes, Kupffer cells and endothelial cells during endotoxemia. Biochem Biophys Res Commun 211:74–82
Essani NA, McGuire GM, Manning AM, Jaeschke H (1996) Endotoxin-induced activation of the nuclear transcription factor κB and expression of E-selectin messenger RNA in hepatocytes, Kupffer cells, and endothelial cells in vivo. J Immunol 156:2956–2963
Essani NA, Bajt ML, Farhood A, Vonderfecht SL, Jaeschke H (1997a) Transcriptional activation of vascular cell adhesion molecule-1 gene in vivo and its role in the pathophysiology of neutrophil-induced liver injury in murine endotoxin shock. J Immunol 158:5941–5948
Essani NA, Fisher MA, Jaeschke H (1997b) Inhibition of NF-kappa B activation by dimethyl sulfoxide correlates with suppression of TNF-α formation, reduced ICAM-1 gene transcription, and protection against endotoxin-induced liver injury. Shock 7:90–96
Eyhorn S, Schlayer HJ, Henninger HP, Dieter P, Hermann R, Woort Menker M, Becker H, Schaefer HE, Decker K (1988) Rat hepatic sinusoidal endothelial cells in monolayer culture. Biochemical and ultrastructural characteristics. J Hepatol 6:23–35
Failli P, DeFranco RMS, Caliguri A, Gentilini A, Romanelli RG, Marra F, Batignani G, Guerra CT, Laffi G, Gentilini P, Pinzani M (2000) Nitrovasodilators inhibit platelet-derived growth factor-induced proliferation and migration of activated human hepatic stellate cells. Gastroenterology 119:479–492
Falasca L, Bergamini A, Serafino A, Balabaud C, Dini L (1996) Human Kupffer cell recognition and phagocytosis of apoptotic peripheral blood lymphocytes. Exp Cell Res 224:152–162
Fausto N (2000) Liver regeneration. J Hepatol 2000; 32(1 Suppl):19–31
Feder LS, Todaro JA, Laskin DL (1993) Characterization of interleukin-1 and interleukin-6 production by hepatic endothelial cells and macrophages. J Leukoc Biol 53:126-132
Fennekohl A, Schieferdecker HL, Jungermann K, Püschel GP (1999) Differential expression of prostanoid receptors in hepatocytes, Kupffer cells, sinusoidal endothelial cells and stellate cells of rat liver. J Hepatol 30:38–47
Fennekohl A, Lucas M, Puschel GP (2000) Induction by interleukin 6 of G(s)-coupled prostaglandin E(2) receptors in rat hepatocytes mediating a prostaglandin E(2)-dependent inhibition of the hepatocyte's acute phase response. Hepatology 31:1128–1134
Fibbi G, Pucci M, Grappone C, Pellegrini G, Salzano R, Casini A, Milani S, Del Rosso M (1999) Functions of the fibrinolytic system in human Ito cells and its control by basic fibroblast and platelet-derived growth factor. Hepatology 29:868–878
Fiete D, Srivastava V, Hindsgaul O. Baenziger JU (1992) A hepatic reticuloendothelial cell receptor specific for SO4. 4GalNAc-β l,4GlcNAc -β1,2Man-α that mediates rapid clearance of lutropin. Cell 67:1103–1110
Filkins JP (1985) Monokines and the metabolic pathophysiology of septic shock. Fed Proc 44:300–304
Fimmel CJ, Brown KE, ONeill R, Kladney RD (1996) Complement C4 protein expression by rat hepatic stellate cells. J Immunol 157:2601–2609
Folch E, Prats N, Hotter G, Lopez S, Gelpi E, Rosello-Catafau J, Closa D (2000) P-selectin expression and Kupffer cell activation in rat acute pancreatitis. Digest Dis Sci 45:1535–1544
Ford-Hutchinson AW, Gresser M, Young RN (1994) 5-Lipoxygenase. Annu Rev Biochem 63:383–417
Fox ES, Thomas P, Broitman SA (1988) Uptake and modification of 125I-lipopolysaccharide by isolated rat Kupffer cells, Hepatology 8:1550–1554
Fox ES, Thomas P, Broitman S.A. (1989) Clearance of gut-derived endotoxins by the liver. Gastroenterology 96:456–461

Fraser R, Dobbs BR, Rogers GWT (1995) Lipoproteins and the liver sieve: the role of the fenestrated sinusoidal endothelium in lipoprotein metabolism, atherosclerosis, and cirrhosis. Hepatology 21:863–874
Freudenberg, M. A., N. Freudenberg C. Galanos (1982) Time course of cellular distribution of endotoxin in liver lungs and kidneys of rats. Br J Exp Pathol 63:56–65
Friedman SL (1993) The cellular basis of hepatic fibrosis. Mechanisms and treatment strategies. N Engl J Med 328:1828–1825
Friedman SL (2000) Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J Biol Chem 275:2247–2250
Friedman SL, Roll F (1987) Isolation and culture of hepatic lipocytes, Kupffer cells, and sinusoidal endothelial cells by density gradient centrifugation with stractan. Anal Biochem 161:207–218
Friedman SL, Arthur MJ (1989) Activation of cultured rat hepatic lipocytes by Kupffer cell conditioned medium. Direct enhancement of matrix synthesis and stimulation of cell proliferation via induction of platelet-derived growth factor receptors. J Clin Invest 84:1780–1785
Friedman SL, Roll F, Boyles J, Bissell D (1985) Hepatic lipocytes. The principal collagen producing cell of normal rat liver. Proc Natl Acad Sci USA 82:8681–8685
Friedman G, Liu L, Friedman SL, Boyles JK (1991) Apolipoprotein is secreted by cultured lipocytes of the rat liver. J Lip Res 3:107–114
Friedman SL, Rockey DC, McGuire RF, Maher JJ, Boyles JK, Yamasaki G (1992) Isolated hepatic lipocytes and Kupffer cells from normal human liver: morphological and functional characteristics in primary culture. Hepatology 15:234–243
Friedman SL, Wei S, Blaner WS (1993) Retinol release by activated rat hepatic lipocytes: regulation by Kupffer cell-conditioned medium and PDGF. Am J Physiol 264:G947–G952
Friedman SL, Yamasaki G, Wong L (1994) Modulation of transforming growth factor-β receptors of rat lipocytes during the hepatic wound healing response. Enhanced binding and reduced gene expression accompany cellular activation in culture and in vivo. J Biol Chem 269:10551–10558
Frizell E, Liu SL, Abraham A, Ozaki I, Eghbali M, Sage EH, Zern MA (1995) Expression of SPARC in normal and fibrotic livers. Hepatology 21:847–854
Fukai F, Suzuki Y, Ohtaki H, Katayama T (1993) Rat hepatocytes generate peptide leukotrienes from leukotriene A4. Arch Biochem Biophys 305:378–384
Fukai F, Suzuki Y, Nishizawa Y, Katayama T (1996) Transcellular biosynthesis of cysteinyl leukotrienes by Kupffer cell-hepatocyte cooperation in rat liver. Cell Biol Int 20:423–428
Fukushige H, Doi Y, Kudo H, Kayashima K, Kiyonaga H, Nagata, Itoh, H (2000) Synthesis and receptor sites of endothelin-l in the rat liver vasculature. Anat Rec 259:437–445
Funaki N, Arii S, Monden K, Tanaka J, Imamura M (1996) HLA-DR antigen and interleukin-2 receptor expression on primary-cultured human hepatic macrophages in relation to liver cirrhosis and hepatitis virus infection. Life Sci 58:551–559
Gabay C, Kushner I (1999) Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 340:448–454
Gabriel A, Kuddus, RH, Rao AS, Watkins WD, Gandhi, CR (1998) Superoxide-induced changes in endothelin (ET) receptors in hepatic stellate cells. J Hepatol 29:614–627
Gabriel A, Kuddus RH, Rao AS, Gandhi CR (1999) Down-regulation of endothelin receptors by transforming growth factor β1 in hepatic stellate cells. J Hepatol 30:440–450
Gaca MDA, Pickering JA, Arthur MJP, Benyon RC (1999) Human and rat hepatic stellate cells produce stem cell factor: a possible mechanism for mast cell recruitment in liver fibrosis. J Hepatol 30:850–858
Gaillard T, Mulsch A, Busse R, Klein H, Decker K (1991) Regulation of nitric oxide production by stimulated rat Kupffer cells. Pathobiology 59:280–283
Gaillard T, Mulsch A, Klein H, Decker K (1992) Regulation by prostaglandin E2 of cytokine-elicited nitric oxide synthesis in rat liver macrophages. Biol Chem Hoppe Seyler 373:897–902
Gallai M, Kovalszky I, Knittel T, Neubauer K, Armbrust T, Ramadori G (1996) Expression of extracellular matrix proteoglycans perlecan and decorin in carbon-tetrachloride-injured rat liver and in isolated liver cells. Am J Pathol 148:1463–1471
Galli A, Crabb D, Price D, Ceni E, Salzano R, Surrenti C, Casini A. (2000) Peroxisome proliferator-activated receptor ã transcriptional regulation is involved in platelet-derived growth factor-induced proliferation of human hepatic stellate cells. Hepatology 31:101–108

Gandhi CR, Stephenson K, Olson MS (1992) A comparative study of endothelin- and platelet-activating-factor-mediated signal transduction and prostaglandin synthesis in rat Kupffer cells. Biochem J 281:485–492
Gandhi RC, Uemura T, Kuddus R (2000) Endotoxin causes up-regulation of endothelin receptors in cultured hepatic stellate cells via nitric oxide-dependent and -independent mechanisms. Br J Pharmacol 131:319–327
Gard AL., White FP, Dutton GR (1985) Extra-neural glial fibrillary acidic protein (GFAP) immunoreactivity in perisinusoidal stellate cells of rat liver. J Neuroimmunol 8:359–375
Gardner Cr, Wasserman AJ, Laskin DL (1991) Liver macrophages mediated cytotoxicyty towards mastocytoma cells involves phagocytosis of tumor targets. Hepatology 14:318–324
Gebhardt R, Mecke D (1983) Hetergeneous distribution of glutamine synthetase among rat liver parenchymal cells in situ and in primary cultures. EMBO J 2:567–570
Geerts A, Bouwens L, Wisse E (1990) Ultrastructure and function of hepatic fat-storing and pit cells. J Electron Microsc Tech 14:247–256
Geerts A, Greenwel P, Cunningham M, De Bleser P, Rogiers V, Wisse E, Rojkind M (1993) Identification of connective tissue gene transcripts in freshly isolated parenchymal, endothelial, Kupffer and fat-storing cells by Northern hybridization analysis. J Hepatol 19:209–213
Geerts A, Niki T., Hellemans K, de Craemer D, van den Berg K, Lazou J M, Stange G, van de Winkel M, de Bleser P (1998) Purification of rat hepatic stellate cells by side scatter activated cell sorting. Hepatology 27:590–598
Geller DA, Nussler AK, Silvio MD, Lowenstein CJ, Shapiro RA, Wang SC, Simmons RL, Billiar TR (1993) Cytokines, endotoxin, and glucocorticoids regulate the expression of inducible nitric oxide synthase in hepatocytes. Proc Natl Acad Sci USA 90:522–526
Gentilini A, Feliers D, Pinzani M, Woodruff K, Abboud S (1998) Characterization and regulation of insulin-like growth factor binding proteins in human hepatic stellate cells. J Cell Physiol 174:240–250
George J, Roulot D, Koteliansky VE, Bissell DM (1999) In vivo inhibition of rat stellate cell activation by soluble transforming growth factor-β type II receptor: a potential new therapy for hepatic fibrosis. Proc Natl Acad Sci USA 96:12719–127124
George J, Wang SS, Sevcsik AM, Sanicola M, Cate RL, Koteliansky VE, Bissell DM (2000) Transforming growth factor-β initiates wound repair in rat liver through induction of the EIIIA-fibronectin splice isoform. Am J Pathol 156:115–124
Gerber DJ, Azuara V, Levraud J-P, Huang SY, Lembezat M-P, Pereira P (1999) IL-4-producing γT cells that express a very restricted TCR repertoire are preferentially localized in liver and spleen. J Immunol 163:3076–3082
Gerbes AL, Vollmar AM, Kiemer AK, Bilzer M (1998) The guanylate cyclase-coupled natriuretic peptide receptor:a new target for prevention of cold ischemia-reperfusion damage of the rat liver. Hepatology 28:1309–1317
Ghyselinck NB, Bavik C, Sapin V, Mark M, Bonnier D, Hindelang C, Dierich A, Nilsson CB, Hakansson H, Sauvant P, Azais-Braesco V, Frasson M, Picaud S, Chambon P (1999) Cellular retinol-binding protein I is essential for vitamin A homeostasis. EMBO J 18:4903–4914
Gjoen T, Seljelid R, Kolset SO (1989) Binding of metastatic colon carcinoma cells to liver macrophages. J Leukoc Biol 45:362-369
Goda N, Suzuki K, Naito M, Takeoka S, Tsuchida E, Ischimura Y, Tamatani T, Suematsu M (1998) Distribution of heme oxygenase isoforms in rat liver: topographic basis for carbon monoxide-mediated microvascular relaxation. J Clin Invest 101:604–612
Gohda E, Yamasaki T, Tsubouchi H, Kurobe M, Sakiyama O, Aoki H, Niidani N, Shin S, Hayashi K, Hashimoto S (1990) Biological and immunological properties of human hepatocyte growth factor from plasma of patients with fulminant hepatic failure. Biochim Biophys Acta 1053:21–26
Goldstein SA, Elwyn DH (1989) The effects of injury and sepsis on fuel utilization. Annu Rev Nutr 9:445–473
Gondo K, Ueno T, Sakamoto M, Sakisaka S, Sata M, Tanikawa K (1993) The endothelin-1 binding site in rat liver tissue: light- and electron-microscopic autoradiographic studies. Gastroenterology 104:1745–1751
Gong W, Roth S, Michel K, Gressner AM (1998) Isoforms and splice variant of transforming growth factor β-binding protein in rat hepatic stellate cells. Gastroenterology 114:352–363

Gonzales-Amaro R, Garcia Monzon C, Garcia-Buey L, Moreno-Otero R, Alonso JL, Yague E, Pivel JP, Lobez-Cabrera M, Fernandez-Ruiz E, Sanchez-Madrid F (1994) Induction of tumor necrosis factor-α production by human hepatocytes in chronic viral hepatitis. J Exp Med 179:841–848
Gorbig MN, Gines P, Bataller R, Nicolas JM, Garcia Ramallo E, Tobias E, Titos E, Rey MJ, Claria J, Arroyo V, Rodes J (1999) Atrial natriuretic peptide antagonizes endothelin-induced calcium increase and cell contraction in cultured human hepatic stellate cells. Hepatology 30:501–509
Gove CD, Hughes RD, Kmiec Z, Noda Y, Williams R (1990) In vivo and in vitro studies on the protective effects of 9β-methylcarbacyclin, a stable prostacyclin analogue, in galactosamine-induced hepatocellular damage. Prostagl Leukotr Essent Fatty Acids 40:73–77
Grande JP (1997) role of transforming growth factor-β in tissue injury and repair. Proc Soc Exp Biol Med 214:27–40
Grant EP, Degano M, Rosat JP, Stenger S, Modlin RL, Wilson IA, Brenner MB (1999) Molecular recognition of lipid antigens by T cell receptors. J Exp Med 189:195–205
Greenwel P, Rubin J, Schwartz M, Hertzberg EL, Rojkind M (1993) Liver fat-storing cell clones obtained from a CCl4-cirrhotic rat are heterogeneous with regard to proliferation, expression of extracellular matrix components, interleukin-6, and connexin 43. Lab Invest 69:210–216
Greenwel P, Iraburu MJ, Reyes Romero M, Meraz Cruz N, Casado E, Solis Herruzo JA, Rojkind M (1995) Induction of an acute phase response in rats stimulates the expression of α1(I) procollagen messenger ribonucleic acid in their livers. Possible role of interleukin-6. Lab Invest 72:83–91
Gressner A (1991) Time-related distribution profiles of sulfated glycosaminoglycans in cells, cell surfaces, and media of cultured rat liver fat-storing cells. Proc Soc Exp Biol Med 196:307–315
Gressner AM (1998) The cell biology of liver fibrogenesis – an imbalance of proliferation, growth arrest and apoptosis of myofibroblasts. Cell Tissue Res 292:447–452
Gressner A, Haarmann R (1988) Hyaluronic acid synthesis and secretion by rat liver fat-storing cells (perisinusoidal lipocytes) in culture. Biochem Biophys Res Comm 151:222–229
Gressner AM, Brenzel A, Vossmeyer T (1993a) Hepatocyte-conditioned medium potentiates insulin-like growth factor (IGF) 1 and 2 stimulated DNA synthesis of cultured fat storing cells. Liver 13:86–94
Gressner AM, Lotfi S, Gressner G, Haltner E, Kropf J (1993b) Synergism between hepatocytes and Kupffer cells in the activation of fat storing cells (perisinusoidal lipocytes). J Hepatol 19:117–132
Grewe M, Gausling R, Gyufko K, Hoffmann R, Decker K (1994) Regulation of the mRNA expression for TNF-α in rat liver macrophages. J Hepatol 20:811–818
Guillouzo A (1998) Liver cell models in in vitro toxicology. Environ Health Perspect 106 Suppl 2:511–532
Hagmann W, Denzlinger C, Keppler D (1985) Production of peptide leukotrienes in endotoxin shock. Eur Biochem Soc Lett 180:309–313
Hagmann W, Steffan AM, Kirn A, Keppler D (1987) Leukotrienes as mediators in frog virus 3-induced hepatitis in rats. Hepatology 7:732–736
Hagmann W, Hacker HJ, Buchholz U (1992) Resident mast cells are the main initiators of anaphylactic leukotriene production in the liver. Hepatology 16:1477–1484
Hamada E, Nishida T, Uchiyama Y, Nakamura J, Isahara K, Kazuo H, Huang T-P, Momoi T, Ito T, Matsuda H (1999) Activation of Kupffer cells and caspase-3 involved in rat hepatocyte apoptosis induced by endotoxin. J Hepatol 30:807–818
Harbrecht BG, Billiar TR, Stadler J, Demetris AJ, Ochoa JB, Curran RD, Simmons RL (1992) Nitric oxide synthesis serves to reduce hepatic damage during acute murine endotoxemia. Crit Care Med 20:1568–1574
Harbrecht BG, McClure EA, Simmons RL, Billiar TR (1995) Prostanoids inhibit Kupffer cell nitric oxide synthesis. J Surg Res 58:625–629
Harbrecht BG, Kim YM, Wirant EM, Shapiro RA, Billiar TR (1996) PGE and LTB_4 inhibit cytokine-stimulated nitric oxide synthase type 2 expression in isolated rat hepatocytes. Prostaglandins 52:103–116
Hardardotirr I, Grunfeld C, Feingold KR (1994) Effects of endotoxin and cytokines on lipid metabolism. Curr Opin Lipidol 5:207–215
Hardonk MJ, Van Goor H, Scherphof GL, Daemen T (1989) Functional heterogeneities between large and small Kupffer cells. In: Wisse E,. Knook DL, Decker K (eds) Cells of the Hepatic Sinusoid. Vol 2, The Kupffer Cell Foundation, Rijswijk, pp 434–438

Harrison RL, Boudreau R (1989) Human hepatic sinusoidal endothelial cells in culture produce von Willebrand factor and contain Weibel-Palade bodies. Liver 9:242–249
Hashimoto N, Watanabe T, Shiratori Y, Ikeda Y, Kato H, Han K, Yamada H, Toda G, Kurokawa K (1995) Prostanoid secretion by rat hepatic sinusoidal endothelial cells and its regulation by exogenous adenosine triphosphate. Hepatology 21:1713–1718
Hasselgren PO (1995) Muscle protein metabolism during sepsis. Biochem Soc Trans 23:1019–1025
Hata K, Zhang XR, Iwatsuki S, Van-Thiel DH, Herberman RB, Whiteside TL (1990) Isolation, phenotyping, and functional analysis of lymphocytes from human liver. Clin Immunol Immunopathol 56:401–419
Haussinger D (1990) Nitrogen metabolism in liver: structural and functional organization and physiological relevance. Biochem J 267:281–290
Heldin P, Pertoft H, Nordlinder H. Heldin CH, Laurent TC (1991) Differential expression of platelet-derived growth factor α and β-receptors on fat-storing cells and endothelial cells in rat liver. Exp Cell Res 193:364–369
Hellemans K, Grinko I, Rombouts K, Schuppan D, Geerts A (1999) All-trans and 9-cis retinoic acid alter rat hepatic stellte cell phenotype differentially. Gut 45:134–142
Hellerbrand C, Jobin C, Licato LL, Sartor RB, Brenner DA (1998) Cytokines induce NF-κB in activated but not in quiescent rat hepatic stellate cells. Am J Physiol 275:G269–G278
Hellerbrand C, Stefanovic B, Giordano F, Elmar R, Burchardt ER, Brenner DA (1999) The role of TGFβ1 in initiating hepatic stellate cell activation in vivo. J Hepatol 30:77–87
Hellman L, Sedsrod B, Sandberg H, Pettersson U (1989) Secretion of coagulant factor VIII activity and antigen by in vitro cultivated rat liver sinusoidal endothelial cells. Br J Haematol 73:348–355
Helyar L, Bundschuh DS, Laskin JD, Laskin DL (1994) Induction of hepatic Ito cell nitric oxide production after acute endotoxemia. Hepatology 20:1509–1515
Hendriks HF, Verhoofstad W, Brouwer A, De Leeuw A, Knook DL (1985) Perisinusoidal fat-storing cells are the main vitamin A storage sites in rat liver. Exp Cell Res 160:138–149
Herbst H, Frey A, Heinrichs O, Milani S, Bechstein WO, Neuhaus P, Schuppan D (1997a) Heterogeneity of liver cells expressing procollagen types I and IV in vivo. Histochem Cell Biol 107:399–409
Herbst H, Wege T, Milani S, Pellegrini G, Orzechowski HD, Bechstein WO, Neuhaus P, Gressner AM, Schuppan D (1997b) Tissue inhibitor of metalloproteinase-1 and -2 RNA expression in rat and human liver fibrosis. Am J Pathol 150:1647–1659
Hernandez-Munoz I, de la Torre P, Sanchez-Alcazar JA, Garcia I, Santiago E, Munoz-Yague MT, Solis-Herruzo JA (1997) Tumor necrosis factor alpha inhibits collagen alpha 1(I) gene expression in rat hepatic stellate cells through a G protein. Gastroenterology 113:625–640
Hespeling U, Jungermann K, Puschel GP (1995a) Feedback-inhibition of glucagon-stimulated glycogenolysis in hepatocyte/Kupffer cell cocultures by glucagon-elicited prostaglandin production in Kupffer cells. Hepatology 22:1577–1583
Hespeling U, Puschel GP, Jungermann K, Gotze O, Zwirner J (1995b) Stimulation of glycogen phosphorylase in rat hepatocytes via prostanoid release from Kupffer cells by recombinant rat anaphylatoxin C5a but not by native human C5a in hepatocyte/Kupffer cell co-cultures. FEBS L 372:108–112
Hewett JA, Roth RA (1993) Hepatic and extrahepatic pathobiology of bacterial lipopolysaccharides. Pharmacol Rev 45:381–411
Hildebrand R, Schleicher A (1986) Image analysis of the histochemical demonstration of glucose-6-phosphatase activity in rat liver. Histochemistry 86:181–190
Hill M, McCallum RE (1992) Identification of tumor necrosis factor as a transcriptional regulator of the phosphoenopyruvate carboxykinase after endotoxin treatment of mice. Infect Immun 60:4040–4050
Hinglais N, Kazatchkine MD, Mandet C, Appay MD, Bariety J (1989) Human liver Kupffer cells express CRl, CR3, and CR4 complement receptor antigens. An immunohistochemical study. Lab Invest 61:509–514
Hironaka K, Sakaida I, Matsumura Y, Kaino S, Miyamoto K, Okita K (2000) Enhanced interstitial collagenase (matrix metalloproteinase-13) production of Kupffer cell by gadolinium chloride prevents pig serum-induced rat liver fibrosis. Biochem Biophys Res Commun 267: 290–295

Hirose M, Watanabe S, Oide H, Kitamura T, Miyazaki A, Sato N (1996) A new function of Ito cells in liver morphogenesis: evidence using a novel morphogenic protein, epimorphin, in vitro. Biochem Biophys Res Commun 225:155–160
Ho JS, Buchweitz JP, Roth RA, Ganey PE (1996) Identification of factors from rat neutrophils responsible for cytotoxicity to isolated hepatocytes. J Leukoc Biol 59:716-724
Hoedemaker RMJ, Morselt HWM, Scherphof GL, Daemen T (1995) Heterogeneity in secretory responses of rat liver macrophages of different size. Liver 15:313–319
Hoffmann R, Henninger HP, Schulze-Specking A, Decker K (1994) Regulation of interleukin-6 receptor expression in rat Kupffer cells: modulation by cytokines, dexamethason and prostaglandin E2. J Hepatol 21:543–550
Horton RA, Knowles RG, Titheradge MA (1994) Endotoxin causes reciprocal changes in hepatic nitric oxide synthesis, gluconeogenesis, and flux through phosphoenolpyruvate carboxykinase. Biochem Biophys Res Commun 204:659–665
Housset C, Rockey DC, Bissel DM (1993) Endothelin receptors in rat liver: lipocytes as a contractile target for endothelin-1. Proc Natl Acad Sci USA 90:9266-9270
Hoyle G, Hill R (1988) Molecular cloning and sequencing of cDNA for a carbohydrate binding receptor unique for Kupffer cells. J Biol Chem 262:7487–7492
Hu PPC, Datto MB, Wang XF (1998) Molecular mechanisms of transforming growth fator-β signalling. Endocr Rev 19:349–363
Huang TP, Nishida T, Kamike W, Kosaka H, Seiyama A, Morimoto Y, Tanaka S, Obunai S, Takei Y, Shiga T, Matsuda H (1997) Role of nitric oxide in oxygen transport in rat liver sinusoids during endotoxemia. Hepatology 26:336–342
Iimuro Y, Yamamoto M, Kohno H, Itakura J, Fujii H, Matsumoto Y (1994) Blockade of liver macrophages by gadolinium chloride reduces lethality in endotoxemic rats – analysis of mechanisms of lethality in endotoxemia. J Leukocyte Biol 55:723–728
Ikeda K, Kawada N, Wang YQ, Kadoya H, Nakatani K, Sato M, Kaneda K (1998) Expression of cellular prion protein in activated hepatic stellate cells. Am J Pathol 153:1695–1700
Ikeda K, Wakahara T, Wang YQ, Kadaya H, Kawada N, Kaneda K (1999) In vitro migratory potential of rat quiescent hepatic stellate cells and its augmentation by cell activation. Hepatology 29:1760–1767
Ikeda H, Yatomi Y, Yanase M, Satoh H, Maekawa H, Ogata I, Ozaki Y, Takuwa Y, Mochida S, Fujiwara K (2000) Biological activities of novel lipid mediator sphingosine 1-phosphate in rat hepatic stellate cells. Am J Physiol Gastrointest Liver Physiol 279:G304–310
Ikejima K, Iimuro Y, Forman DT, Thurman RG (1996) A diet containing glycine improves survival in endotoxin shock in the rat. Am J Physiol 271:G97–103
Ikezawa Y, Yamatani K, Ogawa A, Ohnuma H, Igarashi M, Daimon M, Manaka H, Sasaki H (1998) Effects of glucagon on glycogenolysis and gluconeogenesis are region-specific in periportal and perivenous hepatocytes. J Lab Clin Med 132:547–555
Immenschuh S, Tan M, Ramadori G (1999) Nitric oxide mediates the lipopolysaccharide dependent upregulation of the heme oxygenase-1 gene expression in cultured rat Kupffer cells. J Hepatol 30:61–69
Iraburu MJ, Dominguez-Rosales JA, Fontana L, Auster A, Garcia-Trevijano ER, Covarrubias-Pinedo A, Rivas-Estilla AM, Greenwel P, Rojkind M (2000) Tumor necrosis factor-α down-regulates expression of the α1 (I) collagen gene in rat hepatic stellate cells through a p20 C/EBPβ- and C/EBPδ-dependent mechanism. Hepatology 31:1086–1093
Iredale JP, Murphy G, Hembry RM, Friedman SL, Arthur MJP (1992) Human hepatic lipocytes synthesize tissue inhibitor of metalloproteinases-1: implications for regulation of matrix degradation in liver. J Clin Invest 90:282–287
Iredale JP, Goddard S, Murphy G, Benyon RC, Arthur MJ (1995) Tissue inhibitor of metalloproteinase-1 and interstitial collagenase expression in autoimmune chronic active hepatitis and activated human hepatic lipocytes. Clin Sci (Colch) 89:75–81
Iredale JP, Benyon RC, Pickering J, McCullen M, Northrop M, Pawley S, Hovell C, Arthur MJ (1998) Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. J Clin Invest 102:538–549
Irie S, Tavassoli M (1985) Liver endothelium desialates ceruloplasmin.Biochem Biophys Res Commun 140:94–100

Irie S, Tavassoli M (1989) Desialylation of transferrin by liver endothelium is selective for its triantennan chain. Biochem J 262:491–496
Irving MG, Roll J, Huang S, Bissell DM (1984) Characterization and culture of sinusoidal endothelium from normal rat liver: lipoprotein uptake and collagen phenotype. Gastroenterology 87:1233–1247
Ishiguro S, Arii S, Monden K, Adachi Y, Funaki N, Higashitsuji H, Fujita S, Furutani M, Mise M, Kitao T (1994) Identification of the thromboxane A2 receptor in hepatic sinusoidal endothelial cells and its role in endotoxin-induced liver injury in rats. Hepatology 20:1281–1286
Ito T, Nemoto M (1952) Über die Kupfferschen Sternzellen und die "Fett-speicherungszellen" ("fat storing cells") in der Blutkapillarenwand der menschlichen Leber. Okajima Folia Anat Jpn 24:243
Ito Y, Lukita-Atmadja W, Machen NW, Baker GL, McCuskey RS (2000) High doses of intravenous immunoglobulin G enhance Kupffer cell phagocytic function during the late phase of sepsis and endotoxemia in rats. Shock 13:485–491
Itoh Y, Okanoue T, Enjo F, Sakamoto S, Takami S, Yasui K, Kagawa K, Kashima K (1994) Regulation of hepatocyte albumin and α1-acid glycoprotein secretion by monokines, dexamethasone, and nitric oxide synthase pathway: significance of activated liver nonparenchymal cells. Dig Dis Sci 39:851–860
Iwai M, Gardermann A, Puschel G, Jungermann K (1988a) Potential role for prostaglandin $F_{2\alpha}$, D_2, E_2, and thromboxane A_2 in mediating the metabolic and hemodynamic actions of sympathic nerves in perfused rat liver. Eur J Biochem 175:45–50
Iwai M, Hagmann W, Keppler D, Jungermann K (1988b) Leukotriene C4 metabolism during its action on glucose and lactate balance and flow in perfused rat liver. Biol Chem Hoppe Seyler 369:1131–1136
Iwamoto H, Nakamuta M, Tada S, Sugimoto R, Enjoji M, Nawata H (2000) A p160ROCK-specific inhibitor, Y-27632, attenuates rat hepatic stellate cell growth. J Hepatol 32:762–770
Iwamura S, Enzan H, Saibara T, Onishi S, Yamamoto Y (1995) Hepatic sinusoidal endothelial cells can store and metabolize serum immunoglobulin. Hepatology 22:1456–1461
Jacob AI, Goldberg PK, Bloom N, Degenshein GA, Kozinn PJ (1977) Endotoxin and bacteria in portal blood. Gastroenterology 116:1268–1270
Jaeschke H (1997) Cellular adhesion molecules: regulation and functional significance in the pathogenesis of liver diseases. Am J Physiol 273:G602–611
Jaeschke H (2000) Reactive oxygen and mechanisms of inflammatory liver injury. J Gastroenterol Hepatol 15:718–724
Jaeschke H, Smith CW (1997) Mechanisms of neutrophil-induced parenchymal cell injury. J Leukoc Biol 61:647–653
Jaeschke H, Fisher MA, Lawson JA, Simmons CA, Farhood A, Jones DA (1998) Activation of caspase 3 (CPP32)-like proteases is essential for TNFα-induced hepatic parenchymal cell apoptosis and neutrophil-mediated necrosis in a murine endotoxin shock model. J Immunol 160:3480–3486
Jaeschke H, Ho YS, Fisher MA, Lawson JA, Farhood A (1999) Glutathione peroxidase-deficient mice are more susceptible to neutrophil-mediated hepatic parenchymal cell injury during endotoxemia: importance of an intracellular oxidant stress. Hepatology 29:443–450
Jarnagin WR, Rockey DC, Koteliansky VE, Wang SS, Bissell DM (1994) Expression of variant fibronectins in wound healing: cellular source and biological activity of the EIIIA segment in rat hepatic fibrogenesis. J Cell Biol 127:2037–2048
Jezequel AM, Novelli G, Venturini C, Orlandi F (1984) Quantitative analysis of the perisinusoidal cells in human liver: the lipocytes. Front gastrointest Res 8:85–90
Johansson S, Gustafson S, Pertoft H (1987) Identification of a fibronectin receptor specific for rat liver endothelial cells. Exp Cell Res 172:425–431
Johansson AG, Lovdal T, Magnusson KE, Berg T, Skogh T (1996) Liver cell uptake and degradation of soluble immunoglobulin G immune complexes in vivo and in vitro in rats. Hepatology 24:169–175
Johansson AG, Sundqvist T, Skogh T (2000) IgG immune complex binding to and activation of liver cells. Int Arch Allergy Immunol 121:329–336
Johnson SJ, Hines JE, Burt AD (1992) Macrophage and perisinusoidal cell kinetics in acute liver injury. J Pathol 166:351–358
Johnston DE, Kroenig C (1996) Stimulation of prostaglandin synthesis in cultured liver cells by CCl4. Hepatology 24:677–684

Jonsson JR, Hogan PG, Balderson GA, Ooi LL, Lynch SV, Strong RW (1997) Human liver transplant perfusate: an abundant source of donor liver associated leucocytes. Hepatology 26:1111–1114
Jonsson JR, Edwards-Smith CJ, Catania SC, Morotomi Y, Hogan PG, Clouston AD, Bansal AS, Lynch SV, Strong RW, Powell EE (2000) Expression of cytokines and factors modulating apoptosis by human sinusoidal leukocytes. J Hepatol 32:392–398
Jungermann K (1995) Zonation of metabolism and gene expression in liver. Histochem Cell Biol 103:81–91
Jungermann K, Kietzmann T (1996) Zonation of parenchymal and non parenchymal metabolism in liver. Annu Rev Nutr 16:179–203
Jungermann K, Stumpel F (1999) Role of hepatic, intrahepatic and hepatoenteral nerves in the regulation of carbohydrate metabolism and hemodynamics of the liver and intestine. Hepato-Gastroenterolol 46 Suppl 2:1414–1417
Kamps JAAM, Kruijt JK, Kuiper J, Van Berkel TJC (1991) Uptake and oxidation of human low-density lipoprotein by human liver parenchymal and Kupffer cells in culture. Biochem J 276:135–140
Kan M, Huang J, Mansson P. Yasumitsu H, Carr B, McKeehan W (1989) Heparin-binding growth factor type 1 (acidic fibroblast growth factor): a potential biphasic autocrine and paracrine regulator of hepatocyte regulation. Proc Natl Acad Sci USA 86:7432-7436
Kaneda K, Wake K (1983) Distribution and morphological characteristics of the pit cells in the liver of the rat. Cell Tissue Res 233:485–505
Kaneda K, Pilaro AM, Sayers TJ, Nagashima K, Gonda MA, Ortaldo JR, Wiltrout RH (1994) Quantitative analysis of rod-cored vesicles and dense granules of large granular lymphocytes in the liver, spleen, and peripheral blood of rats. Cell Tissue Res 276:187–195
Kaneda K, Ekataksin W, Sogawa M, Matsumura A, Cho A, Kawada N (1998) Endothelin-1-induced vasoconstriction causes a significant increase in portal pressure of rat liver: localized constrictive effect on the distal segment of preterminal portal venules as revealed by light and electron microscopy and serial reconstruction. Hepatology 27:735–747
Kanemaki T, Kitade H, Kaibori M, Sakitani K, Hiramatsu Y, Kamiyama Y, Ito S, Okumura T (1998) Interleukin 1β and interleukin 6, but not tumor necrosis factor α, inhibit insulin-stimulated glycogen synthesis in rat hepatocytes. Hepatology 27:1296–1303
Kaplan LM (1998) Leptin, obesity, and liver disease. Gastroenterology 115:997–1001
Karck U, Peters T, Decker K (1988) The release of tumor necrosis factor from endotoxin-stimulated rat Kupffer cells regulated by prostaglandin E2 and dexamethasone. J Hepatol 7:352–361
Kawada N, Mizoguchi Y, Shin T, Tsutsui H, Kobayashi K, Morisawa S, Monna T, Yamamoto S (1990) Interferon γ stimulates prostaglandin E2 production by mouse Kupffer cells. Prostagl Leukot Essent Fatty Acids 40:275–279
Kawada N, Klein H, Decker K (1992) Eicosanoid-mediated contractility of hepatic stellate cells. Biochem J 285:367–371
Kawada N, Tran-Thi TA, Klein H, Decker K (1993) The contraction of hepatic stellate (Ito) cells stimulated with vasoactive substances. Possible involvement of endothelin 1 and nitric oxide in the regulation of the sinusoidal tonus. Eur J Biochem 213:815–823
Kawada N, Inoue M (1994) Effect of adrenomedullin on hepatic pericytes (stellate cells) of the rat. FEBS Lett 356:109–113
Kawada N, Seki S, Kuroki T, Inoue M (1998) Regulation of stellate cell proliferation by lipopolysaccharide: role of endogenous nitric oxide. J Gastroenterol Hepatol 13 Suppl:S6–13
Kawada N, Ikeda K, Seki S, Kuroki T (1999) Expression of cyclins D1, D2 and E correlates with proliferation of rat stellate cells in culture. J Hepatology 30:1057–1064
Kawamura T, Seki S, Takeda K, Narita J, Ebe Y, Naito M, Hiraide H, Abo T (1999) Protective effect of NK1.1(+) T cells as well as NK cells against intraperitoneal tumors in mice. Cell Immunol 193:219–225
Kawase T, Shiratori Y, Sugimoto T (1986) Collagen production by liver fat-storing cells in primary culture. Exp Cell Biol 54:183–192
Kawashima R, Mochida S, Matsui A, YouLuTuZ Y, Ishikawa K, Toshima K, Yamanobe F, Inao M, Ikeda H, Ohno A, Nagoshi S, Uede T, Fujiwara K (1999) Expression of osteopontin in Kupffer cells and hepatic macrophages and stellate cells in rat liver after carbon tetrachloride intoxication: a possible factor for macrophage migration into hepatic necrotic areas. Biochem Biophys Res Commun 256:527–531

Keppler D (1994) Leukotrienes and other eicosanoids in liver pathophysiology. In: Arias IM, Boyer JL, Fausto N, Jakoby WB, Schachter DA, Shafritz DA (eds) The Liver: Biology and Pathobiology. Raven Press, New York, pp 1015–1030
Keppler D, König J (1997) Expression and localization of the conjugate export pump encoded by the MRP2 (cMRP/cMOAT) gene in liver. FASEB J 11:509–516
Kiernan F (1833) The anatomy and physiology of the liver. Phil Trans Roy Soc (London) 123:711–770 (cited after Rappaport 1976)
Kimura M, Ogihara M (1998) Effects of insulin-like growth factor I and II on DNA synthesis and proliferation in primary cultures of adult rat hepatocytes. Eur J Pharmacol 354:271–281
Kimura M, Osumi S, Ogihara M (2000) Stimulation of DNA synthesis and proliferation by prostaglandins in primary cultures of adult rat hepatocytes. Eur J Pharm 404:259–271
Kitada T, Seki S, Ikeda K, Natatani K, Sataguchi H, Kawada N, Kadoya H, Kaneda K (2000) Clinicopathological characterization of prion: a novel marker of activated human hepatic stellate cells. J Hepatol 33:751–757
Kitade H, Kanemaki T, Sakitani K, Inoue K, Matsui Y, Kamiya T, Nakagawa M, Hiramatsu Y, Kamiyama Y, Ito S, Okumura T (1996) Regulation of energy metabolism by interleukin-1β, but not by interleukin-6, is mediated by nitric oxide in primary cultured rat hepatocytes. Biochim Biophys Acta 1311:20–26
Klein AS, Cofer JB, Pruett TL, Thuluvath PJ, McGory R, Uber L, Stevenson WC, Baliga P, Burdick JF (1996) Prostaglandin E1 administration following orthotopic liver transplantation: a randomized prospective multicenter trial. Gastroenterology 111:710–715
Kleinherenbrink-Stins MF, van de Boom JH, Schouten D, Roholl PJ, Niels van der Heyde M, Brouwer A, van Berkel TJC, Knook DL (1991) Visualization of the interaction of native and modified lipoproteins with parenchymal, endothelial and Kupffer cells from human liver. Hepatology 14:79–90
Kmiec Z (1994) Prostaglandin cytoprotection of galactosamine-incubated hepatocytes isolated from young and old rats. Ann NY Acad Sci 717:216–225
Kmiec Z, Hughes RD, Moore KP, Sheron N, Gove CD, Williams R (1992) Effects of cysteinyl leukotrienes and tumor necrosis factor-α on isolated rat hepatocytes. Hepato-Gastroenterol 39:270–276
Kmiec Z, Hughes RD, Moore KP, Sheron N, Gove CD, Nouri-Aria KT, Williams R (1993) Effect of supernatants from Kupffer cells stimulated with galactosamine and endotoxin on the function of isolated rat hepatocytes. Hepato-Gastroenterol 40:259–261
Kmiec Z, Hughes RD, Moore KP, Williams R (1997) Effects of eicosanoids secreted by stimulated Kupffer cells on isolated rat hepatocytes. Folia Histochem Cytobiol 35:89–90
Knittel T, Armbrust T, Schwogler S, Schuppan D, Ramadori G (1992a) Distribution and cellular origin of undulin in rat liver. Lab Invest 67:779–787
Knittel T, Fellmer P, Muller L, Ramadori G (1997a) Bone morphogenetic protein-6 is expressed in nonparenchymal liver cells and upregulated by transforming growth factor-β1. Exp Cell Res 232:263–269
Knittel T, Muller L, Saile B, Ramadori G (1997b) Effect of tumour necrosis factor-α on proliferation, activation and protein synthesis of rat hepatic stellate cells. J Hepatol 27:1067–1080
Knittel T, Kobold D, Saile B, Grundmann A, Neubauer K, Piscaglia F, Ramadori G (1999a) Rat liver myofibroblasts and hepatic stellate cells: different cell populations of the fibroblast lineage with fibrogenic potential. Gastroenterology 117:1205–1221
Knittel T, Kobold D, Piscaglia F, Saile B, Neubauer K, Mehde M, Timpl R, Ramadori G (1999b) Localization of liver myofibroblasts and hepatic stellate cells in normal and diseased rat livers: distinct roles of (myo-)fibroblast subpopulations in hepatic tissue repair. Histochem Cell Biol 112:387–401
Knittel T, Mehde M, Kobold D, Saile B, Dinter C, Ramadori G (1999c) Expression patterns of matrix metalloproteinases and their inhibitors in parenchymal and non-parenchymal cells of rat liver: regulation by TNF-α and TGF-β1. J Hepatol 30:48–60
Knolle PA, Gerken G (2000) Local control of the immune response in the liver. Immunol Rev 174:21–34
Knolle PA, Schlaak J, Uhrig A, Kempf P, Meyer zum Buschenfelde KH, Gerken G (1995) Human Kupffer cells secrete IL-10 in response to lipopolysaccharide (LPS) challenge. J Hepatol 22:226–229

Knolle PA, Loser E, Protzer U, Duchmann R, Schmitt E, zum Buschenfelde KH, Rose John S, Gerken G (1997) Regulation of endotoxin-induced IL-6 production in liver sinusoidal endothelial cells and Kupffer cells by IL-10. Clin Exp Immunol 107:555–561

Knolle PA, Schmitt E, Jin S, Germann T, Duchmann R, Hegenbarth S, Gerken G, Lohse AW (1999) Induction of cytokine production in naive CD4(+) T cells by antigen-presenting murine liver sinusoidal endothelial cells but failure to induce differentiation toward Th1 cells. Gastroenterology 116:1428–1440

Knook DL, Seffelaar A, De Leeuw A (1982) Fat-storing cells of the rat liver. Their isolation and purification. Exp Cell Res 139:468–471

Knook DL, Sleyster EC (1977) Isolation and characterization of Kupffer and endothelial cells from the rat liver. Exp Cell Res 99:444–449

Koda H, Okuno M, Imai S, Moriwaki H, Muto Y, Kawada N, Kojima S (1996) Retinoic acid-stimulated liver stellate cells suppress the production of albumin from parenchymal cells via TGF-β. Biochem Biophys Res Commun 221:565–569

Koj A (1996) Initiation of acute phase response and synthesis of cytokines. BBA Biochim Biophys Acta 1317:84–94

Kosai K-H, Matsumoto K, Funakoshi H, Nakamura T (1999) Hepatocyte growth factor prevents endotoxin-induced lethal hepatic failure in mice. Hepatology 30:151–159

Kossmann T, Manthey CL, Brandes ME, Morganti Kossmann MC, Ohura K, Allen JB, Mergenhagen SE, Wahl SM (1992) Kupffer cells express type I TGF-β receptors, migrate to TGF-β and participate in streptococcal cell wall induced hepatic granuloma formation. Growth Factors 7:73–83

Kosugi I, Muro H, Shirasawa H. Ito I (1992) Endocytosis of soluble IgG immune complex and its transport to lysosomes in hepatic sinusoidal endothelial cells. J Hepatol 16:106–114

Kowalski-Saunders PW, Winwood PJ, Arthur MJ, Wright R (1992) Reversible inhibition of albumin production by rat hepatocytes maintained on a laminin-rich gel (Engelbreth-Holm-Swarm) in response to secretory products of Kupffer cells and cytokines. Hepatology 16:733–741

Krause P, Markus PM, Schwartz P, Unthan-Fechner K, Pestel S, Fandrey J, Probst I (2000) Hepatocyte-supported serum-free culture of rat liver sinusoidal endothelial cells. J Hepatol 32:718–726

Krieger M (1997) The other side of scavenger receptors: pattern recognition for host defense. Cur Opin Lipidol 8, 275–280

Kuiper J, De Rijke YB, Zijlstra FJ, Van Waas MP, Van Berkel TJC (1988) The induction of glycogenolysis in the perfused liver by platelet activating factor is mediated by prostaglandin D_2 from Kupffer cells. Biochem Biophys Res Commun 157:1288–1295

Kuiper J, Kamps IAAM, Van Berkel TJC (1989) Identification of the inhibitor of the plasminogen activator as the major protein secreted by endothelial rat liver cells. FEBS Lett 245:229–234

Kupffer K (1876) Uber Sternzellen der Leber. Briefliche Mitteilung an Professor Waldeyer. Arch Mikr Anat 12:253–358

Kuo PC, Abe K, Schroeder RA (2000) Superoxide enhances interleukin 1β-mediated transcription of the hepatocyte-inducible nitric oxide synthase gene. Gastroenterology 118:608–618

Kurose I, Miura S, Higuchi H, Watanabe N, Kamegaya Y, Takaishi M, Tomita K, Fukumura D, Kato S, Ishii H (1996) Increased nitric oxide synthase activity as a cause of mitochondrial dysfunction in rat hepatocytes: roles for tumor necrosis factor α. Hepatology 24:1185–1192

Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman IL, Grompe M (2000) Purified hematopoietic stem cells can differentiate into hepatocytes *in vivo*. Nature Med 6:1229–1234

Laskin DL (1990) Nonparenchymal cells and hepatotoxicity. Semin Liv Dis 11:293–304

Laskin JD, Dokidis A, Gardner CR, Laskin DL (1991) Changes in sulfated proteoglycan production after activation of rat liver macrophages. Hepatology 14:306–3 I 2

Laskin DL, Rodriguez-del-Valle M, Heck DE, Hwang SM, Ohnishi ST, Durham SK, Goller NL, Laskin JD (1995) Hepatic nitric oxide production following acute endotoxemia in rats is mediated by increased inducible nitric oxide synthase gene expression. Hepatology 22:223–234

Lawson JA, Fisher MA, Simmons CA, Farhood A, Jaeschke H (1998) Parenchymal cell apoptosis as a signal for sinusoidal sequestration and transendothelial migration of neutrophils in murine models of endotoxin and Fas-antibody-induced liver injury Hepatology 28:761–767

Lawson JA, Burns AR, Farhood A, Bajt ML, Collins RG, Smith CW, Jaeschke H (2000) Pathophysiologic importance of E- and L-selectin for neutrophil-induced liver injury during endotoxemia in mice. Hepatology 32:900–998
Lee KS, Buck M, Houglum K, Chojkier M (1995) Activation of hepatic stellate cells by TGF-α and collagen type 1 is mediated by oxidative stress through c-myb expression. J Clin Invest 2461–2468
Leist M, Gantner F, Bohlinger I, Tiegs G, Germann PG, Wendel A (1995) Tumor necrosis factor-induced hepatocyte apoptosis precedes liver failure in experimental murine shock models. Am J Pathol 146:1220–1234
LeRoith D (1997) Insulin-like growth factors. N Engl J Med 336:633–640
Levy MT, McCaughan GW, Abbott CA, Park JE, Cunningham AM, Muller E, Rettig WJ, Gorrell MD (1999) Fibroblast activation protein: a cell surface dipeptidyl peptidase and gelatinase expressed by stellate cells at the tissue remodelling interface in human cirrhosis. Hepatology 29:1768–1778
Levy MT, Trojanowska M, Reuben A (2000) Oncostatin M: a cytokine upregulated in human cirrhosis, increases collagen roduction by human hepatic stellate cells. J Hepatol 32:218–226
Leyland H, Gentry J, Arthur MJP, Benyon RC (1996) The plasminogen-activating system in hepatic stellate cells. Hepatology 24:1172–1178
Li J, Bombeck CA, Yang S, Kim YM, Billiar TR (1999) Nitric oxide suppresses apoptosis via interrupting caspase activation and mitochondrial dysfunction in cultured hepatocytes. J Biol Chem 274:17325–17333
Liao J, Keiser JA, Scales WE, Kunkel SL, Kluger MJ (1995) Role of epinephrine in TNF and IL-6 production from isolated perfused rat liver. Am J Physiol 268:R896-R901
Lichtman SN, Wang J, Lemasters JJ (1998) LPS receptor CD14 participates in release of TNF-α in RAW 264.7 and peritoneal cells but not in Kupffer cells. Am J Physiol 275:G39–46
Limmer A, Ohl J, Kurts C, Ljunggren H-G, Reiss Y, Groettrup M, Momburg F, Arnold B, Knolle PA (2000) Efficient presentation of exogenous antigen by liver endothelial cells to CD8+T cells results in antigen-specific T-cell tolerance. Nature Med 6:1348–1354
Lindros KO, Penttila KE (1985) Digitonin-collagenase perfusion for efficient separation of periportal or perivenous hepatocytes. Biochem J 228:757–760
Liu ZZ, Cui S, Billiar TR, Dorko K, Halfter W, Geller DA, Michalopoulos G, Beger HG, Albina J, Nussler AK (1996) Effects of hepatocellular mitogens on cytokine-induced nitric oxide synthesis in human hepatocytes. J Leukoc Biol 60:382–388
Liu P, Xu B, Hock CE, Nagele R, Sun FF, Wong PY (1998a) NO modulates P-selectin and ICAM-1 mRNA expression and hemodynamic alterations in hepatic I/R. Am J Physiol 275:H2191–2198
Liu S, Khemlani LS, Shapiro RA, Johnson ML, Liu K, Geller DA, Watkins SC, Goyert SM, Billiar TR (1998b) Expression of CD14 by hepatocytes: upregulation by cytokines during endotoxemia. Infect Immun 66:5089–5098
Lohse AW, Knolle PA, Bilo K, Uhrig A, Waldmann C, Ibe M, Schmitt E, Gerken G, Meyer Zum Buschenfelde KH (1996) Antigen-presenting function and B7 expression of murine sinusoidal endothelial cells and Kupffer cells. Gastroenterology 110:1175–1181
Loud AV (1968) A quantitative stereological description of the ultrastructure of normal rat liver parenchymal cells. J Cell Biol 37:27–46
Lumsden AB, Henderson JM, Kutner MH (1988) Endotoxin levels measured by a chromogenic assay in portal, hepatic and peripheral venous blood in patients with cirrhosis. Hepatology 8:232–236
Luo D, Vermijlen D, Vanderkerken K, Kuppen PJ, Seynaeve C, Eddouks M, Baekeland M, Wisse E (1999) Involvement of LFA-1 in hepatic NK cell (pit cell)-mediated cytolysis and apoptosis of colon carcinoma cells. J Hepat 31:110–116
Luster MI, Germolec DR, Yoshida T, Kayama F, Thompson M (1994) Endotoxin-induced cytokine gene expression and excretion in the liver. Hepatology 19:480–488
MacDonald HR (1995) NK1.1+ T cell receptor α-β cells: new clues to their origin, specificity and function. J Exp Med 182:633–638
MacPhee PJ, Schmidt EE, Groom AC (1992) Evidence for Kupffer cell migration along liver sinusoids, from high-resolution in vivo microscopy. Am J Physiol 263:G17–23
MacPhee PJ, Schmidt EE, Groom AC (1995) Intermittence of blood flow in liver sinusoids, studied by high-resolution in vivo microscopy.Am J Physiol 269:G692-G698
Magnusson S, Berg T (1989) Extremely rapid endocytosis mediated by the mannose receptor of sinusoidal endothelial rat liver cells. Biochem J 257:651–656

Maher JJ (1993) Cell-specific expression of hepatocyte growth factor in liver. Upregulation in sinusoidal endothelial cells after carbon tetrachloride. J Clin Invest 91: 2244–2252
Maher JJ (1995) Rat hepatocytes and Kupffer cells interact to produce interleukin-8 (CINC) in the setting of ethanol. Am J Physiol 269: G518–523
Maher JJ, Lozier JS, Scott MK(1998) Rat hepatic stellate cells produce cytokine-induced neutrophil chemoattractant in culture and in vivo. Am J Physiol 275:G847–853
Maines MD (1997) The hemeoxygenase system: a regulator od second messenger gases. Annu Rev Toxicol 37:517–554
Mallat A, Preaux AM, Blazejewski S, Rosenbaum J, Dhumeaux D, Mavier P (1995) Interferon α and γ inhibit proliferation and collagen synthesis of human Ito cells in culture. Hepatology 21:1003–1010
Mallat A, Preaux AM, Serradeil Le Gal C, Raufaste D, Gallois C, Brenner DA, Bradham C, Maclouf J, Iourgenko V, Fouassier L, Dhumeaux D, Mavier P, Lotersztajn S (1996) Growth inhibitory properties of endothelin-1 in activated human hepatic stellate cells: a cyclic adenosine monophosphate-mediated pathway. J Clin Invest 98:2771–2778
Mallat A, Gallois C, Tao J, Habib A, Maclouf J, Mavier P, Preaux AM, Lotersztajn S (1998) Platelet-derived growth factor-BB and thrombin generate positive and negative signals for human hepatic stellate cell proliferation. Role of a prostaglandin/cyclic AMP pathway and cross-talk with endothelin receptors. J Biol Chem 273:27300–27305
Mangelsdorf DJ, Evans RM (1995) The RXR heterodimers and orphan receptors. Cell 83:841–850
Marra F, Valente AJ, Pinzani M, Abboud HE (1993) Cultured human liver fat-storing cells produce monocyte chemotactic protein-1. Regulation by proinflammatory cytokines. J Clin Invest 92:1674–1680
Marra F, Choudhury GG, Pinzani M, Abbvud HE (1994) Regulation of platelet-derived growth factor secretion and gene expression in human liver fat-storing cells. Gastroenterology 107:1110–1117
Marra F, Grandaliano G, Valente AJ, Abboud HE (1995) Thrombin stimulates proliferation of liver fat-storing cells and expression of monocyte chemotactic protein-1: potential role in liver injury. Hepatology 22:780–787
Marra F, DeFranco R, Grappone C, Milani S, Pinzani M, Pellegrini G, Laffi G, Gentilini P (1998a) Expression of the thrombin receptor in human liver. Upregulation during acute and chronic disease. Hepatology 27:462–471
Marra F, Pastacaldi S, Romanelli RG, Pinzani M, Ticali P, Carloni V, Laffi G, Gentilini P (1998b) Increased expression of monocyte chemotactic protein-1 during active hepatic fibrogenesis: correlation with monocyte infiltration. Am J Pathol 152:423–430
Marra F, Romanelli RG, Giannini C, Failli P, Pastacaldi S, Arrighi MC, Pinzani M, Laffi G, Montalto P, Gentilini P (1999) Monocyte chemotactic protein-1 as a chemoattractant for human hepatic stellate cells. Hepatology 29:140–148
Matsuda Y, Matsumoto K, Yamada A, Ichida T, Asakura H, Komoriya Y, Nishiyama E, Nakamura T (1997) Preventive and therapeutic effects in rats of hepatocyte growth factor infusion on liver fibrosis/cirrhosis. Hepatology 26:81–89
Matsumoto T, Kawakmi M (1982) The unit-concept of hepatic parenchyma. A re-examination based on angioarchitectural studies. Acta Pathol Jpn 32(Suppl2):285–314
Matsumoto K, Nakamura T (1996) Emerging multipotent aspects of hepatocyte growth factor. J Biochem 119:591–600
Matsuoka M, Tsukamoto H (1990) Stimulation of hepatic lipocyte collagen production by Kupffer cell-derived transforming growth factor β: implication for a pathogenic role in alcoholic liver fibrogenesis. Hepatology 11:599–605
Matsuura T, Nagamori S, Hasumura S, Sujino H, Shimizu K, Niiya M, Hirosawa K (1993) Retinol transport in cultured stellate cells of rat liver: studies by light and electron microscope autoradiography. Exp Cell Res 206:111–118
Matsuura T, Gad MZ, Harrison EH, Ross AC (1997) Lecithin:retinol acyltransferase and retinyl ester hydrolase activities are differentially regulated by retinoids and have distinct distributions between hepatocyte and nonparenchymal cell fractions of rat liver. J Nutr 127:218–224
Matsuura T, Hasumura S, Nagamori S, Murakami K (1999) Retinol esterification activity contributes to retinol transport in stellate cells. Cell Struct Funct 24:111–116

Mawet E, Shiratori Y, Hikiba Y, Takada H, Yoshida H, Okano K, Komatsu Y, Matsumura M, Niwa Y Omata M (1996) Cytokine-induced neutrophil chemoattractant release from hepatocytes is modulated by Kupffer cells. Hepatology 23:353–358

McCloskey TW, Todaro JA, Laskin DL (1992) Lipopolysaccharide treatment of rats alters antigen expression and oxidative metabolism in hepatic macrophages and endothelial cells. Hepatology 16:191–203

McCourt PA, Smedsrod BH, Melkko J, Johansson S (1999) Characterization of a hyaluronan receptor on rat sinusoidal liver endothelial cells and its functional relationship to scavenger receptors. Hepatology 30:1276–1286

McCuskey RS (1999) Hepatic and splanchnic microvascular responses to inflammation and shock. Hepato-Gastroenterol 46 Suppl 2: 1464–1467

McNab G, Reeves JL, Salmi M, Hubscher S, Jalkanen S, Adams DH (1996)Vascular adhesion protein 1 mediates binding of T cells to human hepatic endothelium. Gastroenterology 110:522–528

Mehal WZ, Juedes AE, Crispe N (1999) Selective retention of activated CD8+ T cells by the normal liver. J Immunol 163:3202–3210

Meszaros K, Lang CH, Bagby GJ, Spitzer JJ (1987) Contribution of different organs to increased glucose consumption after endotoxin administration. J Biol Chem 262:10965–10970

Meszaros K, Bojta J, Bautista AP, Lang CH, Spitzer JJ (1991) Glucose utilization by Kupffer cells, endothelial cells, and granulocytes in endotoxemic rat liver. Am J Physiol 260:G7–12

Metzger S, Begleibter N, Barash V, Drize O, Peretz T, Shiloni E, Chajek-Shaul T (1997) Tumor necrosis factor inhibits the transcriptional rate of glucose-6-phosphatase in vivo and in vitro. Metabolism 46:579–583

Meyer DH, Bachem MG, Gressner AM (1990a) Modulation of hepatic lipocyte proteoglycan synthesis and proliferation by Kupffer cell-derived transforming growth factors type β1 and type α. Biochem Biophys Res Commun 171:1122–1129

Meyer DH, Bachem MG, Gressner AM (1990b) Transformed fat storing cells inhibit the proliferation of cultured hepatocytes by secretion of transforming growth factor β. J Hepatol 11:86–91

Meyer D, Krull N, Dreher K, Gressner A (1992) Biglycan and decorin gene expression in normal and fibrotic rat liver: cellular localization and regulatory factors. Hepatology 16:204–216

Michalopoulos GK, Bowen WC, Zajac VF, Beer-Stolz D, Watkins S, Kostrubsky V; Strom SC (1999) Morphogenetic events in mixed cultures of rat hepatocytes and nonparenchymal cells maintained in biological matrices in the presence of hepatocyte growth factor and epidermal growth factor. Hepatology 29: 90–100

Milani S, Herbst H, Schuppan D, Riecken EO, Stein H (1989) Cellular localization of laminin gene transcripts in normal and fibrotic human liver. Am J Pathol 134:1175–1182

Milani S, Herbst H, Schuppan D, Crappvne C, Pellegrini G, Pinzani M, Casini A, Calabró A, Ciancio G, Stefanini F, Burroughs AK, Surrenti C (1994) Differential expression of matrix-metalloproteinase-1 and -2 genes in normal and fibrotic human liver. Am J Pathol 144:528–537

Milosevic N, Schawalder H, Maier P (1999) Kupffer cell-mediated differential down-regulation of cytochrome P450 metabolism in rat hepatocytes. Eur J Pharmacol 368:75–87

Mimura Y, Skisaka S, Harada M, Sata M, Tanikawa K (1995) Role of hepatocytes in direct clearence of lipopolysaccharide in rats. Gastroeneterology 109:1969–1976

Mitaka T, Mizuguchi T, Yokono T Mochizuki Y (1999) Reconstruction of hepatic organoid by rat small hepatocytes and hepatic nonparenchymal cells. Hepatology 29:111–125

Miyamoto M, Emoto M, Brinkmann V, van Rooijen N, Schmits R, Kita E, Kaufmann SH (2000) Cutting edge: contribution of NK cells to the homing of thymic CD4+NKT cells to the liver. J Immunol 165:1729–1732

Miyauchi J, Sasadaira H, Watanabe K, Watanabe Y (1985) Ultrastructural immunocytochemical localization of lysozyme in human monocytes and macrophages. Cell Tiss Res 242:269–277

Mizoguchi Y, Ichikawa Y, Kioka K, Kawada N, Kobayashi K, Yamamoto S (1991) Effects of arachidonic acid metabolites and interleukin-1 on platelet activating factor production by hepatic sinusoidal endothelial cells from mice. J Gastroenterol Hepatol 6:283–288

Mochida S, Ishikawa K, Inao M, Shibuya M, Fujiwara K (1996) Increased expressions of vascular endothelial growth factor and its receptors, flt-1 and KDR/flk-1, in regenerating rat liver. Biochem Biophys Res Commun 226:176–179

Moncada S, Palmer RMJ, Higgs EA (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 43:109–142
Monshouwer M, Witkamp RF, Nujmeijer SM, van Amsterdam JG, van Miert AS (1996) Suppression of cytochrome P450- and UDP glucuronosyl transferase-dependent enzyme activities by proinflammatory cytokines and possible role of nitric oxide in primary cultures of pig hepatocytes. Toxicol Appl Pharmacol 137:237–244
Morgan ET, Thomas KB, Swanson R, Vales T, Hwang J, Wright K (1994) Selective suppression of cytochrome P-450 gene expression by interleukins 1 and 6 in rat liver. Biochem Biophys Acta 1219:475–483
Morikawa A, Kato Y, Sugiyama T, Koide N, Chakravortty D, Yoshida T, Yokochi T (1999) Role of nitric oxide in lipopolysaccharide-induced hepatic injury in D-galactosamine-sensitized mice as an experimental endotoxic shock model. Infect Immun 67:1018–1024
Moshage H, Casini A, Lieber CS (1990) Acetaldehyde selectively stimulates collagen production in cultured rat liver fat-storing cells but not in hepatocytes. Hepatology 12:511–518
Motta PM (1984) The three-dimensional microanatomy of the liver. Arch Histol Jpn 47:1–30
Motomura K, Sakai H, Isobe H, Nawata H (1997) Effects of retinoids on the production of tumour necrosis factor-α and nitric oxide by lipopolysaccharide-stimulated rat Kupffer cells in vitro:evidence for participation of retinoid X receptor signalling pathway. Cell Biochem Funct 15:95–101
Mullhaupt B, Feren A, Fodor E, Jones AL (1994) Liver expression of epidermal growth factor RNA. Rapid increases in immediate-early phase of liver regeneration. J Biol Chem 269:19667–19670
Muro H, Shirasawa H, Maeda M, Nakamura S (1987) Fc receptors of liver sinusoidal endothelium in normal rats and humans. A histologic study with soluble immune complexes. Gastroenterology 93:1078-l085
Muschen M, Warskulat U, Douillard P, Gilbert E, Haussinger D (1998) Regulation of CD95 (APO-1/Fas) receptor and ligand expression by lipopolysaccharide and dexamethasone in parenchymal and nonparenchymal rat liver cells. Hepatology 27:200–208
Musso O, Rehn M, Saarela J, Theret N, Lietard J, Hintikka, Lotrian D, Campion JP, Pihlajaniemi T, Clement B (1998) Collagen XVIII is localized in sinusoids and basement membrane zones and expressed by hepatocytes and activated stellate cells in fibrotic human liver. Hepatology 28:98–107
Mustafa SB, Olson MS (1998) Expression of nitric-oxide synthase in rat Kupffer cells is regulated by cAMP. J Biol Chem 273:5073–5080
Mustafa SB, Howard KM, Olson MS (1996) Platelet-activating factor augments lipopolysaccharide-induced nitric oxide formation by rat Kupffer cells. Hepatology 23:1622–1630
Nagaki M, Naiki T, Brenner DA, Osawa Y, Imose M, Hayashi M, Banno Y, Nakashima S, Moriwaki H (2000) Tumor necrosis factor -α prevents necrosis factor receptor-mediated mouse hepatocyte apoptosis, but not Fas-mediated apoptosis: role of nuclear factor-κB. Hepatology 32:1272–1279
Nagendra AR, Mickelson JK, Smith CW (1997) CD18 integrin and intercellular adhesion molecule-1 (CD54)-dependent neutrophil adhesion to cytokine-stimulated human hepatocytes. Am J Physiol 272:G408-G416
Nagy NE, HolvenKB, Roos N, Senoo H, Kojima N, Norum KR, Blomhoff R (1997) Storage of vitamin A in extrahepatic stellate cells in normal rats. J Lipid Res 38:645-658
Naito M, Hasegawa G, Takahashi K (1997) Development, differentiation, and maturation of Kupffer cells. Micr Res Techn 39:350–364
Nakamura T, Arii S, Monden K, Furutani M, Takeda Y, Imamura M, Tominaga M, Okada Y (1998) Expression of the Na+/Ca2+ exchanger emerges in hepatic stellate cells after activation in association with liver fibrosis. Proc Natl Acad Sci USA 95:5389–5394
Nakamura J, Nishida T, Hayashi K, Kawada N, Ueshima S, Sugiyama Y, Ito T, Sobue K, Matsuda H (1999) Kupffer cell-mediated down regulation of rat hepatic CMOAT/MRP2 gene expression. Biochem Biophys Res Commun 255:143–149
Nakatani K, Seki S, Kawada N, Kobayashi K, Kaneda K (1996) Expression of neural cell adhesion molecule (N-CAM) in perisinusoidal stellate cells of the human liver. Cell Tissue Res 283:159–165
Napoli J, Bishop GA, McGuinness PH, Painter DM, McCaughan GW (1996) Progressive liver injury in chronic hepatitis C infection correlates with increased intrahepatic expression of Th1-associated cytokines. Hepatology 24:759–765
Narumiya S, Sugimoto Y, Ushikubi F (1999) Prostanoid receptors: structures, properties, and functions. Physiol Rev 79:1193–1226

Nathanson MH, Rios Velez L, Burgstahler AD, Mennone A (1999) Communication via gap junctions modulates bile secretion in the isolated perfused rat liver. Gastroenterology 116:1176–1183
Negishi M, Sugimoto Y, Ichikawa A (1995) Molecular mechanisms of diverse actions of prostanoid receptors. Biochim Biophys Acta 1259:109–119
Nelson DR, Lauwers GY, Lau JY, Davis GL (2000) Interleukin 10 treatment reduces fibrosis in patients with chronic hepatitis C: a pilot trial of interferon nonresponders. Gastroenterology 118:655–660
Neubauer K, Knittel T, Aurisch S, Fellmer P, Ramadori G (1996) Glial fibrillary acidic protein – a cell type specific marker for Ito cells in vivo and in vitro. J Hepatol 14:719–730
Neubauer K, Kruger M, Quondamatteo F, Knittel T, Saile B, Ramadori G (1999) Transforming growth factor-β1 stimulates the synthesis of basement membrane proteins laminin, collagen type IV and entactin in rat liver sinusoidal endothelial cells. J Hepatol 31:692–702
Niki T, Pekny M, Hellemans K, Bleser PD, Berg KV, Vaeyens F, Quartier E, Schuit F, Geerts A (1999) Class VI intermediate filament protein nestin is induced during activation of rat hepatic stellate cells. Hepatology 29:520–527
Nishi M, Takeshima H, Houtani T, Nakagawara K, Noda T, Sugimoto T (1999) RhoN, a novel small GTP-binding protein expressed predominantly in neurons and hepatic stellate cells. Brain Res Mol Brain Res 67:74–81
Nishida J, McCuskey RS, McDonnell D, Fox ES (1994) Protective role of NO in hepatic microcirculatory dysfunction during endotoxemia. Am J Physiol 267:G1135–1141
Norris S, Collins C, Doherty DG, Smith F, McEntee G, Traynor O, Nolan N, Hegarty J, O'Farrelly C (1998) Resident human hepatic lymphocytes are phenotypically different from circulating lymphocytes. J Hepatol 28:84–90
Norris S, Doherty DG, Collins C, McEntee G, Traynor O, Hegarty JE, O'Farrelly C (1999) Natural T cells in the human liver: cytotoxic lymphocytes with dual T cell and natural killer cell phenotype and function are phenotypically heterogenous and include Vα24-JαQ and γδ T cell receptor bearing cells. Hum Immunol 60:20–31
Novikoff AB (1959) Cell heterogeneity within the hepatic lobule (staining reactions). J Histochem Cytochem 7:240–244.
Nukina S, Fusaoka T, Thurman RG (1994)Glycogenolytic effect of adenosine involves ATP from hepatocytes and eicosanoids from Kupffer cells. Am J Physiol 266:G99–105
Oberhammer F, Bursch W, Parzefall W, Breit P, Erber E, Stadler M, Schulte-Hermann R (1991) Effect of transforming growth factor β on cell death of cultured rat hepatocytes. Cancer Res 51:2478–2485
Ohata M, Yamauchi M, Takeda K, Toda G, Kamimura S, Motomura K, Xiong S, Tsukamoto H (2000) RAR and RXR expression by Kupffer cells. Exp Molec Pathol 68:13–20
Ohira H, Ueno T, Torimura T, Tanikawa K, Kasukawa R (1995) Leukocyte adhesion molecules in the liver and plasma cytokine levels in endotoxin-induced rat liver injury. Scand J Gastroenterol 30:1027–1035
Oide H, Tateyama M, Wang XE, Hirose M, Itatsu T, Watanabe S, Ochi R, Sato N (1999) Activated stellate (Ito) cells possess voltage-activated calcium current. Biochim Biophys Acta 1418:158–164
Okamura H, Tsutsui H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, Torigoe K, Okura T, Nukada Y, Hattori K (1995) Cloning of a new cytokine that induces IFN-γ production by T cells. Nature 378:88–91
Okumura S, Takei Y, Kawano S, Nagano K, Masuda E, Goto M, Tsuji S, Michida T, Chen SS, Kashiwagi T (1994) Vasoactive effect of endothelin-1 on rat liver in vivo. Hepatology 19:155–161
Okuno M, Moriwaki H, Imai S, Muto Y, Kawada N, Suzuki Y, Kojima S (1997) Retinoids exacerbate rat liver fibrosis by inducing the activation of latent TGF-β in liver stellate cells. Hepatology 26:913–921
Okuno K, Hirai N, Takemoto Y, Kawai I, Yasutomi M (2000) Interleukin-2 as a modulator of 5-fluorouracil in hepatic arterial immunochemotherapy for liver metastases. Hepato-Gastroenterol 47:487–491
Omoto E, Tavassoli M (1990) Purification and partial characterization of ceruloplasmin receptors from rat liver endothelium. Arch Biochem Biophys 282:34–38
Otter M, Kuiper J, Bos R, Rijken DC, van Berkel TJC (1992) Characterization of the interaction both in vitro and in vivo of tissue-type plasminogen activator (t-PA) with rat liver cells. Effects of monoclonal antibodies to t-PA. Biochem J 284:545–550

Ou J, Carlos TM, Watkins SC, Saavedra JE, Keefer LK, Kim YM, Harbrecht BG, Billiar TR (1997) Differential effects of nonselective nitric oxide synthase (NOS) and selective inducible NOS inhibition on hepatic necrosis, apoptosis, ICAM-1 expression, and neutrophil accumulation during endotoxemia. Nitric Oxide 1:404–416

Parillo, JE (1993) Pathogenetic mechanisms of septic shock. N Engl J Med 328:1471–1477

Paul P, Rothman SA, McMahon JT, Gordon AS (1984) Erythropoietin secretion by isolated rat KupfFer cells. Exp Hematol 12:825–830

Pestel S, Schieferdecker HL, Jungermann K (2000) Stimulation of glucose output in the perfused rat liver by anaphylotoxin C5 A and zymosan but not lipopolysaccharide: involvement of prostanoids released from Kupffer cells only in C5 A action. 10th International Symposium on Cells of the Hepatic Sinusoid, Southampton (abstract)

Peters T, Karck U (1990) Interdependence of tumor necrosis factor, prostaglandin E2, and protein synthesis in lipopolysaccharide-exposed Kupffer cells. Eur J Biochem 191:583–589

Peters JM, Rusyn I, Rose ML, Gonzales FJ, Thurman RG (2000) Peroxisome proliferator-activated receptor α is restricted to hepatic parenchymal cells, not Kupffer cells: implications for the mechanism of action of peroxisome proliferators in hepatocarcinogenesis. Carcinogenesis 21:823–826

Peters-Regehr T, Bode JG, Kubitz R, Haussinger D (1999) Organic osmolyte transport in quiescent and activated rat hepatic stellate cells (Ito cells). Hepatology 29:173–180

Pinzani M, Abboud HE, Aron DC (1990) Secretion of insulin-like growth factor-I and binding proteins by rat liver fat-storing cells: regulatory role of platelet-derived growth factor. Endocrinology 127:2343–2349

Pinzani M, Abboud N. Gesualdo L, Abboud S (1992a) Regulation of macrophage colony-stimulating factor in liver fat-storing cells by peptide growth factors. Am J Physiol 262:C876–881

Pinzani M, Gentilini P, Abboud HE (1992b) Phenotypical modulation of liver fat-storing cells by retinoids. Influence on unstimulated and growth factor-induced cell proliferation. J Hepatol 14:211–220

Pinzani M, Failli P, Ruocco C, Casini A, Milani S, Baldi E, Gentilini P (1992c) Fat-storing cells as liver-specific pericytes. Spatial dynamics of agonist-stimulated intracellular calcium transients. J Clin Invest 90:642–646

Pinzani M, Carloni V, Marra F, Riccardi D, Laffi G, Gentilini P (1994) Biosynthesis of platelet-activating factor and its 10-acyl analogue by liver fat-storing cells. Gastroenterology 106:1301–1311

Pinzani M, Gentilini A, Caligiuri A, De-Franco R, Pellegrini G, Milani S, Marra F, Gentilini P (1995) Transforming growth factor-β1 regulates platelet-derived growth factor receptor β subunit in human liver fat-storing cells. Hepatology 21:232–239

Pinzani M, Milani S, De Franco R, Grappone C, Caliguri A, Gentilini P (1996a) Endothelin 1 is overexpressed in human cirrhotic liver and exerts effects on activated hepatic stellate cells. Gastroenterology 110:534–54.

Pinzani M, Milani S, Herbst H, DeFranco R, Grappone C, Gentilini A, Caligiuri A, Pellegrini G, Ngo DV, Romanelli RG, Gentilini P (1996b) Expression of platelet-derived growth factor and its receptors in normal human liver and during active hepatic fibrogenesis. Am J Pathol 148:785–800

Polte T, Abate A, Dennery PA, Schroder H (2000) Heme oxygenase-1 is a cGMP-inducible endothelial protein and mediates the cytoprotective action of nitric oxide. Arterioscler Thromb Vasc Biol 20:1209–1215

Potter JJ, Womack L, Mezey E, Anania FA (1998) Transdifferentiation of rat hepatic stellate cells results in leptin expression. Biochem Biophys Res Commun 244:178–182

Praaning Van Dalen DP, De Leeuw AM, Brouwer A, Knook DL (1987) Rat liver endothelial cells have a greater capacity than Kupffer cells to endocytose N-acetylglucosamine- and mannose-terminated glycoproteins. Hepatology 7:672–679

Puschel GP, Christ B (1994) Inhibition by PGE2 of glucagon-induced increase in phosphoenolpyruvate carboxykinase mRNA and acceleration of mRNA degradation in cultured rat hepatocytes. FEBS Lett 351:353–356

Puschel GP, Kirchner C, Schröder A, Jungermann K (1993) Glycogenolytic and antiglycogenolytic prostaglandin-E_2 actions in rat hepatocytes are mediated via different signalling pathways. Eur J Biochem 218:1083–1089

Puviani AC, Ottolenghi C, Tassinari B, Pazzi P, Morsiani E (1998) An update on high-yield hepatocyte isolation methods and on the potential clinical use of isolated liver cells. Comp Biochem Physiol A Mol Integr Physiol 121:99–109

Qi Z, Atsuchi N, Ooshima A, Takeshita A, Ueno H (1999) Blockade of type β transforming growth factor signaling prevents liver fibrosis and dysfunction in the rat. Proc Natl Acad Sci USA 96:2345–2349

Qu W, Zhong Z, Goto M, Thurman RG (1996) Kupffer cell prostaglandin E2 stimulates parenchymal cell O_2 consumption: alcohol and cell-cell communication. Am J Physiol 270:G574–580

Qu W, Graves LM, Thurman RG (1999) PGE_2 stimulates O_2 uptake in hepatic parenchymal cells: involvement of the cAMP-dependent protein kinase. Am J Physiol 277:G1048–1054

Quistorff B (1985) Gluconeogenesis in periportal and perivenous hepatocytes of rat liver, isolated by a new high-yield digitonin/collagenase perfusion technigue. Biochem J 22:221–226

Racine-Samson L, Rockey DC, Bissell DM (1997) The role of $\alpha 1\beta 1$ in wound contraction. A quantitative analysis of liver myofibroblasts in vivo and in primary culture. J Biol Chem 272:30911–30917

Raddatz D, Henneken M, Armbrust T, Ramadori G (1996) Subcellular distribution of glucocorticoid receptor in cultured rat and human liver-derived cells and cell lines: influence of dexamethasone. Hepatology 24:928–933

Raghow R (1994) The role of extracellular matrix in postinflammatory wound healing and fibrosis. FASEB J 8:823–831

Ramadori G, Dienes HP, Burger R, Meuer S, Rieder H, Meyer zum Buschenfelde KH (1986) Expression of Ia-antigens on guinea pig Kupffer cells: studies with monoclonal antibodies. J Hepatol 2:208–217

Ramadori G, Rieder H, Theiss F, Buschenfelde KHM (1989) Fat storing (lto) cells of rat liver synthesize and secrete apolipoproteins: comparison with hepatocytes. Gastroenterlogy 97:163–172

Ramadori G, Veit T, Schwogler S, Dienes HP, Knittel T, Rieder H Meyer zum Buschenfelde K, (1990) Expression of the gene of the α-smooth muscle-actin isoform in rat liver and in rat fat-storing (Ito) cells. Virchows Arch B Cell Pathol 59:349–357

Ramadori G, Knittel T. Schwogler S, Bieber F, Meyer zum Buschenfelde K (1991a) Dexamethasone modulates α_2-macroglobulin and apolipoprotein E gene expression in cultured rat liver fat-storing cells. Hepatology 14:875–882

Ramadori G, Schwogler S, Veit T, Rieder H, Chiquet-Ehrismann R, Mackie E, Meyer Zum Buschenfelde K (1991b) Tenascin gene expression in rat liver and in rat liver cells. Virchow's Arch B Cell Pathol 60:145–153

Ramadori G, Knittel T, Odenthal M, Schwogler S, Neubauer K, Meyer Zum Buschenfelde K (1992a) Synthesis of cellular fibronectin by rat liver fat-storing (Ito) cells: regulation by cytokines. Gastroenterology 103:1313–1321

Ramadori G, Neubauer K, Odenthal M, Nakamura T. Knittel T, Schwogler S, Meyer Zum Buschenfelde K (1992b) The gene of hepatocyte growth factor is expressed in fat-storing cells of rat liver and is downregulated during cell growth and by transforming growth factor-β. Biochem Biophys Res Commun 183:739–742

Ramm GA, Britton RS, O'Neill R, Bacon BR (1994) Identification and characterization of a receptor for tissue ferritin on activated rat lipocytes. J Clin Invest 94:9–15

Ramm GA, Britton RS, O'NeillR, Blaner WS, Bacon BR (1995) Vitamin A-poor lipocytes:a novel desmin-negative lipocyte subpopulation, which can be activated to myofibroblasts. Am J Physiol 269:G532–541

Rappaport AM (1976) The microcirculatory acinar concept of normal and pathological hepatic structure. Beitr Path 157:215–243

Rappaport AM, Borowy ZJ, Lougheed WM, Lotto WN (1954) Subdivision of hexagonal liver lobules into a structural and functional unit. Anat Rec 119:11–33

Ratziu V, Lalazar A, Wong L, Dang Q, Collins C, Shaulian E, Jensen S, Friedman SL (1998) Zf9, a Kruppel-like transcription factor up-regulated in vivo during early hepatic fibrosis. Proc Natl Acad Sci USA 95:9500-9505

Reeves HL, Thompson MG, Dack CL, Burt AD, Day CP (2000) The role of phosphatidic acid in platelet-derived growth factor-induced proliferation of rat hepatic stellate cells. Hepatology 31:95–100

Reinehr RM, Kubitz R, Peters Regehr T, Bode JG, Haussinger D (1998) Activation of rat hepatic stellate cells in culture is associated with increased sensitivity to endothelin 1. Hepatology 28:1566–1577

Rescan PY, Loreal O, Hassell JR, Yamada Y, Guillouzo A, Clement B (1993) Distribution and origin of the basement membrane component perlecan in rat liver and primary hepatocyte culture. Am J Pathol 142:199–208
Reynaert H, Vaeyens F, Qin H, Hellemans K, Chatterjee N, Urbain D, Geerts A (2000) Functionally active somatostain receptor subtypes 1, 2 and 3 on activated rat hepatic stellate cells. 10th International Symposium on Cells of the Hepatic Sinusoid, Southampton (abstract)
Rieder H, Ramadori G, Dienes HP, Meyer zum Büschenfelde KH (1987) Sinusoidal endothelial cells from guinea pig liver synthesize and secrete cellular fibronectin in vitro.Hepatology 7:856–864
Rieder H, Ramadori G, Hermann E, Meyer zum Büshenfelde KH (1990) Liver sinusoidal endothelial cells induce acute phase protein synthesis ín cultured guinea pig hepatocytes by release of interleukin-6. J Hepatol 11:109
Rieder H, Ramadori G, Meyer zum Buschenfelde KH (1991) Sinusoidal endothelial liver cells in vitro release endothelin – augmentation by transforming growth factor β and Kupfer cell-conditioned media. Klin Wchschr 69:387–391
Rieder H, Armbrust T, Meyer zum Buschenfelde KH, Ramadori G (1993) Contribution of sinusoidal endothelial liver cells to liver fibrosis: expression of transforming growth factor-β 1 receptors and modulation of plasmin generating enzymes by transforming growth factor-β1. Hepatology 18:937–944
Rippe RA, Almounajed G, Brenner DA (1995) SP1 binding activity increases in activated Ito cells. Hepatology 22:241–251
Rizzardini M, Zappone M, Villa P, Gnocchi P, Sironi M, Diomede L, Meazza C, Monshouwer M, Cantoni L (1998) Kupffer cell depletion partially prevents hepatic heme oxygenase 1. Hepatology 27:703–710
Rockey DC, Chung JJ (1995) Inducible nitric oxide synthase in rat hepatic lipocytes and the effect of nitric oxide on lipocyte contractility. J Clin Invest 95:1199–206
Rockey DC, Chung JJ (1996) Regulation of inducible nitric oxide synthase in hepatic sinusoidal endothelial cells. Am J Physiol 271:G260–267
Rockey DC, Chung JJ (1998) Reduced nitric oxide production by endothelial cells in cirrhotic rat liver: endothelial dysfunction in portal hypertension. Gastroenterology 114:344–351
Rockey D, Maher J, Jarnagin W, Gabbiani G, Friedman S (1992) Inhibition of rat hepatic lipocyte activation in culture by interferon-γ. Hepatology 16:776–784
Rockey DC, Chung JJ, McKee CM, Noble PW (1998) Stimulation of inducible nitric oxide synthase in rat liver by hyaluronan fragments. Hepatology 27:86–92
Rojkind M, Greenwel P (1994) The extracellular matrix and the liver. In: Arias IM, Boyer JL, Fausto N, Jakoby WB, Schachter DA, Shafritz DA(eds) The Liver: Biology and Pathology. Raven Press, New York, pp 843–868
Rokita H, Kosz Vnenchak M, Stalinska K, Stachura J (1997) Interleukin 1 receptor antagonist localization exclusively in non-parenchymal cells of the mouse liver. Cytokine 9:249–253
Rosenbaum J, Blazejewski S (1995) Regulation of Ito cell proliferation by soluble factors. J Hepatol 22 (2 Suppl):65–70
Rosenbaum J, Mavier P, Preaux AM, Dhumeaux D (1989) Demonstration of a basic fibroblast growth factor-like molecule in mouse hepatic endothelial cells. Biochem Biophys Res Commun 164:1099–1104
Roskams T, Moshage H, De-Vos R, Guido D, Yap P, Desmet V (1995) Heparan sulfate proteoglycan expression in normal human liver. Hepatology 21:950–958
Roth S, Schurek J, Gressner AM (1997) Expression and release of the latent transforming growth factor β binding protein by hepatocytes from rat liver. Hepatology 25:1398–1405
Roth S, Gong W, Gressner AM (1998a) Expression of different isoforms of TGF-β and the latent TGF-β binding protein (LTBP) by rat Kupffer cells. J Hepatol 29:915–922
Roth S, Michel K, Gressner AM (1998b) (Latent) transforming growth factor β in liver parenchymal cells, its injury-dependent release, and paracrine effects on rat hepatic stellate cells. Hepatology 27:1003–1012
Roth-Eichhorn S, Kuhl K, Gressner AM (1998) Subcellular localization of (latent) transforming growth β and the latent TGF-β binding protein in rat hepatocytes and hepatic stellate cells. Hepatology 28:1588–1596

Rubinstein D, Roska AK, Lipsky PE (1986) Liver sinusoidal lining cells express class II major histocompatibility antigens but are poor stimulators of fresh allogeneic T lymphocytes. J Immunol 137:1803–1810

Ruiter DJ, van der Meulen J, Brouwer A, Hummel MJR, Mauw BJ, van der Ploeg JCM, E Wisse (1981) Uptake by liver cells of endotoxin following its intravenous injection. Lab Invest 45:38–45

Runge D, Runge DM, Jager D, Lubecki KA, Beer-Stolz D, Karathanasis S, Kietzmann T, Strom SC, Jungermann K, Fleig WE, Michalopoulos GK (2000) Serum-free, long-term cultures of human hepatocytes: maintenance of cell morphology, transcription factors, and liver-specific functions. Biochem Biophys Res Commun 269:46–53

Rushfeldt C, Sveinbjornsson B, Seljelid R, Smedsrod B (1999) Early events of hepatic metastasis formation in mice: role of Kupffer and NK-cells in natural and interferon-γ-stimulated defense. J Surg Res 82:209–215

Saad B, Frei K, Scholl FA, Fontana A, Maier P (1995) Hepatocyte-derived interleukin-6 and tumor-necrosis factor mediate the lypoploysaccharide-induced acute-phase response and nitric oxide release by cultured rat hepatocytes. Eur J Biochem 229:349–55

Saez JC (1997) Intercellular gap junctional communication is required for an optimal metabolic response of the functional units of the liver. Hepatology 25:775–776

Saile JG, Ramadori G, Braulke T, Hartmann H (1995) Cellular localization and hormonal regulation of biosynthesis of insulin-like growth factor binding proteins and of the acid-labile subunit within rat liver. Prog Growth Factor Res 6:175–180

Saile B, Knittel T, Matthes N, Schott P, Ramadori G (1997) CD95/CD95L-mediated apoptosis of the hepatic stellate cell. A mechanism terminating uncontrolled hepatic stellate cell proliferation during hepatic tissue repair. Am J Pathol 151:1265–1272

Saile B, Matthes N, Knittel T, Ramadori G (1999) Transforming growth factor β and tumor necrosis factor α inhibit both apoptosis and proliferation of activated rat hepatic stellate cells. Hepatology 30:196–202

Sakaida I, Nagatomi A, Hironaka K, Uchida K, Okita K (1999) Quantitative analysis of liver fibrosis and stellate cell changes in patients with chronic hepatitis C after interferon therapy. Am J Gastroenterol 94:489–496

Sakamoto M, Ueno T, Kin M, Ohira H, Torimura T, Inuzuka S, Sata M, Tanikawa K (1993) Ito cell contraction in response to endothelin-1 and substance P. Hepatology 18:978–983

Sakamoto M, Ueno T, Sugawara H, Torimura T, Tsuji R, Sujaku K, Sata M, Tanikawa K (1997) Relaxing effect of interleukin-1 on rat cultured Ito cells. Hepatology 25:1412–1417

Sanan DA, Fan J, Bensadoun A, Taylor JM (1997) Hepatic lipase is abundant on both hepatocyte and endothelial cell surfaces in the liver. J Lipid Res 38:1002-1013

Sanderson N, Factor V, Nagy P, Kopp J, Kondaiah P, Wakefield L, Roberts AB, Sporn MB, Thorgeirsson SS (1995) Hepatic expression of mature transforming growth factor β1 in transgenic mice results in multiple tissue lesions. Proc Natl Acad Sci USA 92:2572–2576

Sano A, Taylor ME, Leaning MS, Summerfield JA (1990) Uptake and processing of glycoproteins by isolated rat hepatic endothelial and Kupffer cells. J Hepatol 10:211–216

Sarphie TG, D'Souza NB, Deaciuc IV (1996) Kupffer cell inactivation prevents lipopolysaccharide-induced structural changes in the rat liver sinusoid: an electron-microscopic study. Hepatology 23:788–796

Sasse D, Spornitz UM, Maly IP (1992) Liver architecture. Enzyme 46:8–32

Sato K, Yabuki K, Haba T, Maekawa T (1996) Role of Kupffer cells in the induction of tolerance after liver transplantation. J Surg Res 63:433–438

Sato T, Yamamoto H, Sasaki C, Wake K (1998) Maturation of rat dendritic cells during intrahepatic translocation evaluated using monoclonal antibodies and electron microscopy. Cell Tissue Res 294:503–514

Schaffner F, Popper H (1967) Capillarization of hepatic sinusoids in man. Gastroeneterology 44:239–242

Scharf J, Ramadori G, Braulke T, Hartmann H (1996) Synthesis of insulin-like growth factor binding proteins and of the acid-labile subunit in primary cultures of rat hepatocytes, of Kupffer cells, and in cocultures: regulation by insulin, insulinlike growth factor, and growth hormone. Hepatology 23:818–827

Scharf JG, Knittel T, Dombrowski F, Muller L, Saile B, Braulke T, Hartmann H, Ramadori G (1998) Characterization of the IGF axis components in isolated rat hepatic stellate cells. Hepatology 27:1275–1284
Schieferdecker HL, Pestel S, Rothermel E, Puschel GP, Gotze O, Jungermann K (1998) Stimulation by anaphylatoxin C5a of glycogen phosphorylase in rat hepatocytes via prostanoid release from hepatic stellate cells but not sinusoidal endothelial cells. FEBS Lett 434:245–250
Schieferdecker HL, Schlaf G, Koleva M, Gotze O, Jungermann K (2000) Induction of functional anaphylatoxin C5a receptors on hepatocytes by in vivo treatment of rats with IL-6. J Immunol 164:5453–5458
Schlaf G, Schieferdecker HL, Rothermel E, Jungermann K, Gotze O (1999) Differential expression of the C5a receptor on the main cell types of rat liver as demonstrated with a novel monoclonal antibody and by C5a anaphylatoxin-induced Ca2+ release. Lab Invest 79:1287–1297
Schlosser SF, Burgstahler AD, Nathanson MH (1996) Isolated rat hepatocytes can signal to other hepatocytes and bile duct cells by release of nucleotides. Proc Natl Acad Sci USA 93:9948-9953
Schmitt-Graff A, Kruger A, Bochard F, Gabbiani G, Denk H (1991) Modulation of α-smooth muscle actin and desmin expression in perisinusoidal cells of normal and diseased human livers. Am J Pathol 138:1233–1242
Schuftan GG, Bachem MG (1999) α2-macroglobulin reduces paracrine- and autocrine-stimulated matrix synthesis of cultured rat hepatic stellate cells. Eur J Clin Invest 29:519–528
Schuurman B, Heuff G, Beelen RH, Meyer S (1995) Enhanced human Kupffer cell-mediated cytotoxicity after activation of the effector cells and modulation of the target cells by interferon-γ: a mechanistic study at the cellular level. Cell Immunol 165:141–147
Schwoegler S, Odenthal M, Knittel T Meyer zum Büschenfelde KH, Ramadori G (1992) Fat-storing cells of the rat liver synthesize and secrete C1-esterase inhibitor; modulation by cytokines. Hepatology 16:794–802
Schwoegler S, Neubauer K, Knittel T, Chang SF, Ramadori G (1994) Entactin gene expression in normal and fibrotic rat liver and in rat liver cells. Lab Invest 70:525–36
Scoazec JY, Feldmann G (1990) Both macrophages and endothelial cells of the human hepatic sinusoid express the CD4 molecule, a receptor for the human immunodeficiency virus. Hepatology 12:505–510
Scoazec JY, Racine L, Couvelard A, Flejou JF, Feldmann G (1994) Endothelial cell heterogeneity in the normal human liver acinus: in situ immunohistochemical demonstration. Liver 14:113–123
Scott CD, Baxter RC (1986) Production of insulin-like growth factor I and its binding protein in rat hepatocytes cultured from diabetic and insulin-treated diabetic rats. Endocrinology 119:2346–2352
Seglen PO (1976) Preparation of isolated rat liver cells. Methods Cell Biol 13:29–83
Seseke FG, Gardemann A, Jungermann K (1992) Signal propagation via gap junctions, a key step in the regulation of liver metabolism by the sympathetic hepatic nerves. FEBS Lett 301:265–270
Seto Si, Kaido T, Yamaoka S, Yoshikawa A, Arii S, Nakamura T, Niwano M, Imamura M (1998) Hepatocyte growth factor prevents lipopolysaccharide-induced hepatic sinusoidal endothelial cell injury and intrasinusoidal fibrin deposition in rats. J Surg Res 80:194–199
Shah V, Haddad FG, Garcia Cardena G, Frangos JA, Mennone A, Groszmann RJ, Sessa WC (1997) Liver sinusoidal endothelial cells are responsible for nitric oxide modulation of resistance in the hepatic sinusoids. J Clin Invest 100:2923–2930
Shao R, Yan W, Rockey DC (1999) Regulation of endothelin-1 synthesis by endothelin-converting enzyme-1 during wound healing. J Biol Chem 274:3228–3234
Shi J, Kokubo Y, Wake K (1998) Expression of P-selectin on hepatic endothelia and platelets promoting neutrophil removal by liver macrophages. Blood 92:520–528
Shi J, Fujieda H, Kokubo Y, Wake K (1996) Apoptosis of neutrophils and their elimination by Kupffer cells in rat liver. Hepatology 24:1256–1263
Shimada K, Navarro J,. Goeger DE, Mustafa SB, Weigel PH, Weinman SA (1998) Expression and regulation of leukotriene-synthesis enzymes in rat liver cells. Hepatology 28:1275–1281
Shirahama M, Ishibashi H, Tsuchiya Y, Kurokawa S, Okumura Y, Niho Y (1988) Kinetics and parameters of the induction of interleukin 1 secretion by rat Kupffer cells. J Clin Lab Immunol 27:127–132

Shiratori Y, Nakata R, Okano K, Komatsu Y, Shiina S, Kawase T, Sugimoto T, Omata M, Tanaka M (1992) Inhibition of hepatic metastasis of colon carcinoma by asialo GM1-positive cells in the liver. Hepatology 16:469–478
Shiratori Y, Hikiba Y, Mawet E, Niwa Y, Matsumura M, Kato N, Shiina S, Tada M, Komatsu Y, Kawabe T (1994) Modulation of KC/gro protein (interleukin-8 related protein in rodents) release from hepatocytes by biologically active mediators. Biochem Biophys Res Commun 203:1398–1403
Shiratori Y, Ohmura K, Hikiba Y, Matsumura M, Nagura T, Okano K, Kamii K, Omata M (1998) Hepatocyte nitric oxide production is induced by Kupffer cells. Dig Dis Sci 43:1737–1745
Sibille JC, Kondo H, Aisen P (1988) Interactions between isolated hepatocytes and Kupffer cells in iron metabolism: a possible role for ferritin as an iron carrier protein. Hepatology 8:296–301
Simpson KJ, Lukacs NW, Colletti L, Strieter RM, Kunkel SL (1997) Cytokines and the liver. J Hepatol 27:1120–1132
Sjogren K, Liu JL, Blad K, Skrtic S, Vidal O, Wallenius V, LeRoith D, Tornell J, Isaksson OG, Jansson JO, Ohlsson C (1999) Liver-derived insulin-like growth factor I (IGF-I) is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. Proc Natl Acad Sci USA 96:7088-7092
Skouteris GG, Ord MG, Stocken LA (1988) Regulation of the proliferation of primary rat hepatocytes by eicosanoids. J Cell Physiol 135:516–520
Skrtic S, Wallenius V, Ekberg S, Brenzel A, Gressner AM, Jansson JO (1997) Insulin-like growth factors stimulate expression of hepatocyte growth factor but not transforming growth factor-β1 in cultured hepatic stellate cells. Endocrinology 138:4683–4689
Skrtic S, Wallenius V, Ekberg S, Brenzel A, Gressner AM, Jansson JO (1999) Hepatocyte-stimulated expression of hepatocyte growth factor (HGF) in cultured rat hepatic stellate cells. J Hepatol 30:115–124
Skrtic S, Wallenius K, Gressner AM, Jansson JO (2000) Characterization of hepatocyte-derived mitogenic activity on hepatic stellate cells. Liver 20:157–164
Sleyster REC, Knook DL (1982) Relation between localization and function of rat liver Kupffer cells. Lab Invest 47:484–490
Smedsrod B, Pertoft H, Eriksson S, Fraser JRE, Laurent TC (1984) Studies in vitro on the uptake and degradation of sodium hyaluronate in rat liver endothelial cells. Biochem J 223:617–626
Smedsrod B, Pertoft H, Eggertsen G, Sundstrom C (1985) Functional and morphological characterization of cultures of Kupffer cells and liver endothelial cells prepared by means of density separation in Percoll, and selective substrate adherence. Cell Tissue Res 241:639–649
Smedsrod B, Melkko J, Risteli L, Risteli J (1990) Circulating C-terminal propeptide of type I procollagen is cleared mainly via the mannose receptor in liver endothelial cells. Biochem J 271:345–350
Smedsrod B, De Bleser PJ, Braet F, Lovisetti P, Vanderkerken K, Wisse E, Geerts A (1994) Cell biology of liver endothelial and Kupffer cells. Gut 35:1509–1516
Smedsrod B, Melkko J, Araki N, Sano H, Horiuchi S (1997) Advanced glycation end products are eliminated by scavenger-receptor-mediated endocytosis in hepatic sinusoidal Kupffer and endothelial cells. Biochem J 322:567–73
Smith WL, DeWitt DL, Garavito RM (2000) Cyclooxygenases: structural, celellular and molecular aspects. Annu Rev Biochem 69:145–182
Soda R, Tavassoli M, Jacobsen DW (1985) Receptor distribution and the endothelial uptake of transcobalamin II in liver cell suspension. Blood 65:117–123
Soda R, Hardy CL. Kataoka M, Taassoli M (1989) Endothelial mediation is necessary for subsequent hepatocyte uptake of transferrin. Am J Med Sci 297:314–320
Spitzer JA, Mayer AM (1993) Hepatic neutrophil influx: eicosanoid and superoxide formation in endotoxemia. J Surg Res 55:60–67
Spolarics Z (1996) Endotoxin stimulates gene expression of ROS-eliminating pathways in rat hepatic endothelial and Kupffer cells. Am J Physiol 270:G660–666
Spolarics Z (1998) Endotoxemia, pentose cycle, and the oxidant/antioxidant balance in the hepatic sinusoid. J Leukoc Biol 63:34–54
Spolarics Z, Navarro L (1994) Endotoxin stimulates the expression of glucose-6-phosphate dehydrogenase in Kupffer and hepatc endothelial cellls. J Leukoc Biol 56:453–457
Spolarics Z, Wu JX (1997) Tumor necrosis factor-α augments the expression of glucose-6-phosphate dehydrogenase in rat hepatic endothelial and Kupffer cells. Life Sci 60:565–571

Spolarics Z, Bautista AP, Spitzer JJ (1993) Primed pentose cycle activity supports production and elimination of superoxide anion in Kupffer cells from rats treated with endotoxin in vivo. Biochim Biophys Acta 1179:134–140
Sprangers F, Sauerwein HP, Romijn JA, van Woerkom GM, Meijer AJ (1998) Nitric oxide inhibits glycogen synthesis in isolated rat hepatocytes. Biochem J 330:1045–1049
Spray DC, Saez JC, Hertzberg EL, Dermietzel R (1994) Gap junctions in liver. In: Arias IM, Boyer JL, Fausto N, Jakoby WB, Schachter DA, Shafritz DA(eds) The Liver: Biology and Pathology. Raven Press, New York, pp 951–967
Sprenger H, Kaufmann A, Garn H, Lahme B, Gemsa D, Gressner AM (1999) Differential expression of monocyte chemotactic protein-1 (MCP-1) in transforming rat hepatic stellate cells.J Hepatol 30:88–94
Sprenger H, Kaufmann A, Garn H, Lahme B, Gemsa D, Gressner AM (1997) Induction of neutrophil-attracting chemokines in transforming rat hepatic stellate cells. Gastroenterology 113:277–285
Sprenger H, Kaufmann A, Garn H, Lahme B, Gemsa D, Gressner AM (1999) Differential expression of monocyte chemotactic protein-1 (MCP-1) in transforming rat hepatic stellate cells. J Hepatol 30:88–94
Squiers EC, Brunson ME, Salomon DR (1993) Kupffer cells can present alloantigen in vitro: an effect abrogated by gadolinium. J Surg Res 55:571–574
Stachura J, Tarnawski A, Szczudrawa J, Bogda³ J, Mach T, Klimczyk B, Kirchmayer S (1979) Cytoprotective effect of 16, 16 dimethyl prostaglandin E2 and some drugs on an acute galactosamine induced liver damage in rat. Folia Histochem Cytochem 18:311–318
Stadler J, Bentz BG, Harbrecht BG, Di-Silvio M, Curran RD, Billiar TR, Hoffman RA, Simmons RL (1992) Tumor necrosis factor-α inhibits hepatocyte mitochondrial respiration. Ann Surg 216:539–546
Stadler J, Harbrecht BG, Di Silvio M, Curran RD, Jordan ML, Simmons RL, Billiar TR (1993) Endogenous nitric oxide inhibits the synthesis of cyclooxygenase products and interleukin-6 by rat Kupffer cells. J Leukocyte Biol 53:165-172
Stadler J, Trockfeld J, Schmalix WA, Brill T, Siewert JR, Greim H, Doehmer J (1994) Inhibition of cytochromes P4501A by nitric oxide. Proc Natl Acad Sci USA 91:3559-3563
Stadler J, Barton D, Beil-Moeller H, Diekmann S, Hierholzer C, Erhard W, Heidecke CD (1995) Hepatocyte nitric oxide biosynthesis inhibits glucose output and competes with urea synthesis for L-arginine. Am J Physiol 268:G183–188
Stang E, Krause J, Seydel W, Berg T, Roos N (1992) Endocytosis and intracellular processing of tissue-type plasminogen activator by rat liver cells in vivo. Biochem J 282:84l-851
Stefanovic B, Hellerbrand C, Holcik M, Briendl M, Aliebhaber S, Brenner DA (1997) Posttranscriptional regulation of collagen α1(I) mRNA in hepatic stellate cells. Mol Cell Biol 17:5201–5209
Stephenson K, Gupta A, Mustafa SB, Halff GA (1997) Endothelin-stimulated nitric oxide production in the isolated Kupffer cell. J Surg Res 73:149–154
Strain AJ, Crosby HA (2000) Hepatic stem cells. Gut 46:743–745
Strain AJ, Frazer A, Hill DJ, Milner RD (1987) Transforming growth factor β nhibits DNA synthesis in hepatocytes isolated from normal and regenerating rat liver. Biochem Biophys Res Commun 145:436–442
Stumpel F, Ott T, Willecke K, Jungermann K (1998) Connexin 32 gap junctions enhance stimulation of glucose output by glucagon and noradrenaline in mouse liver. Hepatology 28:1616–1620
Su GL, Dorko K, Strom SC, Nussler AK, Wang SC (1999) CD14 expression and production by human hepatocytes. J Hepatol 31:435–442
Su GL, Klein RD, Aminlari A, Zhang HY, Steinstraesser L, Alarcon WH, Remick DG, Wang SC (2000) Kupffer cell activation by lipopolysaccharide in rats: role for lipopolysaccharide binding protein and toll-like receptor 4. Hepatology 31:932–936
Suematsu M, Oda M, Suzuki H, Kaneko H, Watanabe N, Furusho T, Masushige S, Tsuchiya M (1993) Intravital and electron microscopic observation of Ito cells in rat hepatic microcirculation. Microvasc Res 46:28–42
Suematsu M, Goda N, Sano T, Kashiwagi S, Egawa T, Shinoda Y, Ishimura Y (1995) Carbon monoxide: an endogenous modulator of sinusoidal tone in the perfused rat liver. J Clin Invest 96:2431–2437
Suematsu M, Ishimura Y (2000) The heme oxygenase-carbon monoxide system: a regulator of hepatobiliary function. Hepatology 31:3–6

Sugimoto Y, Narumiya S, Ichikawa A (2000) Distribution and function of prostanoid receptors: studies from knockout mice. Progr Lipid Res 39:289–314
Suzuki-Yamamoto T, Yokoi H, Tsuruo Y, Watanabe K, Ishimura K (1999) Identification of prostaglandin F-producing cells in the liver. Histochem Cell Biol 112:451–456
Svegliati-Baroni G, Ridolfi F, Di-Sario A, Casini A, Marucci L, Gaggiotti G, Orlandoni P, Macarri G, Perego L, Benedetti A, Folli F (1999) Insulin and insulin-like growth factor-1 stimulate proliferation and type I collagen accumulation by human hepatic stellate cells: differential effects on signal transduction pathways. Hepatology 29:1743–1751
Sztark F, Dubroca J, Latry P, Quinton A, Balabaud C, Bioulac Sage P (1986) Perisinusoidal cells in patients with normal liver histology. J Hepatol 2:358–369
Takahashi M, Ogasawara K, Takeda K, Hashimoto W, Sakihara H, Kumagai K, Anzai R, Satoh M, Seki S (1996) LPS induces NK1.l+ αβ T cells, with potent cytotoxicity in the liver of mice via induction of IL-12 from Kupffer cells. J Immunol 156:2436–2442
Takeda K, Seki S, Ogasawara K, Anzai R, Hashimoto W, Sugiura K, Takahashi M, Satoh M; Kumagai K (1996) Liver NK1.1+ CD4+ αβ T cells activated by IL-I2 as a major effector in inhibition of experimental tumor metastasis. J Immuno1 156:3366–3373
Takemura S, Kawada N, Hirohashi K, Kinoshita H, Inoue M (1994) Nucleotide receptors in hepatic stellate cells of the rat. FEBS Lett 354:54–56
Takezawa R, Watanabe Y, Akaike T (1995) Direct evidence of macrophage differentiation from bone marrow cells in the liver: a possible origin of Kupffer cells. J Biochem 118:1175–1183
Tao J, Mallat A, Gallois C, Belmadani S, Mery PF, Nhieu JT, Pavoine C, Lotersztajn S (1999) Biological effects of C-type natriuretic peptide in human myofibroblastic hepatic stellate cells. J Biol Chem 274:23761–23769
Tavassoli M, Kishimoto T, Soda R, Kataoka M, Harjes K (1986) Liver endothelium mediates the uptake of iron-transferrin complex by hepatocytes. Exp Cell Res 165:369–379
Terabe M, Shimizu M, Mabuchi A, Matui S, Morikawa H, Kaneda K, Kakiuchi T, Yokomuro K (2000) Unresponsiveness of intrahepatic lymphocytes to bacterial superantigen: rapid development of suppressive Mac-1(high) cells in the mouse liver. Hepatology 32:507–513
Terpstra V, van Berkel TJ (2000) Scavenger receptors on liver Kupffer cells mediate the in vivo uptake of oxidatively damaged red blood cells in mice. Blood 95:2157–2163
Terpstra V, van Amersfoort ES, van Velzen AG, Kuiper J, van Berkel TJ (2000) Hepatic and extrahepatic scavenger receptors: function in relation to disease. Arterioscler Thromb Vasc Biol 20:1860–1872
Teutsch HF (1981) Chemomorphology of liver parenchyma. Qualitative distribution patterns and quantitative sinusoidal profiles of G6Pase, G6PDH and malic enzyme, and of glycogen content. Prog Histochem Cytochem 14/3:1–92
Teutsch HF (1988) Regionality of glucose-6-phosphate hydrolysis in the liver lobule of the rat: metabolic heterogeneity of "portal" and "septal" sinusoids. Hepatology 8:311–317
Teutsch HF, Schuerfeld D, Groezinger E (1999) Three-dimensional reconstruction of parenchymal units in the liver of the rat. Hepatology 29: 494–505
Theise ND, Saxena R, Portmann BC, Thung SN, Yee H, Chiriboga L, Kumar A, Crawford JM (1999) The canals of Hering and hepatic stem cells in humans. Hepatology 30:1425–1433
Theise ND, Badve S, Saxena R, Henegariu O, Sell S, Crawford JM, Krause DS (2000) Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. Hepatology 31:235–240
Theret N, Musso O, L'Helgoualc'h A, Clement B (1997) Activation of matrix metalloproteinase-2 from hepatic stellate cells requires interactions with hepatocytes. Am J Pathol 150:51–58
Thimgan MS, Yee HF Jr (1999) Quantitation of rat hepatic stellate cell contraction:stellate cells' contribution to sinusoidal resistance. Am J Physiol 277:G137–143
Thomas P, Petrick AT, Toth CA, Fox ES, Elting JJ, Steele G, Jr (1992) A peptide sequence on carcinoembryonic antigen binds to a 80kD protein on Kupffer cells. Biochem Biophys Res Commun l88:671–677
Thomas C, Nijenhuis AM, Donge B, Daemen T, Scherphof GL (1995) Tumoricidal response of liver macrophages isolated from rat bearing liver metastases of colon adenocarcinoma. J Leukocyte Biol 57:617–623

Thompson KC, Trowern A, Fowell A, Marathe M, Haycock C, Arthur MJ, Sheron N (1998) Primary rat and mouse hepatic stellate cells express the macrophage inhibitor cytokine interleukin-10 during the course of activation in vitro. Hepatology 28:1518–1524
Thornton AJ, Strieter RM, Lindley I, Baggiolini M, Kunkel SL (1990) Cytokine-induced gene expression of a neutrophil chemotactic factor/IL-8 in human hepatocytes. J Immunol 144:2609–2613
Thornton AJ, Ham J, Kunkel SL (1991) Kupffer cell-derived cytokines induce the synthesis of a leukocyte chemotactic peptide, interleukin-8, in human hepatoma and priman hepatocyte cultures. Hepatology 14:1112–1122
Tiegs G, Wendel A (1988) Leukotriene-mediated liver injury. Biochem Pharmacol 37:2569–2573
Tiegs G, Wolter M, Wendel A (1989) Tumor necrosis factor is a terminal mediator in galactosamine/endotoxin-induced hepatitis in mice. Biochem Pharmacol 38:627–631
Tiggelman AMBC, Boers W, Linthorst C, Brand HS, Sala M, Chamuleau RAFM (1995) lnterleukin-6 production by human liver (myo)fibroblasts in culture: evidence for a regulatory role of LPS, IL-1β and TNFα. J Hepatol 23:295–306
Tiniakos DG, Lee JA, Burt AD (1996) Innervation of the liver: morphology and function. Liver 16:151–160
Tirmenstein MA, Nicholls-Grzemski FA, Schmittgen TD, Zakrajsek BA, Fariss MW (2000) Characterization of nitric oxide production following isolation of rat hepatocytes. Toxicol Sci 53:56–62
Titos E, Claria J, Bataller R, Bosch-Marce M, Gines P, Jimenez W, Arroyo V, Rivera F, Rodes J (2000) Hepatocyte-derived cysteinyl leukotrienes modulate vascular tone in experimental cirrhosis. Gastroenterology 119:794–805
Tomita M, Yamamoto K, Kobashi H, Ohmoto M, Tsuji T (1994) Immunohistochemical phenotyping of liver macrophages in normal and diseased human liver. Hepatology 20:317–325
Tordjmann T, Berthon B, Claret M, Combettes L (1997) Coordinated intercellular calcium waves induced by noradrenaline in rat hepatocytes: dual control by gap junction permeability and agonist. EMBO J 16:5398–5407
Tran-Thi TA, Decker K, Baeuerle P (1995) Differential activation of transcription factors NF-κB and AP-1 in rat liver macrophages. Hepatology 22:613–619
Trauner M, Mennone A, Gigliozzi A, Fraioli F, Boyer J (1998) Nitric oxide and guanosine 3, 5-cyclic monophosphate stimulate bile secretion in isolated rat hepatocyte couplets, but not in isolated bile duct units. Hepatology 28:1621–1628
Trim N, Morgan S, Evans M, Issa R, Fine D, Afford S, Wilkins B, Iredale J (2000) Hepatic stellate cells express the low affinity nerve growth factor receptor p75 and undergo apoptosis in response to nerve growth factor stimulation. Am J Pathol 156:1235–1243
Trop S, Samsonov D, Gotsman I, Alper R, Diment J, Ilani Y (1999) Liver-associated lymphocytes expressing NK.1.1 are essential for induction in a murine model of oral immune tolerance. Hepatology 29:746–755
Tsutsui H, Nakanishi K, Matsui K, Higashino K, Okamura H, Miyazawa Y, Kaneda K (1996) IFNγ -inducing factor up-regulates Fas ligand-mediated cytotoxic activity of murine natural killer cell clones. J Immunol 157:3967–3973
Tsutsui H, Matsui K, Kawada N, Hyodo Y, Hayashi N, Okamura H, Higashino K, Nakanishi K (1997) IL-18 accounts for both TNF-α - and Fas ligand-mediated hepatotoxic pathways in endotoxin-induced liver injury in mice. J Immunol 159:3961–3967
Tsutsumi M, Takada A, Takase S (1987) Characterization of desmin-positive rat liver sinusoidal cells. Hepatology 7:277–284
Tzung SP, Gaines KC. Lance P, Ehrke MJ, Cohen S.A. (1990) Suppression of hepatic lymphokine-activated killer cell induction by murine Kupffer cells and hepatocytes. Hepatology 12:644–652
Ueno T, Tanikawa K (1997) Intralobular innervation and lipocyte contractility in the liver. Nutrition 13:141–148
Ueno H, Sakamoto T, Nakamura T, Qi Z, Astuchi N, Takeshita A, Shimizu K, Ohashi H (2000) A soluble transforming growth factor β receptor expressed in muscle prevents liver fibrogenesis and dysfunction in rats. Hum Gene Ther 11:33–42
Ulevitch, RJ, Tobias PS (1995) Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. Annu Rev Immunol 13:437–457
Umansky V, Rocha M, Schirrmacher V (1996) Liver endothelial cells: participation in host response to lymphoma metastasis. Cancer Metastasis Rev 15:273–279

Van Bossuyt H, DeZanger RB, Wisse E (1988) Cellular and subcellular distribution of injected lippolisaccharide in rat liver and its inactivation by bile salts. J Hepatol 7:325–337
Vanderkerken K, Bouwens L, Wisse E (1990) Characterization of a phenotypically and functionally distinct subset of large granular lymphocytes (pit cells) in rat liver sinusoids. Hepatology 12:70–75
Vanderkerken K, Bouwens L, Neve WD, Berg KVD, Baekeland M, Delens N, Wisse E (1993) Origin and differentiation of hepatic natural killer cells (pit cells). Hepatology 18:919–925
Vanderkerken K, Bouwens L, Van Roijen N, Van den Berg K, Baekeland M, Wisse E (1995) The role of Kupffer cells in the development of hepatic NK (pit) cells. Hepatology 22:283–290
Van Dijk MC, Boers W, Linthorst C, Van Berkel TJ (1992) Role of the scavenger receptor in the uptake of methylamine-activated α2-macroglobulin by rat liver. Biochem J 287:447–455
Van Egmond M, Van Garderen E, Van Spriel AB, Damen CA, Van Amersfoort ES, Van Zandbergen G, Van Hattum, Kuiper J, Van de Winkel (2000) FcαRI-positive liver Kupffer cells: reappraisal of the function of immunoglobulin A in immunity. Nature Med 6:680–685
Vidal-Vanaclocha F, Fantuzzi G, Mendoza L, Fuentes AM, Anasagasti MJ, Martin J, Carrascal T, Walsh P, Reznikov LL, Kim SH, Novick D, Rubinstein M, Dinarello CA (2000) IL-18 regulates IL-1β-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. Proc Natl Acad Sci USA 97:734–739
Van Oosten M, Van De Bilt TE, Van Berkel TIC, Kuiper J (1998) New scavenger receptor-like receptors for the binding of lipopolysaccharide to liver endothelial and Kupffer cells. Infect Immun 66:5107–5112
Van Velzen AG, da Silva RP, Gordon S, van Berkel TJC (1997) Characterization of a receptor for oxidized low-density lipoproteins on rat Kupffer cells: similarity to macrosialin. Biochem J 322:411–415
Vermijlen D, Luo D, Robaye B, Seynaeve C, Baekeland M, Wisse E (1999) Pit cells (hepatic natural killer cells) of the rat induce apoptosis in colon carcinoma cells by the perforin/granzyme pathway. Hepatology 29:51–56
Vickers AE, Lucier GW (1996) Estrogen receptor levels and occupancy in hepatic sinusoidal endothelial and Kupffer cells are enhanced by initiation with diethylnitrosamine and promotion with 17α-ethinylestradiol in rats. Carcinogenesis 17:1235–1242
Vidal-Vanaclocha F, Fantuzzi G, Mendoza L, Fuentes AM, Anasagasti MJ, Martin J, Carrascal T, Walsh P, Reznikov LL, Kim SH, Novick D, Rubinstein M, Dinarello CA (2000) IL-18 regulates IL-1β-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. Proc Natl Acad Sci USA 97:734–739
Villafuerte BC, Koop BL, Pao C-I, Gu L, Birdsong GG, Phillips LS (1994) Coculture of primary rat hepatocytes and non-parenchymal cells permits expression of insulin-like growth factor binding protein-3 in vitro. Endocrinology 134:2044–2050
Voci A, Arvigo M, Massajoli M, Garrone S, Bottazzi C, Demori I, Gallo G (1999) IGF-I production by adult rat hepatocytes is stimulated by transforming growth factor-α and transforming growth factor-β1. Eur J Endocrinol 140:577–582
Vogel S, Gamble MV, Blaner WS (1999) Biosynthesis, absorption, metabolism and transport of retinoids. In: Nau H, Blaner WS (eds) Retinoids: the Biochemical and Molecular Basis of Vitamin A and Retinoid Action. Springer, Berlin, pp 31–95
Volpes R, van-den Oord JJ, De-Vos R, Depla E, De-Ley M, Desmet VJ (1991) Expression of interferon-γ receptor in normal and pathological human liver tissue. J Hepatol 12:195–202
vom Dahl S, Wettstein M, Gerok W, Haussinger D (1990) Stimulation of release of prostaglandin D2 and thromboxane B2 from perfused rat liver by extracellular adenosine.Biochem J 270:39–44
Vyas SK, Leyland H, Gentry J, Arthur MJP (1995) Rat hepatic lipocytes synthesize and secrete transin (stromelysin) in early primary culture. Gastroenterology 109:889–898
Wake K (1971) "Sternzellen" in the liver: perisinusoidal cells with special reference to storage of vitamin A. Am J Anat 132:429–446
Wake K (1980) Perisinusoidal stellate cells (fat-storing cells, interstitial cells, lipocytes), their related structure in and around the liver sinusoids, and vitamin A-storing cells in extrahepatic organs. Int Rev Cytol 66:303–353
Wake K (1988) Liver perivascular cells revealed by gold and silver impregnation methods and electron microscopy. In: Motta P (ed) Biopathology of the Liver. An ultrastructural Approach. Kluwer Academic Publisher, Dordrecht, pp. 23–26

Wake K, Decker K, Kirn A, Knook DL, McCuskey RS, Bouwens L, Wisse E (1989) Cell biology and kinetics of Kupffer cells in the liver. Int Rev Cytol 118:173–229

Wake K, Sato T (1993) Intralobular heterogeneity of perisinusoidal stellate cells in the porcine liver. Cell Tissue Res 273:227–237

Wan YH, Freeswick PD, Khemlani LS, Kispert PH, Wang SC, Su GL, Billiar TR (1995) Role of lipopolysaccharide (LPS), IL-1, IL-6, TNF, and dexamethasone in regulation of LPS-binding protein expression in normal hepatocytes and hepatocytes from LPS-treated rats. Infect Immun 63:2435–2442

Wang JH, Redmond HP, Watson RW, Bouehier-Hayes D (1995a) Role of lipopolysaccharide and tumor necrosis factor-α in induction of hepatocyte necrosis. Am J Physiol 269:G297–304

Wang K, Deutschman CS, Clemens MG, De Maio A (1995b) Reciprocal expression of phosphoenolpyruvate carboxykinase and acute phase genes during acute inflammation. Shock 3:204–209

Wang H, Gao X, Fukumoto S, Tademoto S, Sato K, Hirai K (1998a) Post-isolation inducible nitric oxide synthase gene expression due to collagenase buffer perfusion and characterization of the gene regulation in primary cultured murine hepatocytes. J Biochem 124:892–899

Wang SC, Ohata M, Schrum L, Rippe RA, Tsukamoto H (1998b) Expression of interleukin-10 by in vitro and in vivo activated hepatic stellate cells. J Biol Chem 273:302–308

Wang JH, Redmond HP, Wu QD, Bouchier-Hayes D (1998c) Nitric oxide mediates hepatocyte injury. Am J Physiol 275:G1117–1126

Wang YQ, Ikeda K, Ikebe T, Hirakawa K, Sowa M, Nakatani K, Kawada N, Kaneda K (2000) Inhibition of hepatic stellate cell proliferation and activation by the semisynthetic analogue of fumagillin TNP-470 in rats. Hepatology 32:980–989

Watanabe H, Miyaji C, Kawashi Y, Liai T, Ohtsuka K, Iwanage T, Takahashi-Iwanaga H, Abo T (1995) Relationships between intermediate TCR cells and NK1.1+ T cells in various immune organs. NK1.1+ T cells are present within a population of intermediate TCR cells. J Immunol 155:2972–2983

Watanabe J, Kanai K, Kanamura S (1988) Glucagon receptors in endothelial and Kupffer cells of mouse liver. J Histochem Cytochem 36:1081–1089

Weibel ER, Staubli W, Gnagi HR, Hess FA (1969) Correlated morphometric and biochemical studies on the liver cells. I. Morphometric model, stereologic methods and normal morphometric data for rat liver. J Cell Biol 42:68–91

Weiner F, Blaner W, Czaja M. Shah A, Geerts A (1992) Ito cell expression of a nuclear retinoic acid receptor. Hepatology 15:336–342

Weiss SJ (1989) Tissue destruction by neutrophils. N Engl J Med 320:365–376

West MA, Billiar TR, Curran RD, Hyland BJ, Simmons R (1989) Evidence that rat Kupffer cells stimulate and inhibit hepatocyte protein synthesis in vitro by different mechanisms. Gastroenterology 96:1572–1582

Whalen R, Rockey DC, Friedman SL, Boyer TD (1999) Activation of rat hepatic stellate cells leads to loss of glutathione S-transferases and their enzymatic activity against products of oxidative stress. Hepatology 30:927–933

Wheeler MD, Rose ML, Yamashima S, Enomoto N, Seabra V, Madren J, Thurman RG (2000a) Dietary glycine blunts lung inflammatory cell influx following acute endotoxin. Am J Physiol Lung Cell Mol Physiol 279:L390–398

Wheeler MD, Yamashina S, Rusyn I, Thurman RG (2000b) The use of recombinant adenoviral vectors to target and inactivate Kupffer cells. 10th International Symposium on Cells of the Hepatic Sinusoid, Southampton (abstract)

Williams EJ, Gaca MD, Brigstock DR, Arthur MJP, Benyon RC (2000) Increased expression of connective tissue growth factor in fibrotic human liver and in activated hepatic stellate cells. J Hepatol 32:754–61

Winnock M, Garcia Barcina M, Lukomska B, Huet S, Saric J, Balabaud C, Bioulac Sage P (1995) Human liver-associated lymphocytes: an overview. J Gastroenterol Hepatol 10 Suppl 1:S43–46

Winwood PJ, Schuppan D, Iredale JP, Kawser CA, Docherty AJ, Arthur MJ (1995) Kupffer cell-derived 95-kd type IV collagenase/gelatinase B: characterization and expression in cultured cells. Hepatology 22:304–315

Wisse E (1972) An ultrastructural characterization of the endothelial cell in the rat liver sinusoid under normal and various experimental conditions, as a contribution to the distinction between endothelial and Kupffer cells. J Ultrastruct Res 38:528–562
Wisse E (1974) Observations on the fine structure and peroxidase cytochemistry of normal rat Kupffer cells. J Ultrastr Res 46:393–426.
Wisse E, van't Noordede JM, van der Meulen J, Daems WT (1976) The pit cell: description of a new type of cell occurring in rat liver sinusoids and peripheral blood. Cell Tissue Res 173:423–438
Wisse E, De Zanger RB, Charels K, Van der Smissen P, McCuskey RS (1985) The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. Hepatology 5:683–692
Wisse E, Luo D, Vermijlen D, Kanellopoulou C, De Zanger R, Braet F (1997) On the function of pit cells, the liver-specific natural killer cells. Semin Liver Dis 17:265–286
Wisse E, Braet F, Luo D, Vermijlen D, Eddouks M, Konstandoulaki M, Empsen C, de Zanger RB (1999) Endothelial cells of the hepatic sinusoids: a review. In: Tanikawa K, Ueno T (eds) Liver Diseases and Hepatic Sinusoidal Cells. Springer, Tokyo, pp. 17–55
Wong L, Yamasaki G, Johnson RJ, Friedman SL (1994) Induction of platelet-derived growth factor receptor in rat hepatic lipocytes during cellular activation in vivo and in culture. J Clin Invest 94:1563–1569
Wong J, Johnston B, Lee SS, Bullard DC, Smith CW, Beaudet AL, Kubes P (1997) A minimal role for selectins in the recruitment of leukocytes into the inflamed liver microvasculature. J Clin Invest 99: 2782–2790
Xu Y, Rojkind M, Czaja MJ (1996) Regulation of monocyte chemoatractant protein 1 by cytokines and oxygen free radicals in rat hepatic fat-storing cells. Gastroenterology 110:1870–1877
Yamaguchi R, Yano H, Nakashima Y, Ogasawara S, Higaki K, Akiba J, Hicklin DJ, Kojiro M (2000) Expression and localization of vascular endothelial growth factor receptors in human hepatocellular carcinoma and non-HCC tissues. Oncol Rep 7:725–729
Yamane A, Seetharam L, Yamaguchi S, Gotoh N, Takahashi T, Neufeld G, Shibuya M (1994) A new communication system between hepatocytes and sinusoidal endothelial cells in liver through vascular endothelial growth factor and Flt tyrosine kinase receptor family (Flt-1 and KDR/Flk-1). Oncogene 9:2683–2690
Yamaoka Y (1997) The protective effects of prostaglandin E1 on sinusoidal endothelial cells in xenogeneic pig liver perfusion. J Surg Res 73:28–34
Yan J, Vetvicka V, Xia Y, Hanikyrova M, Mayadas TN, Ross-GD (2000) Critical role of Kupffer cell CR3 (CD11b/CD18) in the clearance of IgM-opsonized erythrocytes or soluble β-glucan. Immunopharmacology 46:39–54
Yee HF Jr (1998) Rho directs activation-associated changes in rat hepatic stellate cell morphology via regulation of the actin cytoskeleton. Hepatology 28:843–850
Yokoi Y, Namihisa T, Kuroda H, Komatsu I, Miyazaki A, Watanabe S, Usui K (1984) Immunocytochemical detection of desmin in fat-storing cells (Ito cells). Hepatology 4:709–7I4
Zhang F, zur Hausen A, Hoffmann R, Grewe M, Decker K (1994a) Rat liver macrophages express the 55 kDa tumor necrosis factor receptor: modulation by interferon-γ, lipopolysaccharide and tumor necrosis factor-α. Biol Chem Hoppe Seyler 375:249–254
Zhang JX, Pegoli WJ, Clemens MG (1994b) Endothelin-1 induces direct constriction of hepatic sinusoids. Am J Physiol 266:G624–632
Zhang B, Calmus Y, Wen L, Sogni P, Lotersztajn S, Houssin D, Weill B (1997) Endothelin-1 induces liver vasoconstriction through both ETA and ETB receptors. J Hepatol 26:1104–1110
Zhu J, Wu J, Frizell E, Liu S-L, Bashey R, Rubin R, Nortom P, Zern MA (1999) Rapamycin inhibits hepatic stellate cell proliferation in vitro and limits fibrogenesis in an vivo model of liver fibrosis. Gastroenterology 117:1198–1204
Zimmermann EM, Li L, Hoyt EC, Pucilowska JB, Lichtman S, Lund PK (2000) Cell-specific localization of insulin-like growth factor binding protein mRNAs in rat liver. Am J Physiol Gastrointest Liver Physiol 278:G447–457
Zindy F, Lamas E, Schmidt S, Kirn A, Brechot C (1992) Expresion of insulin-like growth factor II (IGF-II) and IGF-II, IGF-I and insulin receptors mRNAs in isolated non-parenchymal rat liver cells. J Hepatol 14:30–34

Subject Index