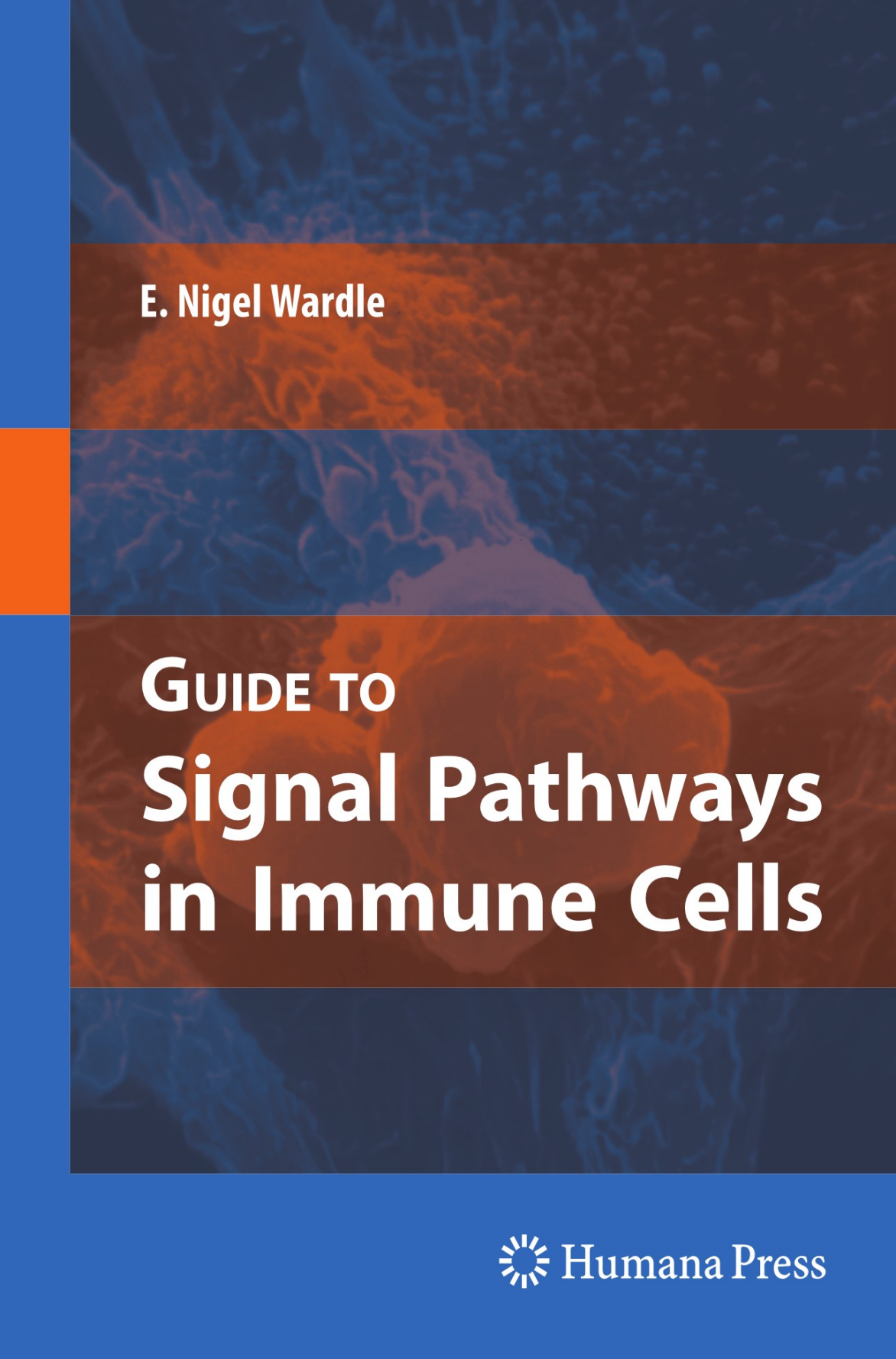


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E. Nigel Wardle

GUIDE TO
Signal Pathways
in Immune Cells

 **Humana Press**

Guide to Signal Pathways in Immune Cells

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Preface

To read current biomedical science, one has to have a working knowledge of how important effector molecules cause transduction of their signal within cells, altering the control of genes. This work aims to provide that basic knowledge for medical readers. Students of immunology or cell biology will note its relevance.

One will learn how platelets, macrophages, neutrophils, T and B lymphocytes and natural killer cells perform their functions and how skin, breast, prostate and colon cancers emerge. The associated diagrams and tables are used to obviate extensive text. Appropriate references to articles and reviews by workers in each field are given so that further consideration can easily be undertaken.

We are all at differing stages of our appreciation of immunology and of pathophysiology. Some persons will have a profound background in biochemistry or molecular biology. Others will have a reminiscence of lectures received years ago. Since this work is principally for clinical doctors, the sections that can be avoided at first reading are marked with an asterisk (*). Always proceed line by line and think of associations that you know. Do you feel comfortable with the statement, “Interleukin 6 stimulates glucose uptake in renal proximal tubular cells, and that action is associated with Stat3, PI3K/Akt, MAPKs and NF- κ B signal pathways”? If not, please read on.

Extracellular signals acting on cells are sensed by two families of cell membrane receptors, the G protein-coupled receptors (GPCRs) and the receptor tyrosine kinases. GPCRs interact with G proteins, which contact second messengers such as inositol triphosphates, Ca^{2+} ions or cyclic nucleotides. They might also activate small G proteins of the Rho or Ras families that lie upstream of the mitogen-activated protein kinase (MAPK) signal cascades. Alternatively, the thirteen families of receptor tyrosine kinases (RTKs) create docking sites for downstream effectors like Ras-MAPKs, PI3 kinase (PI3K) and protein kinase C (PKC). Essentially, signal transduction depends on reversible phosphorylation of proteins. There is phosphorylation by protein kinases and dephosphorylation by phosphatases. So, protein kinases like tyrosine kinases or serine-threonine kinases transfer phosphate to specific amino acids (tyrosine, serine or threonine). Tyrosine kinases are divided into RTKs and nonreceptor tyrosine kinases (NRTKs), which occur as JAKs (Janus kinases), focal adhesion kinase (FAK) or src kinases. The serine-threonine kinases are protein kinase A (PKA), which modulates cell metabolism and ion channel

activity; protein kinase B (Akt), which has a role in glucose metabolism and is an inhibitor of apoptosis; and PKC, which is implicated in cell growth and differentiation. The MAPKs are comprised of extracellular signal-related kinase (ERK), which transduces signals for cellular proliferation; the p38 MAPK; and c-Jun NH₂-terminal kinase (JNK). The MAPKs have downstream targets affecting all aspects of cell function. In addition, there is the nuclear factor kappa B (NF- κ B) pathway, which is crucial for inflammatory and antiapoptotic signalling. In brief, these are the objectives of our cell signalling scrutinies.

E. Nigel Wardle, MD

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Chapter 1

Cellular Activation Processes

Abstract This introduces cell surface receptors and intracellular messengers. Many receptors transmit their initial signal by means of G proteins as G protein-coupled receptors (GPCRs). Obviously, there has to be control at this level, and that is exerted by means of GRKs, G protein-coupled receptor kinases, by regulators of G protein signalling and by means of β -arrestins. However, one now realizes that β -arrestins fulfil important roles in transmitting signals directly into cells.

1.1 Membrane Receptors and Second Messengers

Receptors on cell membranes for hormones, neurotransmitters, chemokines, cytokines, or growth factors are integral membrane proteins that traverse the phospholipid bilayer. When a first messenger like adrenalin occupies a receptor to incite glycogenolysis inside hepatocytes or muscle cells or lipolysis in adipocytes, signal transduction arises by activation of cyclic adenosine monophosphate (cAMP) second messenger, which leads to further intracellular events. As shown in Fig. 1.1, adrenalin or glucagon act on receptors that couple to the adenylyl cyclase (AC) enzyme that creates cAMP, which is a second messenger for the activation of cAMP-dependent protein kinase A (PKA).

Cyclic AMP is the second messenger for glycolysis. Other second messengers are listed in Table 1.1. They activate protein kinases that then phosphorylate serine or threonine residues of enzymes within the cytosol. Such phosphorylation reactions initiate cascades, that amplify the action of the original effector molecules that acted on the cell membrane receptors. AC is a G protein-coupled transmembrane molecule. Lipid rafts are important for cAMP compartmentalization, and there is dimerization/oligomerization of the molecule that helps signalling [1]. The product cAMP acts on PKA, on cAMP gated ion channels, and on guanine nucleotide exchange proteins (exchange proteins activated by cAMP, EPACs), which latter are guanine-nucleotide exchange factors (GEFs) for Rap1/2 small guanine triphosphatases (GTPases), that help cadherin-mediated integrity of epithelia or integrin-mediated cell adhesion. Those A protein kinases and their associated phosphatases are

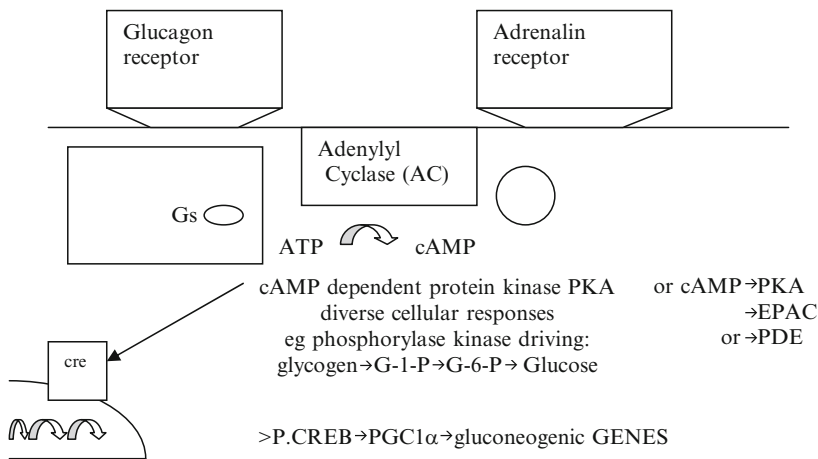


Fig. 1.1 Adrenalin or glucagon causing glycogenolysis. *adrenalin* epinephrine, *cAMP* cyclic adenosine monophosphate *CRE* cyclic AMP response element, *Gs* trimeric G protein that stimulates cAMP release. AC (adenylyl cyclase) is regulated by receptor-linked and non-receptor-linked tyrosine kinases. On the one hand, glucagon may induce glycogenolysis. On the other hand, cAMP that is provoked by an agonist may activate (a) protein kinase A anchored by an A-kinase anchoring protein (*AKAP*), (b) exchange protein activated by cAMP (*EPAC*), or (c) phosphodiesterase (*PDE*)

Table 1.1 Second messengers

Cyclic adenosine monophosphate (cAMP)	Cyclic guanosine monophosphate (cGMP)	
sn 1,2-Diacylglycerol (DAG)	Ceramide	Sphingosine-1-phosphate
Calcium ions	Inositol 3,4,5-triphosphate (IP3)	
Reactive oxygen species (ROS)		
Nitric oxide (NO [•])	Peroxynitrite ONOO ⁻	

compartmentalized by A-kinase anchoring proteins, AKAPs [2]. Any cAMP formed within nuclei will act on cAMP response element-binding protein (CREB; Fig. 4.8). Cyclic guanosine monophosphate (cGMP) is a second messenger analogous to cAMP. There are soluble and membrane forms of guanylyl cyclase that convert guanosine triphosphate (GTP) to cGMP [3].

1.2 Examples of Plasma Membrane Receptors

The type of receptor to which an effector molecule binds might be (a) a G protein-coupled receptor (GPCR) as in Fig. 1.1, (b) a receptor connected to an ion channel, (c) a receptor with tyrosine kinase activity (RTK, receptor tyrosine kinase), (d) a haemopoietin or cytokine receptor. Examples of physiological events triggered by receptor activations appear in Table 1.2.

Table 1.2 Physiological events triggered by receptors

(a) <i>G protein coupled</i>	
Gs activation of adenylyl cyclase	β 2-Adrenergic stimulation by adrenalin, action of glucagons, adrenocorticotrophic hormone (ACTH), vasoactive intestinal peptide (VIP), or human chorionic gonadotropin (HCG)
Gi inhibition of adenylyl cyclase via Go or Gp	α 2-Adrenergic action, muscarinic type 2 cholinergic activation
(i) PtdIns (phosphatidyl-inositol) turnover	
(ii) $\rightarrow$ Ca^{2+} influx	Muscarinic type 2 cholinergic activation
(iii) $\rightarrow$ Guanylyl cyclase	Action of thrombin, immunoglobulin E (IgE), concanavalin A
(b) <i>Ion channel linked</i>	
Na^+ ion channel	Nicotinic acetylcholine receptor
Cl^- ion channel	γ -Aminobutyric acid (GABA) or glycine receptors in brain
(c) <i>Tyrosine kinase linked</i>	
	Growth factors: epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin
(d) <i>Haemopoietin or cytokine receptors</i>	
	Erythropoietin receptor, interleukin 2 (IL-2) receptor

The cell adhesion receptors that we call *integrins* bind the cell to extracellular matrix proteins. Integrins do not have intrinsic enzyme activity. Therefore, they work by association with Src family tyrosine kinases beneath the membrane (Fig. 9.11). Whenever there is sepsis, C5a-dependent expression of surface β 2-integrins on polymorphonuclear leucocytes (PMNs) enables rapid passage of those PMNs through the walls of blood vessels.

The many receptors that mediate their actions via G proteins may concurrently engage with other receptors, like P2Y purinergic receptors and integrins (Fig. 9.11). In Fig. 5.4, we see the receptor for platelet-derived growth factor (PDGF), and it is associated on cell membranes with EDG1, which is the receptor for sphingosine-1-phosphate, a classic GPCR [4].

The remainder of this chapter is only easy reading for those with prior knowledge of pharmacology. However, by the end of the whole work, these aspects are elucidated by other illustrations. Proceed line by line and think.

1.3 G Proteins: Guanine Nucleotide Coupling

A G protein is a GTP-binding protein [4–6]. Each has three subunits $G\alpha$, $G\beta$, and $G\gamma$. The α -unit is 45 kDa, the β -unit is 35 kDa, and γ is only 7 kDa. The G protein subunits are anchored just under cell membranes by their attached lipid molecules, such as palmitate (C16) or myristate (C14). In Fig. 1.1, a signal generated by a hormone binding to its receptor is transmitted via the G protein to a membrane-bound enzyme AC, resulting in generation of a second messenger cAMP. There are at least 15 types of the α -unit. They influence ACs, phospholipases, and ion channels. Thus, there is a $G\alpha_s$ that activates AC, a $G\alpha_i$ that inhibits AC, and a $G\alpha_q$ that activates submembranous phospholipase C. The α -subunit binds guanosine diphosphate (GDP)/GTP

and acts as GTPase for the hydrolysis of: $\text{GTP} + \text{H}_2\text{O} \rightarrow \text{GDP} + \text{Pi}$. Details of G protein functioning are found in biochemical or pharmacological texts [5–7].

In its inactive state each G protein has bound GDP. When its surface receptor is activated, GDP is released in exchange for higher-energy GTP. Then, the intrinsic GTPase activity of the α -unit breaks the G protein trimer into a free α -unit, now bound to GDP, and a β/γ complex that does not dissociate further. So, when ligand encounters the receptor (Fig. 1.1), G protein activation leads to activation of the AC. The free α -subunit diffuses to the AC, to which it binds and causes its activation so that cAMP is released. The energy of the GTP is used to stabilize an activated state of the G protein, and thus $\text{G}\alpha\text{s}$ stimulates the AC by controlling the orientation of its catalytic domains. There is rapid signal termination as the GTP is hydrolyzed to GDP. In other tissues, there can be alternative means of activating the AC [8]. Calcium-calmodulin will activate some isoforms, as can protein kinase C (PKC).

Cholera toxin (CTX) inactivates the α -subunit of G protein. The A1 unit of CTX uses NAD^+ as substrate and catalyses the transfer of adenosine diphosphate (ADP)-ribose to an arginine residue on the α -subunit, stopping its GTPase activity. Modification of the G proteins requires the presence of ARF (ADP ribosylation factor). Modified G proteins render AC permanently activated; thus, when cholera toxin acts on gut epithelium, formation of an excess of cAMP leads to flux of sodium and water into the intestine. Accordingly, there is diarrhoea. Current interest in CTX centres on its ability to inhibit innate immune responses like the production of interferon- γ . When used experimentally, CTX is anti-inflammatory [9]. It can also be used to induce tolerance on mucosae.

A *Gi protein* will inactivate the AC so that cAMP cannot be formed. However a *Gi* protein is inactivated when the pertussis toxin (PTX) transfers ADP-ribose to a cysteine residue. The high intracellular cAMP that is created via PTX ensures ineffectiveness of immune cells. The *Gi* proteins have their use. They function in macrophages to mediate proinflammatory gene expression [10, 11]. Thus leukotriene receptors BLT1 and cysLT1 reduce cAMP and enhance phagocytosis and killing by alveolar macrophages [11], the former by a PTX-sensitive mechanism. LTD4 via $\text{G}\alpha\text{q}$ enhances intracellular calcium [11].

Most extracellular signals, the first messages, such as hormones and growth factors or signals of physical status like thermal injury or oxidative damage, transmit information into the cells by means of these GPCRs. There are 16 genes for $\text{G}\alpha$ units, 5 genes for $\text{G}\beta$ units, and 11 genes for $\text{G}\gamma$ subunits. As indicated in Table 1.2, there is a $\text{G}\alpha\text{s}$ that mediates activation of AC by β -adrenergic stimuli or by glucagon, and there is a $\text{G}\alpha\text{i}$ that mediates the action of acetylcholine in inhibiting AC. A $\text{G}\alpha\text{13}$ mediates thrombin induction of the Na/H^+ ion exchanger of platelets. When (Fig. 9.7) a lymphocyte is moving in response to a chemoattractant, $\text{Gi}\beta\gamma$ subunits at the leading edge coordinate PI3 kinase activation; $\text{G}\alpha\text{13}$ at the rear reduces the sensitivity there to the mediator. $\text{G}\alpha\text{e}$ is geared to the exocytosis of neutrophil azurophilic granules.

Often, growth factor receptors are coupled to G proteins, as when epidermal growth factor (EGF) or fibroblast growth factor (FGF) receptors couple to Gs , insulin receptor and IGF-I/II couple to Gi , and the T cell receptor (CD3 ϵ) couples

to $G\alpha_q/11$ (Chap. 13). In the heart, EGF, via G_s stimulation of AC that increases cellular cAMP levels, enhances myocardial fibre contractility.

GPCRs often activate ion channels by creating macromolecular *signal complexes* at the plasma membrane. β_2 -Adrenergic receptors that have an L-type calcium channel are closely associated with G protein subunits, the enzyme AC and its effector PKA, and the counteracting phosphatase PP2A. cAMP-induced PKA switches the β_2 -adrenergic receptor from G_s to G_i control [12].

Mouse knockout models that lack almost all the known genes for G protein α -subunits have been generated to give insight into G protein signalling pathways. $G\alpha_q$ -deficient platelets fail to aggregate in response to thrombin, ADP, or thromboxane A₂ due to lack of agonist-induced phospholipase C activation (Chap. 3). Mice that lack $G\alpha_q$ thus have increased bleeding times and are protected against thromboembolism. We now have the alternative choice of using small interfering RNA technology.

It has been realized [13] that GPCR complexes that signal from the plasma membrane can yet influence nuclear events; alternatively, parts of a GPCR will sometimes enter nuclei and signal there by direct mechanisms. As an example of the former, when SDF-1 acts on the chemokine receptor CXCR4 (cf. [13]), there is thereby activation of pERK (pERK MAP kinase), which translates from the cytosol into the nuclei, so inducing transcription and cell proliferation. In Sect. 7.5, there is consideration of the action of lysophosphatidic acid (LPA), which, acting via a GPCR, stimulates PKC ζ and RhoA in the cytosol and then these messengers enter the nuclear domain and stimulate the activity of phospholipase D1 [13]. Also, transduced LPA/GPCR can fix onto nuclear membranes so that iNOS (inducible nitric oxide synthase) and cyclooxygenase 2 (Cox-2) can be induced [14]. LPA is known to enhance the proliferation of colon cancer cells.

1.4 Control over GPCRs: G Protein-Coupled Receptor Kinases, Arrestins, Regulators of G Protein Signaling Proteins, and GPCR Interacting Proteins

Since GPCRs are so important for the coupling of messages at the cell membrane to intracellular signals, there have to be precise controls over their activation. First, a GPCR is desensitized when it is phosphorylated by a specific GRK, a *G protein-coupled receptor kinase*. Thus, the α -/ β -adrenergic receptors or chemokine receptors are uncoupled when they are phosphorylated by means of a GRK. GRK action can be illustrated by how the β_1 -adrenergic receptors for heart muscle respond in patients with chronic heart failure [15]. The activated β_1 -adrenergic receptors are desensitized by phosphorylation by means of GRK2 or PKA in response to cAMP production. Yet, paradoxically, the desensitization process worsens heart failure pathophysiology. Accordingly, a GRK antagonist is being developed for use in therapy. Another role for GRK5 is in the desensitization of a GPCR like the β -adrenergic receptor. As reviewed recently [16] elevated GRK2 and GRK5 are relevant to human hypertension.

Second, phosphorylation of a GPCR will often lead to binding of β -arrestin, which promotes G protein-coupled endocytosis of receptors [17–19]. Arrestins carry unwanted receptors into endosomes for subsequent destruction (Fig. 1.2). Yet, the same arrestins also transmit signals to the mitogen-activated protein (MAP) kinase cascades for gene activation [20]. One notable example has been recorded. When nicotine acts on its receptors on endothelial cells or bronchial epithelium, association of β -arrestin with the nAChRs leads to activation of Src kinase and so Raf-1; thus, cell proliferation is promoted [21]. As another example, when PTH acts on its receptors, Src coprecipitates with β -arrestin for there is then scaffolding of c-Src with β -arrestin 2, with the consequence that there can be extracellular signal-related kinases 1 and 2 (ERK1/2) activation [22]. The topic is ripe for discussion [23], albeit at this stage all essential details have not yet been uncovered.

Arrestins were first identified in retinal rods and cones, and then arrestins 2 and 3 were found to regulate 7TM spanning receptors in most tissues [20]. There are class A receptors like the β 2-adrenergic receptors that bind β -arrestin transiently, traffic with it to clathrin-coated pits, and then dissociate. Receptors internalized without arrestin recycle quite rapidly. However, class B receptors, as for angiotensin or vasopressin, bind β -arrestin more tightly and internalize with it. These receptors recycle slowly and contribute to signal transduction [20, 24, 25], as when the chemokine CCL19 acting on its receptor CCR7 activates ERK1/2 [25]. Similarly, attraction of β -arrestin to the receptor for angiotensin II (Fig. 5.10) permits immediate conduction of its signal to the small GTPase RhoA to organize stress fibres [26].

β -Arrestins, by binding MAPKKK (MAP kinase kinase kinase), form scaffolds for other proteins like the MAP kinases. Accordingly, β -AR/scaffold signalling complexes are used (a) for ERK1/ERK2 activation; (b) for Jun N-terminal kinase 3 (JNK3); (c) Akt activation in some cells; (d) activation of cofilin, providing actin filaments for chemotaxis. Importantly, the ability of the GRKs and the arrestins to control GPCR signalling by desensitization means that they can regulate the inflammatory process [27]. Thus β -arrestin 2 controls chemokine interleukin 8 (IL-8) signalling into neutrophils [28], and those IL-8 receptors aid granule release

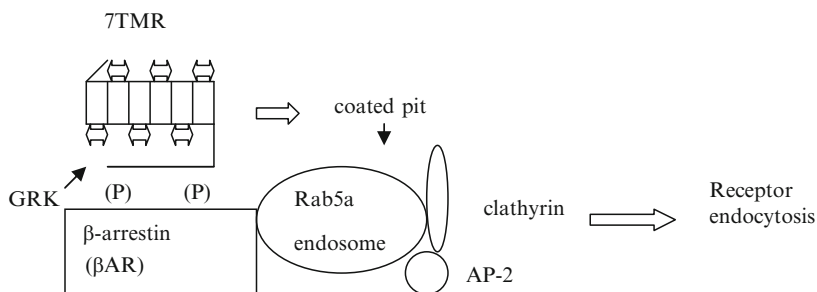


Fig. 1.2 β -Arrestin-mediated clathrin/AP-2 receptor endocytosis

by chemokine CXCR1 [29]. Likewise β -AR1 and β -AR2, by controlling the migration of leukocytes, regulate the development of allergic asthma [30].

Clearly, our knowledge of the G proteins and their regulators impinges on all aspects of pathophysiology [31]. There has been recent emphasis on the fact that β -arrestins 1 and 2 not only desensitize membrane receptors (Fig. 1.2) but also facilitate other forms of signalling, like that required for chemotaxis of cells [32]. Thus, β -arrestin determines dephosphorylation and activation of cofilin so that actin filaments can be formed [33]. β -Arrestin-1 has also been shown to have a prosurvival action in CD4 T cells by upregulation of antiapoptotic bcl-2 [34]. Amazingly, in adipocytes TNFRI stimulation by connecting with TRAF2 and β -AR1 will lead to lipolysis and release of glycerol as a mediator.

In the setting of T lymphocytes, TCR/CD28 ligation at the time of antigen stimulation induces a cAMP-driven PKA type I, attached to an AKAP, to promote the action of Csk, an inhibitory phosphotyrosine kinase that downregulates the T cell receptors (Chap. 13). Yet, β -arrestin and phosphodiesterase 4 (PDE4) are recruited to the lipid rafts of T cells; thus, they will counteract the local production of cAMP at the immune synapse [35]. Previously, the Lefkowitz group [36] had shown that when β 2-adrenergic receptors recruit β -arrestin along with PDE4, local cAMP second messenger is degraded.

One knows only too well that there are deleterious effects on the heart from chronic stimulation of β -adrenergic receptors because there is then Gs-dependent activation of adenylate cyclase. However, the Lefkowitz group has shown how β 1-adrenergic receptors also recruit β -arrestin so that there is transactivation of EGF receptors, which counteract the effects of catecholamine toxicity.

Nelson et al. [37] noted how activated M1 muscarinic receptors bind β -arrestin and thereby recruit diacylglycerolkinase (DGK), so ensuring that local diacylglycerol (DAG) is degraded to phosphatidic acid; accordingly, there is negative regulation of protein kinases C and D. Obviously, β -arrestin can simultaneously recruit different enzymes. Thus, when PDE4 is also co-opted, again there is degradation of cAMP to AMP. Since both DAG and cAMP are second messengers for many diverse receptors, the potential of control by β -arrestin is great.

Third, there are *regulators of G protein signalling* (RGS) proteins [38] that reduce or terminate a signal within milliseconds [39]. There are at least 20 RGS proteins. An RGS is actually a GTPase-activating protein (GAP) (Fig. 5.1), which accelerates the hydrolysis of $G\alpha$ -bound GTP, turning the G protein off. RGS proteins can (a) limit receptor coupling to AC, (b) limit receptor coupling to MAP kinases (Fig. 4.3), and (c) reduce calcium ion mobilization within a cell. An RGS2 is involved in the fine-tuning of blood pressure regulation. RGS2 inhibits signalling through receptors coupled to $Gq\alpha$, whose ligands include most physiological vasoconstrictors [40]. Likewise, Toll-like receptor (TLR) proteins on dendritic cells modulate signalling by altering the levels of RGS proteins [41]. It has become evident that surface receptors on NK (natural killer) cells affect the expression of associated RGS proteins, which will modulate NK cell potential [42].

Vascular smooth muscle cells contract in response to α 1-adrenergic activity (Table 1.2). Conversely stimulation of their β -adrenergic receptors leads to formation

of cAMP and hence smooth muscle relaxation. When studying lymphocytes of persons with essential hypertension, GPCR kinase activity is found to be increased [16, 43]. The consequent reduction of β -adrenergic responsiveness gives an indication of how arteriolar tone is increased in Japanese with hypertension, who show a mutation of G protein $\beta 3S$, which means that there is enhanced G_i signalling and increased $\alpha 2$ -adrenergic responsiveness [44]. It was found that RGS2-deficient mice suffer prolonged vasoconstriction, and thus hypertension, mediated by angiotensin II acting on its AT1 receptors [45].

Finally, it has been found that there can be phosphorylation-independent means of uncoupling GPCRs from their G proteins [46] by *GPCR interacting proteins* (GIPs) [47].

There has been a recent review of the current state of knowledge of GPCRs in macrophages [48], which describes their involvement in (a) cell-cell contact, (b) chemotaxis, and (c) their production of inflammatory mediators. Apart from contact of α - or $\beta\gamma$ G protein units with essential intracellular enzymes, surface receptors often have attached β -arrestin, which then transmits signals to ERK, JNK, or NF- κ B (nuclear factor-kappa B). On some occasions, it is even possible that membrane β -arrestin that is not linked to GPCRs will transmit to the ERK cascade.

References

1. Cooper DMF, Crossthwaite AJ. Higher order organization and regulation of adenylyl cyclases. *Trends Pharm Sci* 2006;27:426–431
2. Donelson Smith F, Langeberg LK, Scott JD. The where's and when's of kinase anchoring. *Trends Biochem Sci* 2006;31:316–323
3. Lucas KA, Pitoria GM, Kazerounian S et al. Guanylyl cyclases and signalling by cyclic GMP. *Pharmacol Rev* 2000;52:375–413
4. Patel TB. Single transmembrane spanning heterotrimeric GPCRs and their signalling cascades. *Pharmacol Rev* 2004;56:371–385
5. (a) Lindner ME, Gilman AG. G proteins. *Sci Am* 1992;July;36–43; (b) Sprang SR. G protein mechanisms. *Annu Rev Biochem* 1997;66:639–678
6. Hamm HE. The many faces of G protein signaling. *J Biol Chem* 1998;273:669–672
7. Morris AJ, Malbon CC. Physiological regulation of G-protein linked signaling. *Physiol Rev* 1999;79:1373–1430
8. Defer N, Mest-Belpomme M, Hamoune J. Tissue specificity and physiological relevance of isoforms of adenylyl cyclase. *Am J Physiol Renal Physiol* 2000;279:F400–F416
9. Lavelle EC, Jarnicki A, McNeela E et al. Effect of cholera toxin on innate and adaptive immunity and application as an immunomodulatory agent. *J Leukoc Biol* 2004;75:756–763
10. Fan H, Williams DL, Zingarelli B et al. Differential regulation of lipopolysaccharide and gram positive bacteria induced cytokine and chemokine production in macrophages by Gai proteins. *Immunology* 2007;122:116–123
11. Peres CM, Aronoff DM, Serzani CH et al. Specific leukotriene receptors couple distinct G proteins to effect alveolar macrophage defense functions. *J Immunol* 2007;179:5454–5461
12. Daak Y, Luttmell LM, Lefkowitz RJ. Switching of coupling of $\beta 2$ -adrenergic receptor to different G proteins by protein kinase A. *Nature* 1997;390:88–90
13. Goetzl EJ. Diverse pathways for nuclear signaling by GPCRs and their ligands. *FASEB J* 2007;21:638–642

14. Gobeil F, Zhu T, Brault S et al. Nitric oxide signaling via nuclearized endothelial nitric oxide synthase modulates expression of the immediate early genes iNOS and mPGES-1. *J Biol Chem* 2006;281:16058–16067
15. Freedman NJ, Lefkowitz RJ. Anti- β 1 adrenergic receptor antibodies and heart failure. *J Clin Invest* 2004;113:1379–1382
16. Harris DM, Cohn HI, Pesant S, Eckhart AD. GPCR signalling in hypertension: role of GRKs. *Clin Science* 2008;115:79–89
17. Gaidarov J, Krupnick JG, Falck JR. Arresting function in G protein coupled receptor endocytosis. *EMBO J* 1999;18:871–881
18. Laporte DA, Oakley RH, Zhang J et al. The β 2-adrenergic receptor:arrestin complex recruits the clathrin adaptor AP-2 during endocytosis. *Proc Natl Acad Sci U S A* 1999;96:3712–3717
19. Shenoy SK, Lefkowitz RJ. Multifaceted roles of β -arrestins in the regulation of seven transmembrane spanning receptor trafficking and signalling. *Biochem J* 2003;375:503–515
20. Ma L, Pei G. β -Arrestin signaling and regulation of transcription. *J Cell Sci* 2007;120:213–218
21. Dasgupta P, Rasyogi S, Pillai S et al. Nicotine induces cell proliferation by β -arrestin activation of Src and Rb-Raf-1 pathways. *J Clin Invest* 2006;116:2208–2217
22. Rey A, Manen S, Rizzoli R, Caverzasio J, Ferrari SL. Proline rich motifs in the PTH related protein receptor mediate scaffolding of c-Src with beta-arrestin2 for ERK1/2 activation. *J Biol Chem* 2006;281:38181–38188
23. Premont RT, Gainetdinov RR. Physiological roles of G-protein coupled receptor kinases and arrestins. *Annu Rev Physiol* 2007;69:511–534
24. Lefkowitz R, Whalen EJ. β -arrestins:traffic cops. *Curr Opin Cell Biol* 2004;16:162–168
25. Kohout TA, Nicholas SL, Perry SJ et al. Differential desensitization, receptor phosphorylation, β -arrestin recruitment and ERK 1:2 activation by the two endogenous ligands for the C-C chemokine receptor 7. *J Biol Chem* 2004;279:23214–23222
26. Barnes WG, Reiter E, Violin JD et al. β -Arrestin-1 and G α q11 coordinately activate RhoA and stress fiber formation following receptor stimulation. *J Biol Chem* 2005;280:8041–8050
27. Vroon A, Neijnen CJ, Kavelaars A. GRKs and arrestins:regulators of migration and inflammation. *J Leukoc Biol* 2006;80:1214–1221
28. Su Y, Raghuvanshi SK, Yu Y et al. Altered CXCR2 signaling in β -arrestin 2 deficient mouse models. *J Immunol* 2005;175:5396–5402
29. Barlic J, Andrews JD, Kelvin AA et al. Regulation of tyrosine kinase activation and granule release through β arrestin by CXCR1. *Nat Immunol* 2000;1:227–223
30. Walker JKL, Fong AM, Lawson BL et al. β Arrestin2 regulates the development of allergic asthma. *J Clin Invest* 2003;112:566–574
31. Hupfeld CJ, Olefsky JM. Regulation of receptor tyrosine kinase signaling by GRKs and beta-arrestins. *Annu Rev Physiol* 2007;69:561–577
32. Defea KA. β -arrestin dependent chemotaxis. *Annu Rev Physiol* 2007;69:535–560
33. Zoudilova M, Kumar P, Ge L et al. Beta-arrestin dependent regulation of the cofilin pathway. *J Biol Chem* 2007;282:20634–20646
34. (a) Shi Yet al. Critical regulation of CD4 T cell survival and autoimmunity by β -arrestin 1. *Nat Immunol* 2007;8:817–824
35. Tasken K, Stokka AJ. The molecular mechanism for cAMP dependent immunoregulation in T cells. *Biochem Soc Trans* 2006;34(4):476–479
36. Baillie GS, Sood A, McPhee I et al. β -Arrestin mediated PDE4 cAMP phosphodiesterase regulates β -adrenoreceptor switching from Gs to Gi. *Proc Natl Acad Sci U S A* 1003;100:940–945
37. Nelson CD, Perry SJ, Regier DS et al. Targeting of diacylglycerol to M1 muscarinic receptors by beta-arrestins. *Science* 2007;315:663–666
38. Siderovski DP, Strockbine B, Behe CI. Whither goes the RGS proteins. *Crit Rev Biochem Mol Biol* 1999;34:215–251
39. Kerl J, Sinnarajah S. RGS2: regulator of G-protein. *Int J Biochem Cell Biol* 2002;34:432–488

40. Persu A. G-proteins: tuning of blood pressure regulation. *J Hypertens* 2005;23:1465–1467
41. Shi G-X, Harrison K, Han S-B, Moratz C, Kehrl JH. Toll-like receptor signaling alters the expression of regulator of G protein signaling proteins in dendritic cells: implications for GPCR signaling. *J Immunol* 2004;172:5175–5184
42. Kueberg L, Rayon JC, Rolstad B, Inngjerdingen M. Expression of regulator of G protein signalling proteins in NK cells. *Immunology* 2005;115:358–365
43. Gros R, Benovic JL, Tan CM, Feldman RD. G protein coupled receptor kinase activity is increased in hypertension. *J Clin Invest* 1997;99:2087–2093
44. Towaza Y. G protein beta3 subunit variant: increasing susceptibility to hypertension in Japanese. *Blood Press* 2001;10:131–134
45. Heximer SP, Knutsen RH, Sun X et al.. Hypertension and prolonged vasoconstrictor signaling in RGS2 deficient mice. *J Clin Invest* 2003;111:445–452
46. Ferguson SSG. Phosphorylation-independent attenuation of GPCR signalling. *Trends Pharm Sci* 2007;28:173–179
47. Bockaert J, Roussignol G, Becamel C et al.. GPCR interacting proteins, GIPS. *Biochem Soc Trans* 2004;32(5):851–855
48. Lattin J, Zidor DA, Schroder K et al. G protein coupled receptor expression, function and signaling in macrophages. *J Leukoc Biol* 2007;82:16–32

Chapter 2

Cell Membrane Receptors and Phospholipids

Abstract Membrane phospholipids, in particular phosphatidylinositol (PtdIns), provide crucial phosphoinositides that activate enzymes like PI3 kinase, which drives the protein kinase B (Akt), cell survival and signal pathway. Also, calcium ions entering through membrane ion channels exert controls over vital enzymes.

2.1 Turnover of Cell Membrane Phosphatidyl-Inositol

The inositol phospholipids are situated in the inner leaflet of the plasma membrane bilayer. They constitute only 1–2% of the total cell phospholipids. By using adenosine triphosphate (ATP), phosphatidyl-inositol (PtdIns) is converted to PtdIns(4)P and then to PtdIns(4,5)P₂. Whenever an external stimulus acts on a receptor that is coupled to phospholipase C (PLC), the substrate PtdIns(4,5)P₂ is hydrolysed to release diacylglycerol (DAG) and myo-inositol (1,4,5)P₃, often indicated as IP₃ or PIP₃. Micromolar concentrations of IP₃ release large amounts of ionic calcium into the cytosol from an ATP-dependent pool on the endoplasmic reticulum (ER) [1]. The various phosphorylation-dephosphorylation reactions normally maintain a small but relatively constant intracellular pool of IP₃. The hydrophobic DAG is left at the plasma membrane (Fig. 2.1) and so is on site for activation of protein kinase C (PKC), which will come up from a submembranous site. PKC is a common means of transmembrane signalling [2], as is the situation when platelets or neutrophils are incited to release their granules, when mast cells release histamine, or when the β -cells of the pancreatic islets release their insulin.

2.1.1 Phospholipase C (PLC)

Usually, PLC is activated by G proteins, either by means of the α -subunit or by the $\beta\gamma$ heterodimer. Yet, only 1% of the cellular pool of PLC is associated with an activated receptor at any one time. There are several isoforms of PLC [2b], and there is a hierarchy of PLC subtypes that are used in cell signalling (Fig. 2.2) [4, 5].

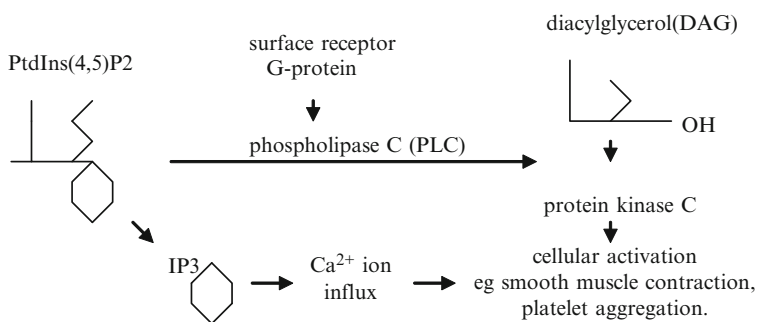


Fig. 2.1 Inositol phospholipids and cellular activation. Diacylglycerol (DAG) is a cone-shaped lipid that can induce negative concave curvature on lipid bilayers, promoting fluidisation. DAG binds to C1 domains in molecules like protein kinase C (PKC), protein kinase D (PKD) and Ras-GRP, activating them. Various DAG-binding proteins are regulated by PKC [3]. DAG signals are terminated by DAG kinase, which forms phosphatidic acid (PtdOH). As indicated, PKC induces a variety of important physiological reactions [2a, 2b]

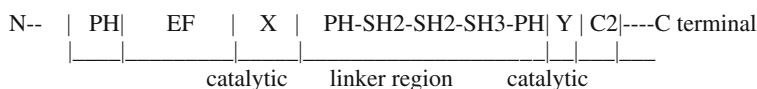


Fig. 2.2 The structure of phospholipase C (PLC). PLC γ 1 and PLC γ 2 have 50% identity of their amino acids. Both have Src-homology domain 2 (SH2) and Src-homology domain 3 (SH3) domains in a linker region between two pleckstrin homology (PH) domains. PLC γ 1 is ubiquitous, whilst PLC γ 2 is expressed in the spleen and thymus. PLC β , - δ , - ϵ do not have a linker region

Generally, β -PLC isoforms are G protein regulated, whilst the γ -forms PLC γ 1 and PLC γ 2 are stimulated by receptors with protein tyrosine kinase activity such as PDGFR or EGFR. G proteins induce PLC γ 1 phosphorylation in platelets, smooth muscle, and mesangial cells. PLC γ 1 phosphorylation occurs at three sites: Tyr771, Tyr783, and Tyr1254. PIP3 will activate PLC γ isoforms also, and calcium ions can activate PLC δ .

Antigen binding to immunoreceptors activates PLC. There is PLC γ 1 in T cells and mast cells, and PLC γ 2 in B cells. Lymphocyte chemokine receptors activate PLC β .

2.1.2 Diacylglycerol

Diacylglycerol (DAG) stimulates protein kinases C and D, aiding proliferation and survival of cells and promoting immune cell signalling and cytoskeletal or vesicle rearrangements. DAG stimulates DAG kinases, which phosphorylate DAG to produce phosphatidic acid. In T cells, DAG aids RasGRP, which is the guanine-nucleotide exchange factor (GEF) for Ras.

	regulatory PS DAG binding Ca ²⁺				catalytic ATP	
conventional	N-	PSE	C1A	C1B C2	kinase	-C terminal
novel	N-		PSE	C1A C1B	kinase	-C
atypical	N-----			PSE C1 like	kinase	-C

Fig. 2.3 Structure of protein kinase C (*PKC*). All *PKC* isoenzymes have a conserved kinase domain that is auto-inhibited by the pseudosubstrate domain (*PSE*), which blocks access to the active site. Conventional isozymes have a C1 domain that binds diacylglycerol (*DAG*) or phorbol esters and a C2 domain that binds phosphatidyl-serine (*PS*). Novel isoenzymes have a functional C1 domain and a nonfunctional C2-like domain that has no residues for calcium binding. Atypical *PKCs* are deficient in C1/C2; hence, they are *DAG* independent and calcium independent

2.1.3 Protein Kinase C

There are various *PKC* isoenzymes [6, 7]. The conventional *PKC* isoenzymes α , β 1, β II, and γ require Ca^{2+} ions and are activated by *DAG* or phorbol ester. Novel isoenzymes *PKC* δ , ϵ , θ , or η are calcium ion independent, but they are activated by *DAG* or phorbol ester. The atypical *PKC* ζ and λ (*PKC* ι) are neither Ca^{2+} nor *DAG* sensitive (Fig. 2.3).

PKC α is implicated in cell differentiation, proliferation, or apoptosis, which means that it is a key coordinator in differing cell compartments [8]. *PKC* α helps to promote an immunoglobulin G (IgG)-stimulated respiratory burst [9], and *PKCs* δ/ϵ are implicated in mediating phagocytosis. *PKC* β helps motility of T lymphocytes and raft formation by B cells. *PKC* θ and β have particular functions in B cells. In T cells, *PKC* θ translocates into lipid rafts within the immunological synapse, and it then helps to induce formation of interleukin 2 (IL-2) [7]. In activated T lymphocytes, *PKC* θ not only drives cell activation but also downregulates directly engaged T cell receptors (TCRs), and *PKC* α downregulates the nonengaged TCRs [10]. In mast cells, *PKC* θ activates extracellular signal-related kinase (ERK) and therefore discharge of the granules [11, 12]. In mast cells or platelets, *PKC* δ is a negative regulator of granule discharge. Generally, *PKC* δ is a multitask kinase that, via mitogen-activated protein (MAP) kinase activation, can promote cell proliferation, as in breast tissue, and yet it will also induce cell apoptosis [13]. Indeed, it ranks as a tumour suppressor gene [13].

2.2 The Phosphatidyl-Inositol Phosphates

The various inositol phosphates are well described [14–16]. Generally, they induce intracellular mobilisation of Ca^{2+} ions. *Ins*(1,4,5)*P*3 will release large amounts of ionic Ca^{2+} from the pool in the ER [1]. So, when there is α 1-adrenergic stimulation of vascular smooth muscle cells, that raises Ca_i toward $10^{-6}M$ via influx through plasma membrane ion channels, and concurrently *IP*3 releases calcium that is

bound on the internal ER store. Hence, there will be actin-myosin interaction and thereby smooth muscle contraction [16].

Figure 2.4 represents the phosphoinositide syntheses. All phosphoinositides are located on the cytosolic leaflet of the plasma membrane and of intracellular organelles. PI(4)P and PI(4,5)P₂ are the major products. The products Ins(1,4,5)P₃ and Ins(3,4,5)P₃ are important second messengers in immune cells. In epithelial cells, Ins(3,4,5)P₃ regulates the formation of the basolateral membranes [17]. Phosphatidylinositol phosphate kinases [PI3/5PKs] use substrates PtdIns(4)P, PtdIns(5)P, and PtdIns(3)P to generate PI4, $\leftarrow$ 5P₂, and PI3,4, $\leftarrow$ 5P₃. Those PIPKs (phosphatidylinositol phosphate kinases) are controlled either by growth factors or when chemoattractants act by G proteins [17, 18a, 18b]. All three PIPK isoforms physically associate with Rho and Rac1 guanosine triphosphatases (GTPases).

Originally, it was known that PtdIns(4,5)P₂ is cleaved by PLC to IP₃(Ins 1,4,5P₃) and DAG as in Figs. 2.1 and 2.4. Then, it was realised that PtdIns(4,5)P₂ is substrate for other phosphoinositide kinases, so that several other inositol lipid messengers are produced [14], and they influence cell growth, actin fibre formation, cell motility, endocytosis, and exocytosis [17, 19].

One can now be more precise about the functions of the basic phosphoinositides: PtdIns(3,4,5)P₃, PtdIns(4,5)P₂, and PtdIns(3)P [18]. Rapid synthesis of PtdIns(3,4,5)P₃ in response to growth factor stimulation of PI3K at the plasma membrane means that it is available to control the attachment of proteins to membranes. PtdIns(3,4,5)P₃ has a major role in exocytosis. This PIP₃ is also produced on the ER, in the Golgi [17], and within nuclei. The targets of PIP₃ are often GEFs and GTPase-activating proteins (GAPs) for small GTPases. PIPs activate fibrils for actin polymerisation and depolymerisation. Thus, PIP₃ induces membrane ruffling; when platelet-derived growth factor (PDGF) or insulin is acting, it is the activator at the leading edge of migrating neutrophils, and it is localised at sites for neutrophil or macrophage phagocytosis. PIPs are concerned also with membrane trafficking. PIPs regulate signalling elements like C phospholipases, the guanosine triphosphate (GTP)-binding

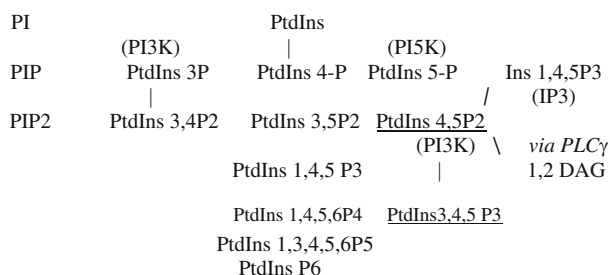


Fig. 2.4 Phosphoinositide syntheses. Observe that initially there are three different monophosphoinositides phosphorylated at position 3, 4 or 5 of the inositol ring. In turn, they are phosphorylated further to form the PIP₂s as PI(3,4)P₂, PI(3,5)P₂ and PI(4,5)P₂ and hence the PIP₃ termed PI(3,4,5)P₃. Guanosine triphosphatase (*GTPase*) effectors often coordinate synthesis and turnover of PIs. In turn, the phosphoinositides regulate recruitment of GTPase-activating proteins (*GAPs*) and guanine-nucleotide exchange factors (*GEFs*) to membranes, and their GTPases function as co-receptors with PIs for recruitment of cytosolic proteins [17]

proteins, and protein kinases. PtdIns(3,4,5)P3 induces specific enzymes for signalling, like (a) AGC serine-threonine protein kinases, (B) Tec family tyrosine kinases, and (C) the Rho family GTPases.

Product PtdIns(4,5)P2 is also concentrated at the plasma membrane, in lipid rafts, or in membrane ruffles. The level of PIP2 in a cell membrane can be regulated by β 2-integrin through the small G protein called ARF6 and the action of PI5K. PtdIns(4,5)P2 directs endocytosis through its ability to anchor coat proteins to the plasma membrane [19]. PtdIns(4,5)P2 will regulate (a) proteins involved in vesicle trafficking like the Ras GTPases Rab and ARF1, (b) Golgi function and secretion, (c) assembly of actin microfilaments, or (d) the action of ERM proteins, like ezrin/radixin/ moesin, which act as cross-linkers between the plasma membrane and the cytoskeleton.

Other phosphoinositides that regulate internal movements of proteins along membranes [19, 20] are PtdIns(4)P in the Golgi [21], PtdIns(3)P within early endosomes, and PtdIns(3,5)-biphosphate on late compartments of endocytic organelles. PI3P-binding modules [22–24] with FYVE and PX domains (Fig. 5.6) are present in various endosomal proteins. In the lower left part of Fig. 2.4, observe Ins(1,3,4,5,6)P5. This is converted by a receptor-regulated 1-phosphatase to yield Ins(3,4,5,6)P4. IP4 at 1–10 μ M helps CaM kinase II-dependent activation of membrane chloride channels. IP4 also controls acidification of vesicles. In thymocytes, IP4 aids calcium signalling. Note in Fig. 2.5 a physiological scheme [5] of the phosphoinositide signalling pathways and their control by phosphatases. Note how PtdIns(3,4,5)P3 is degraded rapidly by PTEN phosphatase or the SHIP Src homology inositol polyphosphate 5-phosphatase. This means that PTEN (Fig. 2.6) is a negative regulator [21] of PI3K signalling. Loss of PTEN can lead to the development of tumours. PTEN is absent from various cancers [25], which actually helps expression of hypoxia-inducing factor (HIF). In prostate cancer, lack of PTEN means that aberrant activation of PI3K-Akt helps cell invasiveness.

2.3 Phosphoinositide 3 Kinases

PtdIns 3 kinase (i.e. PI3K) synthesises PtdIns(3,4,5)P3 in response to stimulation of receptor protein tyrosine kinases, G protein-coupled receptors (GPCRs), or cytokine receptors. PI3K is a protein serine kinase and a lipid kinase. It has a p110 catalytic

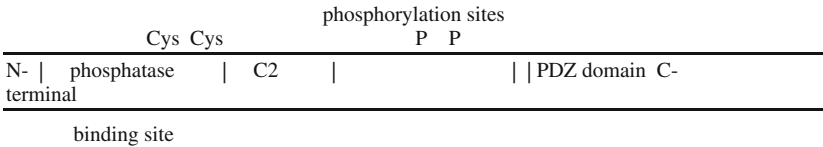


Fig. 2.5 Phosphoinositide signalling pathways. *Phosphorylation by phosphoinositide 3 kinase. **Dephosphorylations by PTEN or SHIP control the PIP3 level. *PTEN* phosphatase and tensin homologue phosphatase that hydrolyses 3P-inositol p-lipids. C1-TEN is analogous to PTEN. *SHIP* Src homology inositol polyphosphate 5-phosphatase. Note that Jurkat cells have unusual signal transduction for they lack PTEN and SHIP enzymes. Lack of PTEN can explain exaggerated chemotactic responses to chemokine SDF-1 α

unit (PI3Kc), which interacts with ATP and with the head group of PI4,5P2 and a p85 regulator unit at the N-terminal PI3Kr. The domains in between (Fig. 2.7) are a PI3Ka accessory domain, a C2(PKC homology domain 2), and a Ras- or Rac-binding domain. PI3Ks are required for control of cell growth, survival and proliferation and glucose uptake [28]. Cell growth and proliferation are controlled by PI3K working in conjunction with mTOR [29] (Fig. 11.6). PI3K determines actin cytoskeleton remodelling and vesicle movements [30–32], just as PI3K γ determines the T cell immunological synapses. PI3K action is integral to the movement of leucocyte [33].

There are various PI3Ks [30] categorised as class I, II or III. Receptors on immune cells activate the class IA/class IB PI3Ks that convert PtdIns(4,5)P2 to the second messenger PtdIns(3,4,5)P3, which can then be hydrolysed by PTEN or SHP phosphatases. Class I PI3Ks are the only kinases that generate PIP3. Class II PI3Ks convert PI to PI(3)P to PtdIns(3,4)P2. Class III PI3Ks are involved in phagocytosis. PI(3)P is produced within endosomes.

There are details on the structure of PI3Ks to be clarified. Mammalian cells encode three isoforms of the PI3Kr regulatory unit, which are varied further by

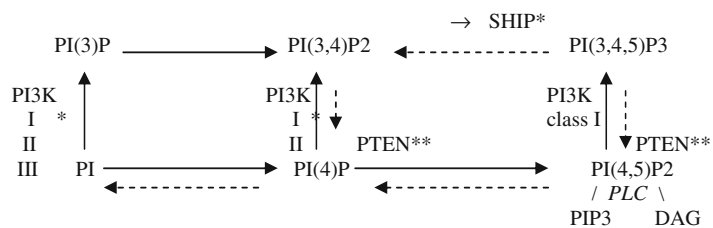


Fig. 2.6 The structure of protein tyrosine phosphatase (*PTEN*) phosphatase. Owing to its active cysteine residues, PTEN is readily oxidised and inactivated. The enzyme PTEN limits the pool of PIP3 (cf. Fig. 2.5). This means that PTEN has proapoptotic effects. Conversely, PTEN^{null} mice have autoimmune lymphoproliferative syndrome. PTEN governs the proliferation rate and number of intestinal stem cells. In PTEN deficiency, intestinal polyposis occurs [26]. Interestingly, in T cells PTEN-mediated regulation of PI3 kinase imposes a requirement for CD28 costimulation for complete cell activation [27]

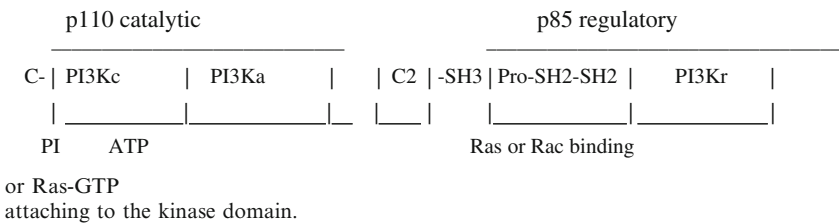


Fig. 2.7 Structure of PI3 kinase (*PI3K*) class I. The PI3K molecule attaches by two Src-homology domain 2 (*SH2*) domains to phosphorylated tyrosine proteins beneath the plasma membrane. Thus, PI3K makes multiple interactions with the Src-homology domain 3 (*SH3*) of Src family kinases, and with guanosine triphosphatase (GTP)-bound Ras. These then help to regulate PI3K activity. The PI3Ks provide 3-phosphoinositide substrates for complex signal transduction networks, for cytoskeletal reorganisation and for trafficking of vesicles [17, 18]

alternative splicing yielding PI3K α 1 85 α , 55 α or 50 α ; PI3K α 2 p85 β ; PI3K α 3 p55 γ . The three class Ia catalytic units p110 α , p110 β and p110 γ interact with any of the regulatory subunits. Each controls the metabolic state of the cell and so its growth or proliferation [29, 30]. A class Ia PI3K (Fig. 2.7) has a p85 regulator unit and a binding or catalytic unit (α , β or δ). A class Ia PI3K will be activated by a receptor with intrinsic tyrosine kinase activity. Full activation of a Ia PI3K requires occupancy of both Src-homology domain 2 (SH2) domains of the regulatory unit by tyrosine phosphopeptides and binding of Ras-GTP to the catalytic subunit. A class Ib PI3K has a p101 regulator and a p110 γ catalytic unit. Class Ib PI3Ks are highly expressed by cells of the immune system [31]. These are activated by the $\beta\gamma$ of GPCRs, which include the receptors for the chemokines.

Whilst p100 α and - β PI3Ks are present in all cells, p110 γ / δ are restricted to leucocytes. PI3K γ is expressed in cells of haemopoietic origin, so it is involved in inflammation [31]. PI3K γ is activated rapidly in innate immune cells like neutrophils and macrophages that confront pathogens. Induction of reactive O species is accompanied by activation of PI3K [32]. Knockout of PI3K γ in mice protects them from rheumatoid arthritis or asthma, and its blockade helps the glomerulonephritis of lupus mice. PI3K catalytic unit p110 γ transmits the antigen receptor signal of T and B lymphocytes [31]. In T cells, the costimulatory molecule CD28 activates p110 δ PI3K, and ICOS activates the p110 β unit of PI3K [33]. So, PI(3,4,5)P₃ determines the threshold for T cell activation. Low PIP₃ imposed by PTEN actually determines the need for costimulation, but that enables self-tolerance. Both increased and decreased activity of PI3K can manifest as an autoimmune phenotype in mice [34]. PI3K p110 δ is integral to T cells, including T regulatory lymphocytes and B cells and the mast cells.

PI3K is at the leading edge of moving leucocytes (Fig. 9.7) [35]. The PI3K aids migration of macrophages in response to MCP-1 or CSF-1 [36]. PI3K γ p110 aids chemotaxis of T cells [37], and p110 δ is important for B cell homing [37]. p110 α PI3K is the determinant of monocyte functions: phagocytosis, the oxidative burst and cytokine secretion. PI3K γ occurs at low concentration in endothelial and smooth muscle cells and in cardiomyocytes. PI3K controls cell fate decisions at many points in B cell development and activation. PI3Ks help to attract B lymphocytes to developmental sites like lymph nodes [38]. On B cells, CD19 is a major effector of PI3K activation. Indeed, cross-linking of CD19 with the BCR increases the sensitivity of B cell responses by three to four orders of magnitude.

2.3.1 PI3K Effector Proteins

The 3-phosphoinositide products PI(3)P, PI(3,4)P₂ or PI(3,4,5)P₃ bind to modules on various proteins, activating them. Thus, they bind to *pleckstrin homology* (PH) domains of proteins to either modify their activity or induce localisation of the protein to a specific part of the plasma membrane where activation will occur [20]. So, PI3K attracts to cell membranes PH-domain-bearing enzymes like protein kinase B (PKB) (Akt) and the 3-phosphoinositide-dependent kinase 1 (PDK1) that

phosphorylates kinases. The *phox homology* (PX) domain of proteins also binds 3-phosphoinositides. Generally, proteins with PH or PX domains are involved in signal transduction (Fig. 5.6). The FYVE domain that binds PtdIns(3)P is found on proteins implicated in vesicle and protein trafficking [39].

As stressed already, isoforms of the PI3K family regulate (a) cell survival, (b) growth or proliferation of cells, (c) glucose metabolism, (d) cytoskeletal organisation and membrane trafficking, as is required for spreading and motility of leucocytes [40]. Essentially, PI3K activates downstream targets such as p70 ribosomal S6 kinase, PKB(Akt) [41] or Rho family GTPases that affect actin fibres or vesicle trafficking. The lipid products of PI3K also activate PKC [42].

In neutrophils, there are PI3K α , β and δ with the p85/100 structure, and there is PI3K γ as the sole isoform to be activated by GPCRs [43]. PI3K γ forms PI(3,4,5)P₃ in response to chemoattractants; thus, there is attraction of PH proteins to the leading edge of moving cells [44]. The PH proteins include PLC, Vav, Dok, Akt, inducible tyrosine kinase (Itk) and protein kinase D. So, PI3K γ steers neutrophils [45]. PI3K γ of neutrophils is much concerned in inflammation of the airways. Obviously, small molecule inhibitors are being assessed for their therapeutic efficacy [33]. Wortmannin and LY294002 are nonspecific inhibitors, but specific inhibitors of PI3K γ are being developed [46].

The granulocyte-macrophage colony-stimulating factor (GM-CSF) induces neutrophilia, and it delays apoptosis of cells. Its action explains how neutrophils survive during sepsis. PI3K γ contributes to this [47–49] since PI3K stimulates Akt, the survival kinase (Fig. 2.8). Furthermore, Akt activates NF- κ B (nuclear factor-kappa B) signalling [50].

T lymphocytes express p100 α and p110 δ . Both catalytic units play their role in signalling [51]. PI3K γ helps to induce synthesis of IL-2 [52], which makes lymphocytes proliferate. The p85 regulator is in control.

Toll-like receptor (TLR) signals activate PI3K, which modulates GSK3 β and so creates negative regulation [53]!

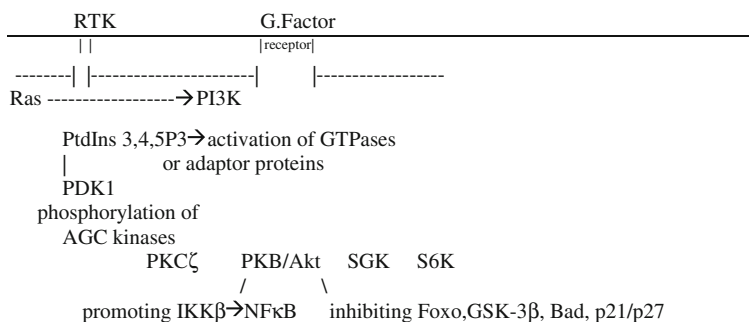


Fig. 2.8 PI-3 kinase (*PI3K*)/Akt signalling pathway. *PKB* protein kinase B, *PKC* ζ atypical PKC isoform, *SGK* serum and glucocorticoid-induced protein kinase, *S6K* p90 ribosomal S6kinase

2.4 Protein Kinase B (Survival Kinase Akt)

The cell survival kinase Akt (PKB) is recruited to the plasma membrane through binding of its PH domain to PI(3,4,5)triphosphate. That is followed by phosphorylation on its threonine 308 by PDK-1 and on serine 473 by another kinase or by a neighbouring Akt. Akt-1 monitors growth and survival of cells. In normal metabolism, PKB mediates the effects of insulin on glucose transport, glycogen and protein synthesis. It is Akt-2 that regulates metabolism. Akt activity counteracts potential cell apoptosis by maintaining positive energy balance through the promotion of glucose uptake and glycolysis.

That the activation of PKB is downstream of PI3K is proven by the fact that an inhibitor of PI3K like wortmannin or expression of a dominant-negative regulatory subunit of PI3K inhibits the activation of PKB in cells by all agonists tested.

Not surprisingly, there are isoforms that create family members of PKB. They are described in relation to their action on cell migration [54].

2.5 Calcium Ions as Second Messenger

Following activation of the TCR, IP₃ releases Ca²⁺ from ER internal stores (1), and when they are depleted, there is a prolonged Ca²⁺ influx via store-operated CRACs (Ca²⁺ release activated Ca²⁺ channels) (2) in the membrane. The diffusible mediator STIM (stromal interaction molecule) connects ER store depletion to entry of calcium via the CRACs [55, 56]. One can compare the inhibitory effects of nitric oxide (NO) and cyclic guanosine monophosphate (cGMP)-dependent protein kinases in T cells or platelets with their action in relaxing vascular smooth muscle [57]. There is also a regulator of calmodulin signalling (RCS) [58]. Positive selection of T cells in thymus depends on Ca²⁺ elevation.

The basal Ca²⁺ in cells is less than 100 nM, but it can rise to 1 μM following TCR stimulation. This causes rounding and immobility of T cells and thus adherence of CD4T cells to the antigen-presenting cells. Since cytosolic Ca²⁺ concentrations are 10,000-fold lower than in the ER lumen, release of the ER store causes a large elevation of Ca_i, which is followed by a low wave derived from influx of external Ca²⁺ ions. Lack of Ca²⁺ influx impairs IL-2 production and T cell proliferation. One will ask how Ca_i can account for such varied actions as smooth muscle contraction, secretion of granules, metabolic effects and cell growth? Its effects on enzyme systems and genes are relevant. Berridge [59] explained how response diversity can be achieved by Ca²⁺ ion influxes of differing amplitude and different spatial or temporal modes. The Ca²⁺ ion signal is often a regular oscillation whose frequency changes in response to the intensity of the incoming signal. Imaging of T cell responses can show transient peaks of Ca_i, repetitive oscillations, or sustained elevation in the range 200 nM to more than 1 μM [60]. Sustained Ca²⁺ is required for cytokine gene transcription, as for tumor necrosis factor-α (TNFα).

Ca^{2+} is released from internal ER stores by IP_3 acting on IP_3Rs or in excitable cells via ryanodine receptors. Differing sensitivities of the three InsP_3R isoforms to regulation by Ca^{2+} allow fine-tuning of the temporal and spatial aspects of the signal [61]. $\text{Ins}(1,4,5)\text{P}_3$ empties the intracellular Ca^{2+} stores, leading to Ca^{2+} influx through CRAC channels in the plasma membrane as in Fig. 2.9 [62, 63]. A concerted interplay between plasma membrane Ca^{2+} and K^+ channels and membrane potential promotes the sustained Ca^{2+} signalling required for TCR-mediated cell activation and gene transcription [61]. Prolonged CRAC activity is reinforced by internal Ca^{2+} release from mitochondria via the ADP ribosyl cyclase, which forms NAADP (nicotinic acid adenine dinucleotide phosphate) from NADP (nicotinamide adenine dinucleotide phosphate) [64]. High-frequency Ca^{2+} oscillations for 1–2 h are required to keep transcription factor NFAT primed to induce IL-2 production. Internal Ca^{2+} activates calmodulin (CaM) and so calcineurin, which enables NFATc to move into nuclei to act on the IL-2 promoters (Fig. 13.5). There NFAT acts along with AP-1 (activator protein), which is stimulated by Ca^{2+} ion-activated JNK (Jun N-terminal kinase). Sphingosine or ceramide inhibit the CRACs.

The multiple forms of the IP_3Rs are downregulated by internal Ca^{2+} [60]. The $\text{PtdIns}/\text{Ca}^{2+}$ signal is sustained by simultaneous involvement of PLC via the tyrosine kinase of the receptor and by PI3K. $\text{PLC}\delta$ isoforms can amplify and extend the Ca^{2+} signals. Furthermore, in T lymphocytes Ras-GRP1 is activated in the Golgi by DAG and Ca^{2+} ions, and its action is accompanied by increases of Ca_i . Concurrently, a Ras GAP called Capri acts at the plasma membrane to mediate short-lived bursts of Ca^{2+} signalling [65]. In B lymphocytes, Ca^{2+} ion influx occurs through DHP (dihydropyridine)-sensitive channels, and the CD20 is a Ca^{2+} channel. As will be explained in Chap. 16, engagement of $\text{Fc}\gamma\text{RIIb}$ receptors has a negative effect on Ca^{2+} influx. Th-2 lymphocytes also use DHP receptors for Ca^{2+} influx, and they are blocked by nifedipine [66]. The TCR stimulates formation of cGMP, which induces protein kinase G, which controls the DHP channels and cytokine release [67]. In leucocytes, transient receptor potential (TRP) channels could be used as targets for pharmacological blockade. A greater appreciation of oxolipid arachidonic acid derivatives, such as 5HETE, 20HETE, and 5,6EET, which enable Ca^{2+} entry into lymphocytes, will surely lead to alternative means of control [68].

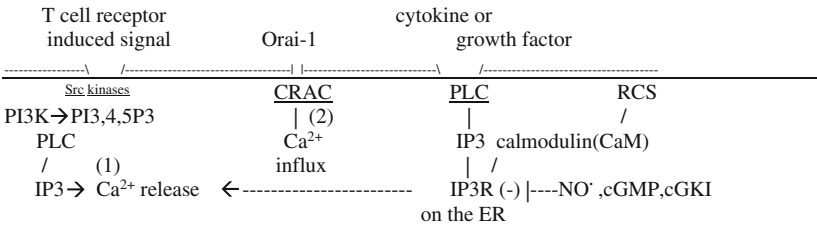


Fig. 2.9 Calcium ion mobilisation in T lymphocytes. CRAC Ca^{2+} release activated Ca^{2+} channel (with Orai-1 pore-forming subunit), cGMP cyclic guanosine monophosphate, cGKI cGMP kinase I

2.6 Desensitisation of Membrane Receptors

Induced signals must soon be toned down or switched off. Following α -adrenergic stimulation, there is *receptor desensitisation* by the GRKs that phosphorylate the receptor, by second messenger kinases like PKC or protein kinase A (PKA). As explained in Chap. 1, arrestins [69] bind to phosphorylated receptors and aid the process of receptor internalisation (**Fig. 1.2**). Desensitisation of membrane receptors that are linked to PLC is a general requirement [70]. So, there is rapid desensitisation of all receptors that activate PLC to a constant level. Accordingly, the effect on cytosolic calcium is that there is an early peak followed by a sustained plateau.

References

1. Berridge HJ, Irvine RF. Inositol triphosphate, a novel second messenger in cellular signal transduction. *Nature* 1984;312:315–322
2. (a) Rebecchi MJ, Pentyla SN. Structure, function and control of phosphatidylinositol specific phospholipase C. *Physiol Rev* 2000;80:1291–1235; (b) Cockcroft S, Thomas G. Inositol lipid specific phospholipase C isoenzymes and their regulation by receptors. *Biochem J* 1992;288:1–14
3. Spitzer M, Cantrell DA. Protein kinase C and beyond. *Nat Immunol* 2004;5:785–790
4. Rhee SG. Regulation of phosphoinositide specific phospholipase C. *Annu Rev Biochem* 2001;70:281–312
5. van Haesebroeck B, Leever SJ, Ahmadi K et al. Synthesis and function of 3-phosphatidylinositol phospholipids. *Annu Rev Biochem* 2001;70:535–602
6. Review. . Protein kinase C isoenzymes: divergence in signal transduction. *Biochem J* 1993;291:329–343
7. Tan S-L, Parker PJ. Diverse roles of protein kinase C in immune cell signaling. *Biochem J* 2003;376:545–552
8. Nakashima S. Protein kinase C α : regulation and biological function. *J Biochem* 2002;132:669–675
9. Larsen EC, Digennaro JA, Saito N et al. Differential requirement for classic and novel protein kinase C isoforms in respiratory burst and phagocytosis. *J Immunol* 2000;165:2809–2017
10. Von Essen M, Nielsen MW, Bonefeld CM et al. Protein kinases C PKC α and PKC θ are the major PKC isotypes in T cell receptor down-regulation. *J Immunol* 2006;176:7502–7510
11. Altman A, Villalba M. Protein kinase C θ . *J Biochem* 2002;132:841–846
12. Liu Y, Graham C, Parravicini V et al. PKC- θ is expressed in mast cells and is involved in Fc ϵ receptor I signaling. *J Leukocyte Biol* 2001;69:831–846
13. Jackson DN, Foster DA. The enigmatic protein kinase C δ : complex roles in cell proliferation and survival. *FASEB J* 2004;18:627–636
14. Majerus PW. Inositol phosphates. *Annu Rev Biochem* 1992;61:225–250
15. Santarius M, Lee CH, Anderson RA. Supervised membrane swimming: small G-protein lifeguards regulate PIPK signalling and monitor intracellular PtdIns(4,5)P₂ pools. *Biochem J* 2006;398:1–13
16. Forder J, Scriabne A, Rasmussen H. Plasma membrane calcium flux, protein kinase C activation and smooth muscle contraction. *J Pharmacol Exp Ther* 1985;235:267–273
17. Di Paolo G, De Camilli P. Phosphoinositides in cell regulation and membrane dynamics. *Nature* 2006;443:651–657

18. (a) Niggli V. Regulation of protein activities by phosphoinositide phosphates. *Annu Rev Cell Dev Biol* 2005;21:57–79; (b) Czech MP. Dynamics of phosphoinositides in membrane retrieval and insertion. *Annu Rev Physiol* 2003;65:791–815
19. Sato M, Ueda Y, Takagi T, Umezawa Y. Production of PtdInsP3 at endomembranes is triggered by receptor endocytosis. *Nat Cell Biol* 2003;5:1016–1020
20. Rameh LE, Arvidsson A-K, Carraway KL et al. A comparative analysis of the phosphoinositide binding specificity of pleckstrin homology domains. *J Biol Chem* 1997;272:22059–22066
21. Leslie NR, Downes CP. PTEN function. *Biochem J* 2004;382:1–11
22. Roth MG. Phosphoinositides in constitutive membrane Traffic. *Physiol Rev* 2004;84:699–730
23. Matteis MA, Godi A. PI-loting membrane traffic. *Nat Cell Biol* 2004;6:487–492
24. Shin HW, Nakayama K. Dual control of membrane targeting by PtdIns(4)P. *Trends Biochem Sci* 2004;29:513–515
25. Assano T, Yao Y, Zhu J et al. The PI3kinase/Akt signaling pathway is activated due to aberrant PTEN expression in pancreatic cancer cells. *Oncogene* 2004;23:8571–8580
26. He XC, Yin T, Grindley JC et al. PTEN deficient intestinal stem cells initiate intestinal polyposis. *Nature Genet* 2007;39:189–198
27. Buckler JL, Walsh OT, Porrett PM et al. T cell requirement for CD28 costimulation is due to negative regulation of T cell receptor signals by PTEN. *J Immunol* 2006;177:4262–4266
28. Frauwirth KA, Thompson CB. Regulation of T lymphocyte metabolism. *J Immunol* 2004;172:4661–4665
29. Richardson CJ, Schalm SS, Blenis J. PI-3kinase and TOR:PIKTORING cell growth. *Semin Cell Dev Biol* 2004;15:147–159
30. Wymann MP, Zvelebil M, Laffargue M. PI-3kinase signalling-which way to target? *Trends Pharmacol Sci* 2002;24:366–373
31. (a) Deane JA, Fruman DA. Phosphoinositide 3-kinase:diverse roles in immune cell activation. *Annu Rev Immunol* 2004;22:563–598; (b) Icazar IA, Marques M, Kumar A et al. PI3-kinase γ participates in T cell receptor induced T cell activation. *J Exp Med* 2007;204:2977–2988
32. Ito K, Caramori G, Adcock IM. Therapeutic potential of PI3-kinase inhibitors in inflammatory respiratory disease. *J Pharmacol Exp Ther* 2007;321:1–8
33. Parry RV, Riley JL, Ward SG. Signalling to suit function: tailoring PI3kinase during T cell activation. *Trend Immunol* 2007;28:161–169
34. Oak JS, Fruman DA. Role of phosphoinositide 3-kinase signaling in autoimmunity. *Autoimmunity* 2007;40(6):433–441
35. Procko E, McColl SR. Leukocytes on the move with PI3kinase and its downstream effectors. *BioEssays* 2005;27:153–163
36. Jones GE, Prigmore E, Calvez R et al. Requirement for PI3kinase in macrophage migration to MCP-1 and CSF-1. *Exp Cell Res* 2003;290:120–131
37. Reif K, Okkenhaug K, Sasaki T et al. Differentiation roles for PI3kinases p110 γ and p110 δ in lymphocyte chemotaxis and homing. *J Immunol* 2004;173:2236–2240
38. Ortolano S, Huang IY, Han SB, Kehrl JH. Role of PI3-kinases, Brutons tyrosine kinase and Jun kinases in B lymphocyte chemotaxis and homing. *Eur J Immunol* 2006;36:1285–1295
39. Fruman DA, Rameh LE, Cantley LC. Phosphoinositide binding domains: embracing 3-phosphate. *Cell* 1999;97:817–820
40. Van Haesebroeck B, Waterfield MD. Signaling by distinct classes of PI3kinases. *Exp Cell Res* 1999;253:239–254
41. Coffey PJ, Jin J, Woodgett JR. Protein kinase B(Akt):a multifunctional mediator of PI3-kinase activation. *Biochem J* 1998;335:1–13
42. Toker A, Cantley LC. Signalling through the lipid products of PI3kinase. *Nature* 1997;387:673–676
43. Wymann MP, Bjorklof K, Calvez R et al. PI3kinase γ :a key modulator in inflammation and allergy. *Biochem Soc Trans* 2003;31:275–280
44. Stephens L, Ellson C, Hawkins P. Role of PI3kinase in leukocyte chemotaxis and phagocytosis. *Curr Op Cell Biol* 2002;14:203–213

45. Sadhu C, Masinivsky B, Dick K et al. Essential role of PI3kinase δ in neutrophil directional movement. *J Immunol* 2003;170:2647–2654
46. Ward SG, Finan P. Isoform-specific PI3kinase inhibitors as therapeutic agents. *Curr Opin Pharmacol* 2003;3:426–434
47. Klein JB, Ranc MJ, Scherzer JA et al. GM-CSF delays neutrophil apoptosis through PI3kinase and ERK pathway. *J Immunol* 2000;163:4286–4291
48. Yum H-K, Arcaroli J, Kupfner J et al. Involvement of PI3kinases in neutrophil activation and development of acute lung injury. *J Immunol* 2001;167:6601–6608
49. Hirsch E, Katanaev V, Garlanda C, Azzolino O et al. Central role for G-protein coupled PI 3kinase in inflammation. *Science* 2000;287:1049–1053
50. Tanaka H, Fujita N, Tsuruo T. 3Phosphoinositide-dependent protein kinase-1 mediated I κ Bkinase β phosphorylation activates NF κ B signaling. *J Biol Chem* 2005;280:40965–40973
51. Ward SG, June CH, Olive D. PI 3-kinase: a pivotal pathway in T cell activation. *Immunol Today* 1996;17:187–198
52. Eder AM, Dominiguez L, Frank TF et al. PI 3-kinase regulation of a T cell receptor mediated interleukin 2 gene expression in normal T cells. *J Biol Chem* 1998; 273: 28025–28031
53. Hazeki K, Nogorikawa K, Hazeki O. Role of phosphoinositide 3-kinase in innate immunity. *Biol Pharm Bull* 2007;30(9):1617–1623
54. Stambolic V, Woodgett JR. Functional distinction of protein kinase B/Akt isoforms defined by their influence on cell migration. *Trend Cell Biol* 2006;16(9):461–466
55. Zhang SL, Yu Y, Roos J et al. STIM1 is a calcium sensor that activates CRAC channels. *Nature* 2005;437:902–905
56. Gallo EM, Cante-Barrett K, Crabtree GR. Lymphocyte calcium signaling from membrane to nucleus. *Nature Immunol* 2006;7:25–31
57. Schlossman J, Feil R, Hofmann T. Signaling through nitric oxide and cGMP dependent protein kinases. *Ann Med* 2003;35(1):21–27
58. Rakhelin SV, Olson PA, Nishi A et al. A network of control mediated by regulator of calmodulin dependent signaling. *Science* 2004;306:698–701
59. Berridge MJ. AM and FM of calcium signalling. *Nature* 1997;386:759–760
60. Feske S. Calcium signalling in lymphocyte activation. *Nat Rev Immunol* 07;7:690–702
61. Yule DI. Subtype specific regulation of inositol 1,4,5 triphosphate receptors. Controlling agents in time and space. *J Gen Physiol* 2001;117:431–434
62. Taylor CW. Store operated Ca²⁺ entry: a STIMulating stOrai. *Trends Biochem Sci* 2006; 31:597–601
63. Panyi G, Vannosi G, Bodnor A et al. Looking through ion channels: recharged events in T cell signaling. *Trends Immunol* 2004;25:565–569
64. Ayub K, Hallett MB. The mitochondrial ADPR link between Ca²⁺ store release and Ca²⁺ influx channel opening in immune cells. *FASEB J* 2004;18:1335–1338
65. Di Fiore PP. Life on Mars, cellular speaking. *Nature* 2003;424:624–625
66. Savignac M, Gomes B, Gallard A et al. Dihydropyridine receptors are selective markers of Th-2 cells. *J Immunol* 2004;172:5206–5212
67. Gomes B, Savignac M, Cabral MD et al. Cyclic GMP/protein kinase G pathway controls dihydropyridine channels. *J Biol Chem* 2006;281:12421–12427
68. Zhu P, Liu X, Labelle E, Freedman B. Mechanisms of hypotonicity induced calcium signaling. *J Immunol* 2005;175:4981–4989
69. Lefkowitz RJ, Whalen EJ. β -Arrestins. *Curr Opin Cell Biol* 2004;16:162–168
70. Wojcikiewicz R, Tobin AB, Nahorski SR. Desensitization of cell signalling mediated by phosphoinositidase C. *Trends Pharmacol Sci* 1993;14:279–228

Chapter 3

Platelet Aggregation and Its Control

Abstract Platelet aggregation exemplifies various cellular activation processes. Adhesion of platelets to collagen is by means of an integrin and a signal transduction pathway. Nitric oxide helps to control platelets and other cells of the immune system. It is produced by nitric oxide synthases.

3.1 Platelet Aggregation

The primary function of platelets is to ensure the integrity of the capillary walls by the formation of aggregates at any site of endothelial cell denudation. Adhesion of platelets is followed by their aggregation and a “release reaction” of subcellular granules, whose mediators cause local vasoconstriction and formation of a blood clot. In fact, the second function of platelets is provision of a surface for thrombin generation, that is, a site for conversion of factor X to Xa and of prothrombin to thrombin, so that fibrinogen will be converted to fibrin strands. Bonding of “tissue factor” thromboplastin drives this process, as does externalization of phosphatidyl-serine, which creates a catalytic surface on the surfaces of platelets for the activation of coagulation.

The “first phase” of platelet aggregation is triggered by various surface-acting agents, such as adrenalin acting on α_2 -receptors, serotonin, vasopressin, small amounts of adenosine diphosphate (ADP), collagen, free fatty acids like arachidonate (C20:4), thrombin, antigen-antibody complexes, and endotoxin. Platelet-activating factor (PAF) is the most powerful aggregating agent since it works at concentrations of 10–100 pmol. Information on these means of aggregation is mainly derived from in vitro studies [1]. Unless one works in haematology, there are many aspects of the physiology to digest. Table 3.1 summarizes mechanisms of platelet aggregation, mediated by integrin $\alpha_{IIb}\beta_3$ binding of fibrinogen and von Willebrand factor, leading to activation of tyrosine kinase Syk and thus cell activation (Fig. 3.1).

Adrenalin acts on platelet α_2 -receptors to stimulate turnover of phosphoinositides, inducing entry of Ca^{2+} ions into platelets [2]. Similarly, when collagen bonds to platelet $\alpha_2\beta_1$ -receptors, activation of phospholipase C (PLC) leads to membrane phosphatidyl-inositol (PtdIns) hydrolysis, protein kinase C (PKC) is

Table 3.1 *Mechanisms of platelet activation*

<i>Agonists:</i> ADP via P2Y ₁₂ receptors. Thrombin, serotonin, PAF, TxA ₂ , adrenalin, SDF-1
Von Willebrand factor
Fibrinogen acting on GPIIb/IIIa(α 2 β 3) receptors
Collagen + von Willebrand factor $\rightarrow$ GPIb/V/IX receptors
Collagen acting on integrin α 2 β 1 + GPVI
Laminin acting on integrin α 6 β 1 + GPVI
<i>Mechanisms^a</i>
Calcium ion entry
Activation of tyrosine kinases like Syk, of phospholipases C, and PI3K $\rightarrow$ PIP ₂
Actin cytoskeleton remodelling initiated from G α 12/13 determined by RhoA $\rightarrow$ MLCK
The final pathway for all agonists is activation of integrin glycoprotein IIb/IIIa(α 2 β 3), resulting in release of
α -granules with von Willebrand factor, GPIIb/IIIa, P-selectins, PDGF
Dense granules containing serotonin and ADP
Lysosomes containing cathepsin and elastase
Platelet aggregation clearly exemplifies processes ^a like activation of phospholipase C and of PI3K, as discussed in Chap. 2, that lead to entry of calcium ions and so the activation of actin filament-mediated discharge of granules. All this is preceded by platelet shape change caused by thrombin and thromboxane A ₂ , acting via G α 13-coupled receptors to activate RhoA and thereby myosin light-chain kinase (MLCK)

ADP adenosine diphosphate, *MLCK* myosin light-chain kinase, *PAF* platelet-activating factor, *PDGF* platelet-derived growth factor, *TxA₂* thromboxane A₂

activated, and there is then Ca²⁺ ion influx [3]. Calcium entry is accompanied by entry of Na⁺ ions by the membrane Na⁺/H⁺ ion exchanger and by the action of thromboxane A₂ (TxA₂). PAF induces hydrolysis of membrane PtdIns and activation of PKC, and it initiates synthesis of thromboxanes. The TxA₂ then sustains platelet aggregation since the secondary release of ADP leads to inhibition of adenylyl cyclase, producing cyclic adenosine monophosphate (cAMP), which would oppose aggregation [4]. In fact, prostacyclin activates adenylyl cyclase and boosts cAMP.

Platelets contain representatives of all G protein families [5]: Gs, Gi, Gq, and G12. When thrombin acts via G α 12/13, there is cdc/Rac guanosine triphosphatase (GTPase) activation of cytoskeletal actin reorganization to effect change of cell shape and migration. Protease-activated receptors (PARs) 1 and 4 for thrombin are coupled to Gq and G α 12/13 G protein-coupled receptors (GPCRs), as are the activated TxA₂ receptors. ADP induces the activation of Gq, G α ₁₂, and Gi via P2Y₁ and P2Y₁₂ metabopurinic receptors. Gq activates PLC (types β 2 and β 3) and leads to activation of phospholipase A₂ and cyclooxygenase 1 so that TxA₂ is produced. The G α ₁₂ activates PI3 kinase (PI3K) and thus Akt concurrently; Rap1.Gi inhibits the adenylyl cyclase.

ADP release is integral to the “second phase” of the aggregation process [6–8]. The sequence is platelet adhesion $\rightarrow$ release reaction $\rightarrow$ ADP-mediated sustained aggregation $\rightarrow$ formation of TxA₂. Yet, platelet activation is limited by (a) endothelium-derived prostacyclin (PGI₂); (b) nitric oxide (NO), which raises intraplatelet cAMP; and (c) the membrane CD39, which hydrolyses ADP to inactive AMP, from which there is formation of adenosine, an inhibitor of platelet aggregation [9]. As the reader will know, there is much concern about the potential of nonsteroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase 2 (Cox-2) inhibitor drugs to

cause strokes and heart attacks. NSAIDs inhibit the Cox-1 enzyme of endothelial cells, so that prostacyclin formation is thwarted. So, when platelets continue to produce TxA₂, they show enhanced aggregability. In obese women, there is a correlation between rates of formation of F₂-isoprostanes and TxA₂ (Table 3.2).

With respect to the role of ADP, purinergic receptors are displayed on platelets. Their P₂X receptors are ligand-gated Ca²⁺ ion channels. Their P₂Y receptors for ADP are of two types [10]: the P₂Y₁ linked to Gα_q, which invoke shape change, and the P₂Y₁₂, which cause sustained aggregation involving exposure of membrane GPIIb/IIIa receptors and concurrent inhibition of adenylyl cyclase, decreasing cAMP levels. ADP releases TxA₂, which further sustains aggregation, this time with the help of integrin αIIbβ₃ (GPIIb/IIIa), which links activated platelets through fibrinogen bridges.

Since ADP performs a crucial role in the formation of stable platelet aggregates, the purinergic receptors operate to maintain the balance between resting and activation. Desensitization is part of the control. Accordingly, G protein-coupled receptor kinases (GRKs) can downregulate P₂Y₁₂ receptors and isoforms of PKC (i.e. classic PKCα and novel PKCδ) will restrain P₂Y₁ receptors [14].

Thrombin-induced platelet aggregation is effected by proteolytically activated receptors [15]. Human platelets express PAR1 and PAR4, whereas endothelial cells have PAR1 and PAR3. PAR1 is the primary mediator, whilst PAR4 has a backup role. The consequence is inhibition of adenylyl cyclase via Gα_i, and PLCβ activation leads to an internal rise of Ca²⁺ ions (via Gα_q) and Gα_{12/13}-induced actin reorganization, which entails activation of Rho and thereby p160Rock, and together with Ca²⁺ ions, it activates myosin light-chain kinase. Along with PLC hydrolysis of membrane PtdIns, there is activation of PKC [16] (cf. Fig. 2.1), and its target is the 47-kDa pleckstrin (platelet and leucocyte kinase substrate). There is also PI3K activation [17], so PIP₂ becomes available, and the Rac-GTPase causes uncapping of gelsolin from the actin filaments. At thrombin concentrations of 0.1–1.0 nM, the release of ADP, serotonin, and fibrinogen are triggered. The high-affinity receptors for thrombin lead to exposure of the surface glycoprotein Ib. Then, there is binding

Table 3.2 *Events during ADP-induced platelet aggregation*

-
1. First phase: Rapid influx of calcium ions via ATP gated P₂X₁ ion channels [11]
 2. Second phase: ADP acting on P₂Y₁ receptors via Gα_q causes phospholipase C activation, mobilisation of Ca²⁺ ion stores and shape change, so there is transient aggregation of platelets. Localisation of adhesion receptor GPIb-IX-V in lipid rafts is required [12], and GPVI is crucial
 3. Via ADP receptor P₂Y₁₂, there is expression of GPIIb/IIIa receptors for fibrinogen, and alkalisation of platelet cytoplasm via Na⁺/H⁺ antiporters. Integrin activation is helped by platelet NADPHox ROS formation [13], so platelet aggregation is sustained
 4. Aggregation is aided by P₂Y₁₂-mediated *inhibition of adenylyl cyclase*, stopping cAMP formation, which would oppose Ca²⁺ ion mobilization
 5. Formation of TxA₂ enhances intrinsic ADP release and reinforces inhibition of adenylyl cyclase. ADP is an amplifier of platelet activation, as are TxA₂ and PAF
-

ADP adenosine diphosphate, *ATP* adenosine triphosphate, *cAMP* cyclic adenosine monophosphate, *ROS* reactive oxygen species, *TxA₂* thromboxane A₂

of von Willebrand factor, with consequent exposure of GPIIb/IIIa molecules that bind plasma fibrinogen. By its activation of Akt, thrombin stimulates phosphodiesterase 3A (PDE3A), thereby lowering intraplatelet cAMP, which occurs also by thrombin inhibition of adenylyl cyclase.

Collagen-induced platelet adhesion, acting via GPIa/IIa (integrin $\alpha 2\beta 1$), and GPVI (Fig. 3.1) provokes ADP and TxA₂ release. At low doses of collagen, there is release of ATP, which by its action on P2X₁ receptors activates PKC and thence extracellular signal-related kinase (ERK) kinase, which leads to the release of granules. At higher levels of collagen exposure, there is $\alpha IIb\beta 3$ integrin (GPIIb/IIIa) outside in signal transduction to be added [18, 19]. In vivo semaphorin 3A from endothelial cells will reduce such Rac1-dependent actin rearrangement [20].

Clinicians are always interested to know how to inhibit the aggregation of platelets [21]. Firstly, adenosine, prostacyclin, prostaglandin E₁ (PGE₁) and PGD₂ will inhibit platelet aggregation because they all stimulate adenylyl cyclase or otherwise lead to a rise of intraplatelet cAMP. Secondly, NO[•], arising from vascular endothelial cells, activates platelet guanylyl cyclase so that cyclic guanosine monophosphate (cGMP) is formed, and a cGMP-dependent protein kinase inhibits platelet aggregation. The cyclic nucleotides cAMP and cGMP both stop activation of the Na⁺/H⁺ antiport of platelets [22]. NO[•] donor drugs that elevate cGMP are now available [23]. Their action stimulates vasodilator-stimulated phosphoprotein (VASP), a regulator of actin-cytoskeletal reorganization. The use of aspirin is standard. It works by preventing the formation of TxA₂ (cf. Fig. 6.2b), as is evident by a fall of the stable metabolite 11-dehydro-thromboxane B₂. Yet, aspirin does not prevent aggregation of platelets by subendothelial collagen. Furthermore, over 20% of the population have “aspirin resistance”. Flow cytometric measurement of the levels of VASP phosphorylation-dephosphorylation turn out to be proportionate to light transmission aggregometry for measurements of drug inhibition of the P2Y₁₂ ADP receptors [24].

3.2 Platelet Adhesion to Collagen

Platelet adhesion is now well understood [25]. When subendothelial collagen binds to platelet $\alpha 2\beta 1$ integrins, low-affinity receptors GPVI are activated [26], and they associate with the Fc receptor γ -chain dimers, so that activation of Syk src-kinase leads to PLC $\gamma 2$ activation; thus, there is formation of IP₃ and diacylglycerol (DAG). Calcium ion entry and DAG action cause platelet activation. Soon, there is exposure of GPIIb/IIIa on the platelet surface. Once thromboxane is formed, ADP will be released, and aggregation is supported. There has been recent work on an alternative pathway by which CalDAG-GEF activates Ras-GRP [27]. Since mouse platelets lacking CalDAG-GEF1 show compromised integrin-dependent aggregation, as Rap1 is not activated, this pathway also is important.

Unexplained in Fig. 3.1 is how exposure of integrins like $\alpha 2\beta 1$ and $\alpha IIb\beta III$ occurs [28]. Details of the platelet integrins have been presented [29] Platelet activation/aggregation means fibrinogen adherence to $\alpha IIb\beta III$ (GPIIb/IIIa). Both via Ca²⁺ ions and via DAG → PKC, there is stimulation of the monomeric GTPase

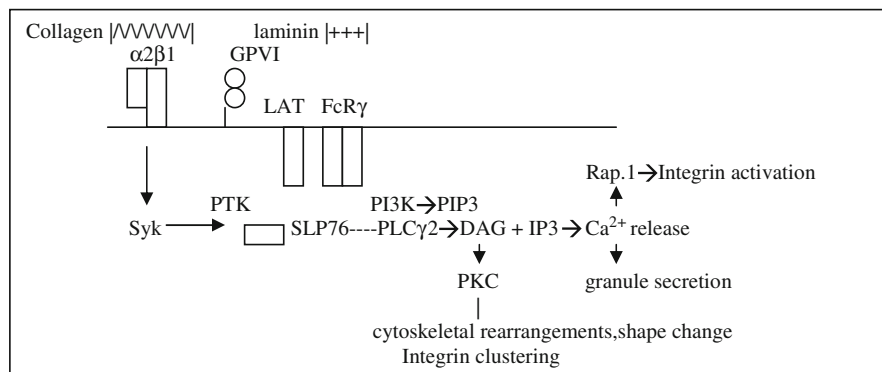


Fig. 3.1 Integrin $\alpha 2 \beta 1$ and associated GPVI in collagen adhesion. Syk is a (splenic) tyrosine kinase. SLP76 adaptor converts integrin signals [28] via extracellular signal-related kinase (ERK) activation to platelet spreading and activation. GPVI and GPIb signals convert $\beta 1 / \beta 3$ integrins to their high-affinity state and so release adenosine diphosphate (ADP) and thromboxane A₂ (TxA₂) [31]. Lipid rafts are crucial for signaling via GPVI and the FcR γ receptor [33], just as for antigen receptor signalling in T and B cells. PECAM1 inhibits platelet responses to collagen [33]. The effect of laminin stimulation via integrin $\alpha 6 \beta 1$ and GPVI is similar to that by collagen [34]

Rap1, which in turn stimulates RIAM adaptor protein and so the ERM protein Talin, which induces integrin activation. Platelet adhesion depends on $\alpha 2 \beta 1$ /GPVI and GPIb-IX-V adherence to collagen and $\alpha 6 \beta 1$ onto laminin.

Exposure of platelets to ADP causes the expression of Lox-1 receptors (for oxLDL) via PKC activation. There is then inside-out signalling that leads to display of $\alpha \text{IIb} \beta 3$ receptors [30]. The process is offset by exposure of platelets to aspirin and pravastatin.

In sum, the vascular endothelium controls the adhesion/aggregation of platelets by three means: (a) formation of prostacyclin from arachidonic acid by means of Cox-1, (b) the endothelial ecto-adenosine diphosphatase (ecto-ADPase) pathway, and (c) the formation of NO from L-arginine.

All this is relevant when one considers the importance of platelets in atherogenesis [35].

3.3 Platelets and Immunity

Not surprisingly, platelets participate as immune cells [36]. They can engulf bacteria, and they produce microbicidal proteins. By release of their lipid mediators (12-HETE and TxA₂), they deliver substrates for activation of polymorphonuclear leucocytes (PMNs). They release chemokines that recruit other immune cells. They release interleukin 1 (IL-1), which activates endothelium for inflammation. A role for IL-1 β in atherosclerosis has long been proposed. P-selectin of platelets and endothelial cells initiates adhesion of platelets and mediates the adhesion of platelets to monocyte-macrophages, neutrophils and lymphocytes. Platelet factor 4 (PF4) enhances monocyte adhesion. PF4 also induces expression of E-selectin by

endothelial cells. When CD40L is expressed on the membrane of activated platelets, it stabilizes arterial thrombi. Release of CD40 ligand from platelets induces inflammatory responses in endothelium [36].

3.4 Constitutive and Inducible NO Synthetases

$\text{NO}^\bullet$ is formed from the guanidine group of L-arginine by a family of enzymes called NO synthases (NOS). All use nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen as cosubstrates [37, 38] for the reaction $\text{L-Arg} \rightarrow \text{N-OH-L-arginine} \rightarrow \text{L-Citrulline} + \text{NO}^\bullet$. NOS occurs in three distinct isoforms: (a) constitutive neuronal NOS (NOSI or nNOS), (b) inducible NOS (NOSII or iNOS), (c) constitutive endothelial NOS (NOSIII or eNOS) (Fig. 3.2).

Constitutive NOS (cNOS or eNOS) of endothelial cells is a Ca^{2+} -calmodulin-requiring enzyme that is attached under the plasma membrane of endothelial cells by myristoyl and palmitoyl tails. It produces small amounts of $\text{NO}^\bullet$ for short periods. Its activity is stimulated by hsp90, calmodulin, and kinases Akt and protein kinase A. Caveolin-1 in the membrane negatively regulates its function. Such locally generated NO regulates compartmentalized protein S-nitrosylation within cells [42]. In turn, that can provoke protein glutathiolation [43]. By its activation of guanylyl cyclase, and thereby cGMP-dependent protein kinase [44], the endothelial $\text{NO}^\bullet$ leads to *vasodilation, and it reduces adhesion of platelets and leucocytes*. Indeed, endothelial-derived $\text{NO}^\bullet$ will be anti-inflammatory [45], and moderate amounts of NO are anti-apoptotic. Nevertheless, ordinarily, in the microcirculation $\text{NO}^\bullet$ is not produced by the endothelial cells but is carried there by haemoglobin [46].

iNOS is stimulated within macrophages by proinflammatory cytokines to a constant activated state, independently of calcium. Such iNOS produces large nanomolar (nM) amounts of $\text{NO}^\bullet$ over a long time. This $\text{NO}^\bullet$ has a role in defence against oxidative stress for it inactivates superoxide. Nevertheless, too much $\text{NO}^\bullet$ generated

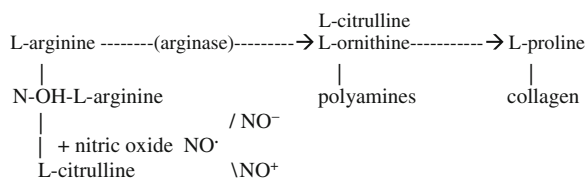


Fig. 3.2 The metabolism of L-arginine. Nitric oxide (NO) gains an electron to form nitroxyl anion NO^- or loses an electron to form the nitrosonium ion NO^+ . It can become nitrite NO_2^- or nitrate NO_3^- or be autooxidized in an aqueous environment to dinitrogen trioxide N_2O_3 . In blood, $\text{NO}^\bullet$ forms nitrosothiols RSNO , and there is a substantial pool of $\text{NO}^\bullet$ as nitrated fatty acids, such as linoleate [39]. Nitrite is also a significant source of $\text{NO}^\bullet$ [40]. Nitrated fatty acids (*nitroalkenes*) inhibit the secretion of proinflammatory cytokines by macrophages, and they stop tumor necrosis factor- α ($\text{TNF}\alpha$) induction of VCAM1 and therefore rolling and adhesion of monocytes [41]

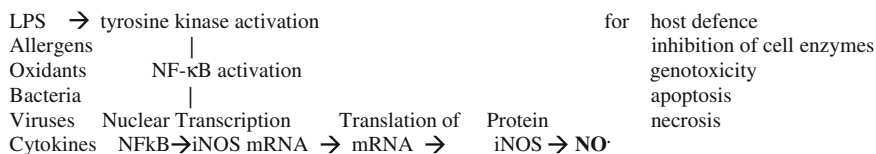


Fig. 3.3 Signal transduction leading to expression of inducible nitric oxide synthetase. Details of transcription factor nuclear factor-kappa B ($\text{NF-}\kappa\text{B}$) are in Chap. 11

by *iNOS* is often damaging to tissues [47, 48]. There are potential controls. At the initiation of an inflammatory response, eNOS-derived NO augments nuclear factor-kappa B ($\text{NF-}\kappa\text{B}$) activity and so facilitates iNOS expression. Thereafter, high NO levels have a negative effect on $\text{NF-}\kappa\text{B}$, so iNOS transcription is inhibited [49]. Furthermore, the cyclopentenone prostaglandin 15-deoxy-PGJ2 inhibits interferon- γ ($\text{IFN}\gamma$) induction of iNOS [50].

Tissue damage arises as a consequence of $\text{NO}\cdot$ interacting with free radicals or the transition metals and its ability to cause nitration or nitrosation of proteins. In fact, $\text{NO}\cdot$ is utilised to effect damage by cytotoxic T cells or within vacuoles when macrophages kill microorganisms. Whenever $\text{NO}\cdot$ interacts in vivo with superoxide anions O_2^- , the local formation of peroxynitrite ONOO^- causes tissue damage of the endothelial cells at sites of inflammation. Thus, iNOS accounts for immune-mediated tissue injury. Peroxynitrite will activate thromboxane synthetase and the Cox enzymes, but it inhibits the synthesis of prostacyclin. Yet, one should point out that CO_2 can moderate the formation of ONOO^- .

Peroxynitrite ONOO^- can account for apoptosis of endothelial cells, macrophages, fibroblasts, neutrophils [51, 52] or lymphocytes [53], and it causes aggregation of platelets. Yet, early on in inflammation $\text{NO}\cdot$ is antiapoptotic by its inhibition of caspases and by its induction of stress proteins. In fact, to support immune defence the formation of $\text{NO}\cdot$ must be rapid, but thereafter its formation has to be shut down to avoid tissue damage. Actually (Fig. 3.2), there is $\text{NF-}\kappa\text{B}$ control of the iNOS gene, and the biphasic $\text{NF-}\kappa\text{B}$ response can account for the changing responses of macrophages [54] (Fig. 3.3).

3.5 Nitric Oxide and the Immune System

iNOS regulates approximately 200 genes that are relevant to inflammation, infection or apoptosis. $\text{NO}\cdot$ influences a variety of processes [55], like (a) the differentiation, proliferation and apoptosis of immune cells, (b) expression of costimulatory and adhesion molecules for lymphocytes, (c) production of cytokines, (d) deposition of extracellular matrix components and (e) antimicrobial activity or tumoricidal capacity of leucocytes. The proinflammatory cytokines $\text{IFN}\gamma$ and tumor necrosis factor- α ($\text{TNF}\alpha$) can stimulate iNOS in macrophages, dendritic cells or NK cells. So will

IL-12/IL-18 acting on macrophages since they lead to the production of IFN γ . Macrophage-derived NO $\cdot$ downregulates activation of T lymphocytes via its control of the Jak3/Stat5 signalling pathway [56]. Generally, local concentrations of NO $\cdot$ will suppress formation of Th1 cells [57], but they support production of Th2 cells, as is the case in allergies. Yet, using in vitro studies Niedbala et al. [55] showed that low NO concentrations (5 μ M) acting via cGMP formation promote production of Th1 lymphocytes. Furthermore, NO achieves this by enhancing the expression of IL-12 R β 2 receptors [58]. These are murine cells. It certainly looks as though NO fine-tunes T lymphocyte responses. NO can even induce Treg cells [56].

Accordingly, NO $\cdot$ can be utilised to inhibit the recruitment of lymphocytes at sites, and it will inhibit proliferation of T or B cells [59] and antibody production. NO $\cdot$ can up- or downregulate chemokines and cytokines since it affects the signal cascades and transcription factors in various ways. The chemokines IL-8, MCP-1, MIP-1, and M-CSF are promoted by NO. NO $\cdot$ activates enzymes crucial to inflammation like phospholipase A2 and Cox-2 cyclooxygenase, and it activates plasminogen activator and the metalloproteinases. NO $\cdot$ itself promotes the activity of iNOS, but it also induces peroxiredoxins that are protective [60].

Inevitably, there will be references in this text to the numerous actions of NO $\cdot$. Thus, NO $\cdot$ can inhibit immunoglobulin E (IgE)-mediated degranulation of mast cells (Chap. 6) [61]. In asthma, the eosinophils are a source of NO $\cdot$ -derived oxidants [62], and exhaled NO is used to monitor asthma and chronic obstructive pulmonary disease (COPD). NO regulates normal epithelial cell function, and the respiratory epithelium is a major source of NO [63, 64].

Murine macrophages readily form large amounts of NO $\cdot$. Yet, human macrophages show little formation of NO $\cdot$ in vitro, and in vivo IFN γ stimulation has to be sustained to boost formation of NO $\cdot$. In fact, human macrophages can be induced to upregulate iNOS [65], and human neutrophils show iNOS upregulation. Murine macrophages are controlled by the balance of Th1 cytokines that induce iNOS and Th2 cytokines that induce arginase. Arginase (Fig. 3.2) leads to the formation of L-ornithine, which can lead to formation of polyamines [66, 67] and so cell proliferation. Alternatively, when there are pathological granulomata, formation of L-proline serves to boost local collagen formation [68].

References

1. Yee DL, Sun C W, Bergeron AL et al. Aggregometry detects platelet hyperactivity in healthy individuals. *Blood* 2005;106:2723–2729
2. Siess W. Inositol phospholipids metabolism and platelet function. *Biochem Pharmacol* 1986;35:3184–3187
3. Karniguian A, Grelac F, Levy-Toledone S et al. Collagen induced platelet activation involves protein kinase C pathway. *Biochem J* 1990;268:325–331
4. Paul BZS, Jin JG, Kunapuli SP. Molecular mechanism of thromboxane A2 induced platelet aggregation. *J Biol Chem* 1999;274:29108–29114
5. Offermanns S. Activation of platelet function through G protein-coupled receptors. *Circ Res* 2006;99:1293–1304

6. Jurk K, Kehrel BE. Platelets: physiology and biochemistry. *Semin Thromb Hemost* 2005;31:381–392
7. Jackson SP, Nesbitt WS, Kulkarni S. Signaling events underlying thrombus formation. *J Thromb Haemost* 2003;1:1602–1612
8. Gachet C. ADP receptors of platelets. *Thromb Haemost* 2001;86:222–232
9. Birk AV, Broekman J, Gladek EM et al. Role of extracellular ATP metabolism in regulation of platelet reactivity. *J Lab Clin Med* 2002;140:166–175
10. Dorsam RT, Kunapuli SP. Central role of P2X1 receptors in platelet activation. *J Clin Invest* 2004;113:340–345
11. Oury C, Toth-Zsamboki E, Vermeylen J, Hoylaerts FF. P2X1 mediated activation of ERK2 contributes to platelet secretion and aggregation by collagen. *Blood* 2002;100:2499–2500
12. Shrimpton CN, Borthakur G, Larrucea S et al. Localization of the adhesion receptor GP Ib-IX-V complex to lipid rafts. *J Exp Med* 2002;196:1057–1066
13. Begonja AJ, Gambaryan S, Geiger J et al. Platelet NADPHox generated ROS production regulates α IIb β 3 integrin activation. *Blood* 2005;106:2757–2760
14. Mundell SJ, Jones ML, Hardy AR et al. Distinct roles for PKC isoforms in regulating platelet purinergic receptor function. *Mol Pharm* 2006;70:1132–1134
15. Bahou WF. Attacked from within, blood thins. *Nat Med* 2002;8:1082–1083
16. Clark EA, Shattil SJ, Brugge JS. Regulation of protein tyrosine kinases in platelets. *Trends Biochem Sci* 1994;19:464–469
17. Rittenhouse SE. Phosphoinositide-3-kinase and platelet function. *Blood* 1995;88:4401–4414
18. Alberio L, Dale GL. Platelet-collagen interactions: membrane receptors and intra-cellular signalling pathways. *Eur J Clin Invest* 1999;29:1066–1075
19. Gassama-Diagne A, Yu W, ter Beest M et al. Phosphatidylinositol 3,4,5 triphosphate regulates formation of the basolateral membrane in epithelial cells. *Nat Cell Biol* 2006 8:963–970
20. Kashiwagi H, Shiraga M, Kato H et al. Negative regulation of platelet function by semaphorin 3A of endothelial cells. *Blood* 2005;106:913–921
21. Manus AJ, Safier LB. Thromboregulation: multicellular modulation of platelet reactivity in hemostasis and thrombosis. *FASEB J* 1993;7:516–522
22. Kimura M, Lasker N, Aviv A. Cyclic nucleotides attenuate thrombin evoked alterations in parameters of the platelet Na⁺/H⁺ antiport. *J Clin Invest* 1992;89:1121–1127
23. Yamamoto T, Bing R. Nitric oxide donors. *Proc Soc Exp Biol Med* 2000;225:200–206
24. Pampuch A, Cerletti C, de Gaetano G. Comparison of VASP-phosphorylation assay to light-transmission aggregometry in assessing inhibition of the platelet ADP P2Y₁₂ receptor. *Thromb Haemost* 2006;96:767–773
25. Judd BA, Myung PS, Leng L et al. Hematopoietic reconstitution of SLP76 corrects hemostasis and platelet signaling through α IIb β 3 and collagen receptors. *Proc Natl Acad Sci U S A* 2000;97:12056–12061
26. Han J et al. Reconstructing and deconstructing agonist induced activation of integrin α IIb β 3. *Curr Biol* 2006;16:1796–1806
27. Crittenden JR, Bergmeier W, Zhang Y et al. CalDAG-GEF1 integrates signaling for platelet aggregation and thrombus formation. *Nat Med* 2004;10:982–986
28. Nieswandt B, Watson SP. Platelet-collagen interaction: is GP VI the central receptor? *Blood* 2003;102:446–449
29. Kasirer-Friede A, Kahn ML, Shattil SJ. Platelet integrins and immunoreceptors. *Immunol Rev* 2007;218:247–264
30. Marwoli MR, Hu C-P, Mohandas B et al. Modulation of ADP induced platelet activation by aspirin and pravastatin: role of Lox-1, nitric oxide, oxidative stress and inside-out integrin signaling. *J Pharmacol Exp Ther* 2007;322:1324–1332
31. Cho M, Liu J, Pestina TI et al. The roles of α IIb β 3 mediated outside-in signal transduction, TxA₂ and ADP in collagen induced platelet aggregation. *Blood* 2003;101:2646–2651
32. Bodin S, Viala C, Ragab A, Payrastre B. A role of lipid rafts in organization of a key Fc γ RIIa-mediated signaling pathway in platelets. *Thromb Haemost* 2003;89:318–330
33. Patil S, Newman DK, Newman PJ. Platelet endothelial cell adhesion molecule-1 serves as an inhibitory receptor that modulates platelet responses to collagen. *Blood* 2001;97:1727–1732

34. Inoue O, Suzuki-Inoue K, McCarty OJT et al. Laminin stimulates spreading of platelets through integrin $\alpha 6 \beta 1$ dependent activation of GPIIb/IIIa. *Blood* 2006;107:1405–1412
35. Davi G, Patrono C. Platelet activation and atherothrombosis. *N Engl J Med* 2007;357:2482
36. von Hundelshausen P, Weber C. Platelets as immune cells: bridging inflammation and cardiovascular disease. *Circ Res* 2007;100:27–40
37. Moncada S, Higgs A. The L-arginine nitric oxide pathway. *N Engl J Med* 1993;329:2002–2012
38. Anggard A. Nitric oxide. *Lancet* 1994;343:1199–1206
39. Kalyanaraman B. Nitrated lipids: a class of cell signaling molecules. *Proc Natl Acad Sci U S A* 2004;101:11527–11528
40. Cosby K, Partoviks KS, Crawford JH et al. Nitrite reduction to NO by deoxy-haemoglobin vasodilates the human circulation. *Nat Med* 2003;9:1498–1505
41. Cui T, Schopfer FJ, Zhang J et al. Nitrated fatty acids: endogenous anti-inflammatory signaling mediators. *J Biol Chem* 2006;281:35686–35698
42. Iwakiri Y, Satoh H, Chatterjee S et al. NO synthase generates NO locally to regulate compartmentalized protein S-nitrosylation. *Proc Natl Acad Sci U S A* 2006;103:19777–19782
43. Hill BG, Bhatnager A. Role of glutathiolation in regulation of protein function. *IUBMB Life* 2007;59:21–26
44. Hofmann F. The biology of cyclic GMP-dependent protein kinases. *J Biol Chem* 2005;280(1):1–4
45. Paul-Clark MJ, Gilroy DW, Willis D, Willoughby DA, Tomlinson A. Nitric oxide synthase inhibitors have opposite effects on acute inflammation depending on route of inflammation. *J Immunol* 2001;166:1169–1177
46. Singel DJ, Stamler JS. Blood traffic control. *Nature* 2004;439:297
47. Piedrafito D, Liew FY. Nitric oxide. *Rev Med Microbiol* 1998;9:179–189
48. Sass G, Koreber K, Bang R et al. Inducible nitric oxide synthase is critical for immune mediated liver injury in mice. *J Clin Invest* 2001;171:979–988
49. Villar IC, Francis S, Webb A et al. Novel aspects of endothelium-dependent regulation of vascular tone. *Kidney Int* 2006;70:840–853
50. Chen C-W, Chang Y-H, Tsi C-J, Lin W-W. Inhibition of IFN γ mediated iNOS induction by 15 deoxy-prostaglandin J2. *J Immunol* 2003;171:979–988
51. Rubbo H, Rodi R, Trujillo M et al. Nitric oxide regulation of superoxide and peroxynitrite dependent lipid peroxidation. *J Biol Chem* 1994;269:26066–26077
52. Messmer UK, Brune B. Nitric oxide induced apoptosis: p53 dependent and p53 independent signalling pathways. *Biochem J* 1996;319:299–305
53. Bronte V, Kasic T, Gri G et al. Boosting antitumor responses of T lymphocytes infiltrating human prostate cancers. *J Exp Med* 2005;201:1257–1268
54. Connelly L, Palacios-Callender M, Ameixa C et al. Biphasic regulation of NF κ B activity underlies pro- and anti-inflammatory actions of nitric oxide. *J Immunol* 2001;166:3873–3881
55. Bogdan C. Nitric oxide and the immune response. *Nat Immunol* 2001;2:907–916
56. Bingisser RM, Tilbrook PA, Holy PG, Kees UR. Macrophage derived nitric oxide regulates T cell activation via reversible disruption of the Jak3/Stat5 signaling pathway. *J Immunol* 1998;160:5729–5734
57. Mahidhara RS, Hoffman RA, Huang S et al. Nitric oxide mediated inhibition of caspase dependent T lymphocyte proliferation. *J Leukoc Biol* 2003;74:403–411
58. Niedbala W, Cai B, Liew FY. Role of nitric oxide in the regulation of T cell functions. *Ann Rheum Disease* 2006;65(Suppl 11):iii37–iii40
59. Vig M, Srivastava S, Kampal U et al. Inducible NO synthase in T cells regulates T cell death and immune memory. *J Clin Invest* 2004;113:1734–1742
60. Diet A, Abbas K, Bouton C et al. Regulation of peroxiredoxins by nitric oxide in macrophages. *J Biol Chem* 2007;282:36199–36205
61. Eastmond NC, Banks EMS, Coleman JW. Nitric oxide inhibits IgE-mediated degranulation of mast cells. *J Immunol* 1997;159:1444–1448
62. MacPherson JC, Comhair SA, Erzurum SC, Klein DF et al. Eosinophils are a major source of nitric oxide-derived oxidants in severe asthma. *J Immunol* 2001;166:5763–5770

63. Bove PF, van der Vliet A. Nitric oxide and reactive N species in airway epithelial signaling and inflammation. *Free Radic Biol Med* 2006;41:515–527
64. Ricciardolo FLM, Sterk PJ, Gaston B, Folkerts G. Nitric oxide in health and disease of the respiratory system. *Physiol Rev* 2004;84(3):731–765
65. Marriott HM, Ali F, Read RC et al. Nitric oxide levels regulate macrophage commitment to apoptosis or necrosis during pneumococcal infection. *FASEB J* 2004;18:1126–1128
66. Wallace HM, Fraser AV, Hughes AA. A perspective of polyamine metabolism. *Biochem J* 2003;376:1–14
67. Bronte V, Zanovello P. Regulation of immune responses by L-arginine metabolism. *Nat Rev Immunol* 2005;5:641–654
68. Hesse M, Modolell M, La Flamme AC et al. Differential regulation of NOS2 and arginase-1 by type 1:2 cytokines: granulomatous pathology is shaped by the pattern of l-arginine metabolism. *J Immunol* 2001;167:6533–6544

Chapter 4

Introduction to Signalling Cascades

Abstract There are various signal cascades that lead to the activation of genes. An outline is given of the mitogen-activated protein kinases (MAPKs), specifically the extracellular signal-related kinase (ERK) cascade and the opposing proapoptotic p38 and Jun N-terminal kinase (JNK) cascades. There are illustrations of other activation systems. Gene control is now realised to involve chromatin-remodelling epigenetic changes.

4.1 Serine/Threonine Kinases

The serine/threonine kinases use adenosine triphosphate (ATP) to phosphorylate Ser or Thr residues. Several are involved in transmitting signals to downstream substrate targets. Examples appear in Table 4.1 on page 60.

Details of the function of these enzymes are set out in standard works [1–3]. For example, p21-activated kinase (PAK), which has a p21-binding domain and a kinase domain, connects guanosine triphosphatases (GTPases) like Rac or cdc42 to cytoskeletal dynamics (Figs. 4.3, 9.3, 9.5).

4.2 Mitogen-Activated Protein Kinases and Signalling Cascades

When growth factors or cytokines act on cells, they trigger signal cascades that use extracellular signal-related kinases (ERKs), now known as mitogen-activated protein kinases (MAPKs). MAPKs are activated on tyrosine and threonine residues by the dual-specificity MAPK kinases (MAPKKs). Those MAPKKs are also phosphorylated and activated by serine- and threonine-specific MAPKK kinases (MAPKKKs). Accordingly, the activation sequence for MAPK is depicted as a three-kinase module [4, 5] (Fig. 4.1).

Each kinase cassette leads to the release of a transcription factor (TF), or set of TFs [6, 7], that influences gene activation [8]. The initial signal that activates the pathway can be a receptor tyrosine kinase (RTK) of, say, epidermal growth factor receptor (EGFR), or a G protein-coupled receptor (GPCR) for a G protein that leads to the MAPK cascade. Only transient activation of MAPK is required for mitogenesis, whereas sustained activation of MAPKs is necessary during cell differentiation.

Cell activation by epidermal growth factor (EGF) is a typical example of how the cascades are used. Binding of EGF to its receptor (Fig. 4.2) causes receptor dimerization and so an autophosphorylation that creates phosphotyrosines. Those phosphotyrosines then offer binding sites for the one –SH2 (Src-homology domain 2) domain

Generic	MAPK-KK-----MAPK-K-----MAPKinase
Generally	MEK kinase -----MKK-----p44/p42MAPK
Example	Raf -----MEK 1:2 -----ERK1/2

Fig. 4.1 A three-kinase module for mitogen-activated protein kinase (MAPK) activation

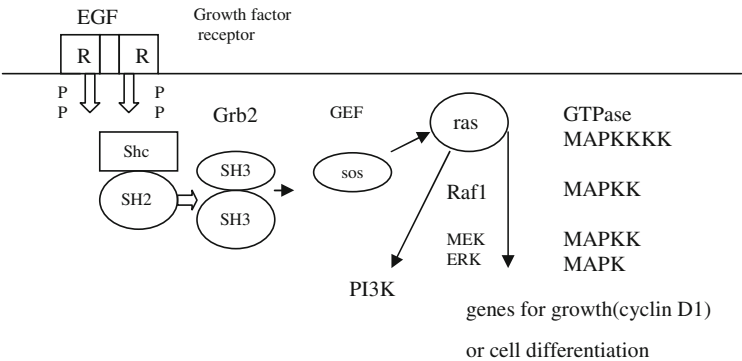


Fig. 4.2 How epidermal growth factor (*EGF*) activates its receptor and hence mitogen-activated protein kinase (*MAPK*). The phosphatase *PTP1* regulates the *EGF* and *PDGF* receptors. The *MAPKs* are controlled by non-receptor phosphatases like *HePTP* (*PTPN7*). *EGFR* signalling is enhanced by adherence of cells to fibronectin. *Grb2* growth factor receptor-bound protein, *Sos* son of sevenless, *GEF* for *Ras*. *Ras* proteins undergo cycles of guanosine triphosphate (*GTP*) loading by *Sos* and then hydrolysis by the guanosine triphosphatase-activating protein (*GAPs*) like *p120* and *neurofibromin* (Fig. 5.1b). *Ras* action via extracellular signal-related kinase (*ERK*) leads to induction of cyclin D1 of the cell cycle, suppression of cyclin-dependent protein kinase (*CDK*) inhibitors and promotion of *c-Myc*. Such *EGFR* signalling occurs subsequent to endocytosis of the receptors. There is dynamin-dependent internalization into endosomes, and there is clathrin-coated pit internalization into late endosomes that causes *MAPK* activation and subsequent mitosis. The endocytic scaffold protein intersectin (*ITSN*) cooperates with *EGFR* in cell growth and signalling. *MAPK* peaks within 5 min (together with *Akt* activation) and then at 5 h. Naturally, there will be controls; thus, protein tyrosine phosphatase *TCP.TP* reduces *EGFR* phosphorylation. Furthermore *Cbl* protein is recruited to the activated *EGFR* via *ITSN* [11], it ubiquitinates both the *EGFR* and itself, and they undergo degradation in lysosomes. One can also note that there is a cholesterol-regulated scaffold protein oxysterol binding protein (*OBSP*) that coordinates *ERK1/2* activation and control of phosphatases like *PP2A* [12]

and two –SH3 (Src-homology domain 3) domains of growth factor receptor-bound protein 2 (Grb2), and for the adaptor Shc, which has –SH2 and phosphotyrosine-binding domain (PTB) domains. The Grb-2 associates with Sos (Son of Sevenless), a guanine-nucleotide exchange factor (GEF) for Ras GTPase. Hence, the sequence Receptor → Shc(scaffold) → Grb2(adaptor) → Sos(GEF) complex shown in Fig. 4.3 causes activation of Ras, which leads to activation of Raf, which is an MAPKKK of the MAPK cascade [9]. Conversely, the adaptor Cbl protein leads to the endocytosis and destruction of receptors by ubiquitination [10].

There are several MAPK cascades [13], such as those for ERK, Jun N-terminal kinase (JNK) and p38 MAPK (Fig. 4.3). Each MAPK has an N-terminal that is for signal reception and a C-terminal that is involved in signal delivery. Each MAPK is activated by dual phosphorylation of a threonine and tyrosine residue in a three-amino acid activation domain, so that activity is enhanced by 1,000. The vital tripeptide activation sequence for an ERK is Thr-Glu-Tyr, for a JNK it is Thr-Pro-Tyr and for p38 it is Thr-Gly-Tyr.

Growth factors stimulate the ERK cascade, whilst lipopolysaccharide (LPS), physiological stresses and proinflammatory cytokines like interleukin 1 (IL-1) or tumour necrosis factor-α (TNFα) stimulate the stress kinases JNK/SAPKs (stress-activated protein kinases) and p38 MAPKs. Note also in **Fig. 4.3** that Ras, Rho, Rap small GTP-binding proteins serve as intermediaries between growth factor receptors and the MEK signal pathway. Rho and Rac also activate the SAPK/JNK stress path and the p38 MAPK pathways. Depending on the cell type, nitric

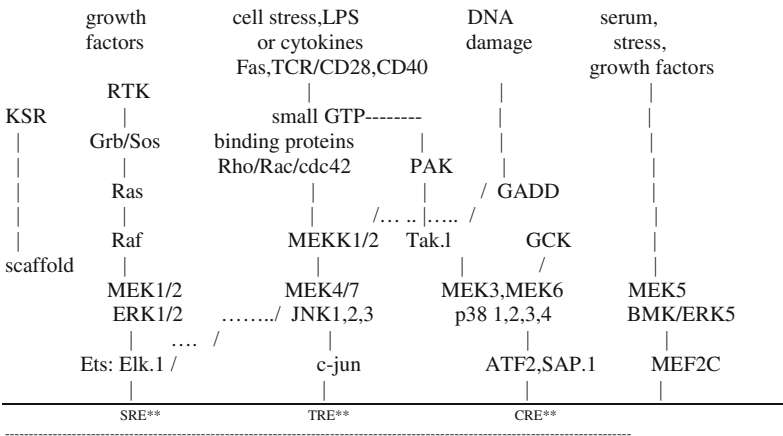


Fig. 4.3 Mitogen-activated protein kinase (MAPK) cascades. There are actually extracellular signal-related kinases (ERKs) 1:2 and 3, 4, 5, 6, 7 and 8. *BMK(ERK5)* big mitogen-activated protein kinase (activated by MEK5), *PAK* p21-activated protein kinase, *ATF2* activating transcription factor 2, *SAP1* stress-activated protein 1, ****SRE** serum response element → cyclin D, ****TRE** tetradecanoylphorbol acetate (TPA) response element, ****CRE** cyclic adenosine monophosphate (cAMP) response element, *GCK* germinal centre kinase, *GADD* growth arrest and DNA damage-inducible gene, *KSR* kinase suppressor of Ras, *cdc42(GTPase)* cell division cycle 42. Jun N-terminal kinase (*JNK*) connects to Elk1; *GADD45* to MEK1/2; *GCK* to MEK6

oxide (NO) may stimulate the various MAPKs. A rapid increase of growth factor EGF induces transient ERK activation, whilst the EGF concentration determines sustained ERK activation [14].

The specificity of the MAPKs is due to preference for phosphorylation of specific ternary complex factors (TCFs) [15]. They have names like Net-b, Elk-1, ATF2 (activating transcription factor 2), and SAP1. They induce transcription of genes by interacting with response elements SRE/TRE/CRE (serum response element/tetradecanoylphorbol acetate response element/cAMP response element) (Fig. 4.3). A network of immediate early gene products (Fos, jun, Egr-1, Myc) responds to the amplitude and duration of MAPK [16] and helps to explain how ERK signals can result in distinct biological outcomes [17]. ERK is essentially involved in cell survival. However, depending on the cell type and environmental condition (oxidative stress?), ERK can facilitate apoptosis [18].

The duration of ERK activation helps determine cell decisions. EGFR activation to cause transient ERK activation causes cell proliferation. There is negative-feedback control. Nerve growth factor via TrkA receptors causes sustained ERK activation and terminal differentiation (of neurones). In this case, positive feedback sustains the process [19].

ERKs, which will be anchored to MEK in the cytoplasm of resting cells, translocate into nuclei on mitogen stimulation. The process is rapid and reversible. However, with long-term MAPK stimulation, p42/p44 MAPKs progressively accumulate in cell nuclei in inactive form. This represents signal termination.

4.2.1 Organisation by Scaffold Proteins

Scrutiny of Fig. 4.3 reveals that cross talk between pathways is needed for coordinated responses by cells. Thus, the growth factor-induced mitogenic path and cellular stress/cytokine pathways [20], acting through ERK and JNK kinases, converge on TF (transcription factor) Elk-1. Such convergence explains the integration of cellular responses to extracellular stimuli.

Regulation of these cascades is achieved by scaffold proteins [21–24], by protein kinase insulators, and by specific docking sites. A scaffold can direct a single input into several signal pathways. Scaffolds prevent cross talk that is not required. A scaffold can enhance or inhibit a signal process [25]. Interaction of signal protein kinases with such scaffold molecules creates *signalling modules*. Thus, scaffold MP1 binds MEK1 and ERK1/2, and scaffold JIP2 binds MLK3 and MKK6, which are driven by Rac GTPase to activate p38 MAPK. Scaffold JIP1 binds MLK3/MKK6 so that Rac GTPase can activate the JNK. The MP1 scaffold binds to MEK1 and ERK1. Kinase suppressor of Ras (KSR) is a scaffold for the ERK1/2 signalling module [26] and for the MAPKKKs. It has a positive effect on ERK activation and thus formation of inflammatory cytokines in experimental arthritis [26]. β -Arrestin (Fig. 1.2) connects with GPCRs and internalises receptors, but also acts as a scaffold for components of the ERK pathway (Chap. 1, Ref. [24]). The caveolins that line the surface invaginations, called *caveolae*, where signal molecules are concentrated

(Fig. 5.5), are scaffolds in which caveolins function as negative regulators (e.g. for endothelial nitric oxide synthase [eNOS]).

The protein IQGAP is a scaffold protein with multiple interacting domains [27] that links components of signal cascades. It is upstream of ERK1/2, F-actin, or the Rac1 and cdc42 GTPases. IQGAP can scaffold for the ERK2 pathway when the response of the cell is to EGF (Fig. 4.2). In platelets, IQGAP stimulates Ca^{2+} /calmodulin and so release of prothrombinase; at the same time by other interactions with Rho GTPases like Rac1 and cdc42, it signals for actin polymerisation.

In Chap. 13 concerning T lymphocytes, adaptor protein Cbp is mentioned as a scaffold protein that binds Csk (C-terminal Src kinase), which is a negative regulator of Src family tyrosine kinases. In membrane ruffles, Csk negatively regulates growth factor EGF, and in polymorphonuclear leucocytes (PMNs) it stops adhesion molecule expression, so that PMNs can move but not adhere locally.

Another scaffold that regulates T cell activation is ADAP (Fig. 13.4). ADAP controls optimal T cell proliferation, expression of prosurvival Bcl-XL, and T cell cytokine production.

4.2.2 Redox Regulation of Signal Transduction

Cell signalling involves posttranslational modification of proteins at redox centres, which are reduced by gaining electrons or oxidised by losing electrons. A common redox reaction is the breaking or formation of a protein disulphide bridge ($-\text{S}-\text{S}-$) [28]. Usually, the amino acid that is implicated is cysteine, and it is modified by reactive oxygen species (ROS) or by reactive nitrogen species (RNS). ROS promote the signalling cascades at multiple steps, so that $\text{ROS}/\text{H}_2\text{O}_2$ has a considerable influence on signal pathways, particularly as H_2O_2 is not efficiently destroyed by periredoxins, as one would expect. For example, when platelet-derived growth factor (PDGF) is acting on its receptors, there is transient formation of H_2O_2 , which aids the activation of Src kinases and PI3 kinase (PI3K) [29]. The Src kinases stimulate $\text{Ras} \rightarrow \text{Rac}$ and so the various MAPKs [30]. Since the pathway phosphatases are inactivated when their cysteines are oxidised by ROS [31], there is this additional reinforcement of signal transduction.

S-Nitrosylation by NO is a versatile redox-based signalling mechanism [32]. Its effects can be inhibitory (e.g. on nuclear factor kappa B [NF- κ B] and on JNK) [33]. At physiological levels, superoxide facilitates such transmission, but high superoxide concentrations disrupt such transmission since superoxide reacts with the same cysteine thiol molecules.

4.2.3 *c-Jun* N-terminal Kinases

In *Drosophila* *c-Jun* N-terminal kinases (JNKs) signalling is concerned with morphogenesis, namely, movement and fusion of epidermal cell sheets. Targeted dis-

ruption of the JNK loci in mice has given insight into the functions of JNKs. In humans, JNK is SAPK [34]. Its cassette is downstream of small GTPases Rho, Rac and cdc42, showing that specificity can arise from alternative biochemical routes. There are scaffolds for the JNKs such as JIP1 and MEKK [35]. JNKs determine apoptosis in response to UV light, ceramide, superoxide anions or NO [36]. JNK activates AP-1 (activator protein 1) (i.e., c-Jun, cFos) (Fig. 4.4). Yet, JNK is inhibited by NF- κ B [37]. The nature of the death signal and the cell type are determinants of whether JNK will cause cell apoptosis [38]. Often, JNK mediates cell survival. Nevertheless, JNK can antagonise Akt [39]. JNK2 has a significant role in cerebral malaria [40]. Indeed, whilst JNK1 supports CD8⁺ cytotoxic T lymphocytes for tumour surveillance, JNK2 negatively regulates proliferation of antigen-specific CD8⁺ T cells [41]. JNK also plays a part in IL-1 cytokine-induced expression of metalloproteinases, as is revealed by the use of a specific inhibitor [42].

4.2.4 p38 MAP Kinases

The p38 MAPK pathway responds to environmental stresses like heat shock, UV light, irradiation, osmotic stress and proinflammatory cytokines and mitogens. p38 MAPK is involved in transcriptional regulation of cells, production of cytokines, determination of apoptosis [43] and cytoskeletal reorganisation. In effect, p38 controls inflammatory responses and cell proliferation [44]. It can be a tumour suppressor [44b]. There are six p38 MAPK isoforms. p38 α is expressed ubiquitously, and there are high levels in white cells. p38 β is found in brain and heart, p38 γ in muscle, and p38 δ in lungs, kidneys, gut and endocrine organs. Monocytes contain p38 α and macrophages p38 α and γ . The agent SB203580 is a widely used pyridinyl imidazole compound that specifically inhibits p38 α and p38 β .

There are various physiological roles for p38 MAPK. p38 MAPK has a negative influence on the MEK1/2 and ERK1/2 of the main MAPK pathway [5]. In neutrophils, there is p38 enhancement of phospholipase A2 release or arachidonate, which is then metabolized via Cox (cyclooxygenase) or lipoxygenase pathways

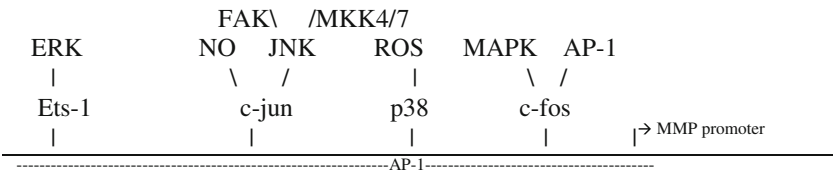


Fig. 4.4 Jun N-terminal kinase (*JNK*) and activator protein 1 (*AP-1*) activation of metalloproteinase. Focal adhesion kinase (*FAK*) is a phosphotyrosine kinase (*PTK*) that is activated by adhesion to extracellular fibronectin. *FAK* links to the Src-homology domain 2 (*SH2*) domain of Src kinases. The mitogen-activated protein kinase (*MAPK*) phosphatase family acts as negative regulators of JNK signalling

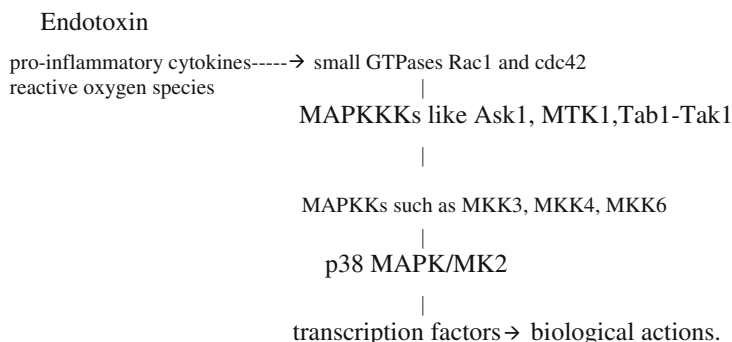


Fig. 4.5 The p38 MAPK transcription factor pathway. Details of the p38- mitogen-activated protein kinase (*MAPK*) transcription factor pathway. p38 α activation by means of TAB1 $\rightarrow$ Tak1 explains important immunological events [45]

(Fig. 6.2b). p38 MAPK enhances Cox-2 expression. p38 MAPK induces iNOS (inducible NOS) activity, and according to the amount that is formed, NO $\cdot$ will enhance or reduce inflammation. Third, p38 action within neutrophils is elevated when they are treated with granulocyte-macrophage colony-stimulating factor (GM-CSF) or TNF α to enhance their respiratory burst activity. p38 action exposes cryptic NF- κ B binding sites in chromatin as part of the response to inflammation [46]. In rheumatoid arthritis, p38 regulates production of IL-1 and TNF α . p38 upregulates cytokine production (a) by direct phosphorylation of TFs, (b) by promoting increased translation of messenger RNAs (mRNAs) that have AREs (adenylate/uridylate-rich elements) and so are phosphorylated by ARE-binding proteins [47]. The use of p38 MAPK inhibitors demonstrates that rheumatoid synovial fibroblast output of IL-6 and IL-8 is curtailed, and that the formation of metalloproteinases MMP-1 and MMP-3 is reduced [48].

By using a p38 MAPK inhibitor, it has been ascertained that p38 must normally downregulate macrophage production of the powerful IL-12 [49]. Yet, p38 MAPK is implicated in monocyte maturation to dendritic cells [50]. p38 MAPK is instrumental in the response of cells (such as basophils) to chemokines [51]. p38 mediates MCP-1 production by monocytes in response to rhinovirus [52].

The p38 MAPKs are involved in the migration of endothelial, epithelial cells, and smooth muscle cells, as one can determine by the application of inhibitors. Some cytoskeletal proteins are substrates for p38 MAPK, and as shown in Fig. 4.4, p38 MAPK influences formation of metalloproteinases [53].

p38 MAPK is important for the functioning of T lymphocytes. First, it aids thymocyte DN to DP transitions; second, it helps to promote the differentiation of Th1 and Th2 cells; third, it aids production of interferon- γ (IFN γ); and finally, it helps cellular proliferation. MKK3 is the main upstream activator of p38 in CD4 $^{+}$ T cells, and MEKK4 is above that (Table 12.12). It causes phosphorylations of the p38 Thr180 and Tyr182. However, an alternative means of p38 activation in T cells has come to light. Zap70 of the T cell receptor complex can also phosphorylate Tyr323

of the p38, and there is then autophosphorylation. This alternative p38 pathway is subject to negative regulation by GADD45 α (growth arrest and DNA damage-inducible gene 45 α) [54, 55]. Yet, GADD45 α of dendritic cells has a positive effect on p38 and hence on Th1 cell polarization [56].

Dodeller et al. [55], using a p38 MAPK inhibitor and a dominant-negative mutant of p38 in vitro, have proven also that p38 MAPK induces formation of IL-10 and the Th2 cytokines IL-4/5/13 in primary naïve and memory T lymphocytes.

4.2.5 *The ERK Cassette*

One can trace pathways from the GPCRs or from receptor tyrosine kinases via Ras to Raf1-MEK-ERK to the phosphorylation of a group of TFs that turn on the rapid-response genes (Fig. 4.3). MAPKs facilitate gene transcription by regulating the structure of chromatin. Chromatin has to be unravelled before the DNA can be transcribed. The MAPKs activate enzymes Rsk-2 and MSK1, which phosphorylate the histone H3. MAPK activates carbamyl phosphate synthetase [57] so that pyrimidine-building units are produced for RNA and DNA synthesis. In addition, MAPK induces translation by enhancing the ability of translation initiation factor EIF-4E to recruit protein-synthesizing ribosomes (Fig. 11.6). Growth of cells is tightly controlled by cyclin-dependent protein kinases (CDKs) (Chap. 17). Activation of the ERK path increases transcription of cyclin D1 gene and the formation of cyclin D1-cdk4 complexes [58] (Fig. 17.1).

4.2.6 *Cascade Control by Phosphatases*

MAPKs have to be controlled. MAPK activation necessitates phosphorylation on a threonine and tyrosine residue located within the activation loop of kinase sub-domain VII. This process is reversible, even in the presence of activating stimuli by means of serine-threonine phosphatases like PP2A, PP2B (calcineurin), and PP2C, the protein tyrosine phosphatase PTPN7 (HePTP) and PTPRR, and the members of the dual-specificity phosphatase (DUSP) family, meaning MAPK phosphatases that dephosphorylate phosphothreonine and phosphotyrosine residues. PP2A, which counteracts many protein kinases, is the most abundant cell enzyme, forming 0.3% of cell protein. The ERKs undergo rapid inactivation via threonine dephosphorylation by the PP ser/thr protein phosphatase and in T cells by HePTP (Table 13.1). PP2B calcineurin has a 60-kDa catalytic subunit A and a 19-kDa calcium-binding subunit B. Isoform Cn A α is widely distributed in tissues, whilst Cn A β is found in lymphoid cells and mediates the immune response. Calcineurin controls various TFs like NFAT (nuclear factor of activated T cells), NF- κ B, c-fos, and Elk1.

Growth factors or cellular stresses induce the DUSP genes [59], and these enzymes generally bind to MAPKs, with the result that their catalytic domain

is activated [60]. Note then that scaffolds serve to protect MAPKs against dephosphorylation. Scaffolds protect the fidelity of the MAPKs, and they exert spatiotemporal control [61, 62].

There are now 13 named MAPK phosphatases. MAP kinase phosphatase 1 (MKP-1) (DUSP1) is a more potent inhibitor of p38 MAPK and JNK than of ERK in macrophages. Accordingly, it attenuates the biosynthesis of cytokines like TNF α and IL-6, and it is a critical regulator of immune responses [63]. Mice that are MKP-1 knockouts show hypersensitivity to LPS and die of multiorgan failure. Yet, the MKP-1 protects against oxidative stress-induced apoptosis of cells [64]. MKP-5 (DUSP10) also inactivates p38 and JNK but not ERK [65]. PP2A, which is inhibited by okadaic acid, is a key regulator of the JNKs [66] and thus of AP-1 activation. Protein phosphatase 5 controls Raf-1 (Fig. 4.3) [67]. In T and B cells, there is DUSP-2 (Pac1), which reduces cytokine production and thus protects against inflammatory arthritis.

As emphasized, phosphatases are inactivated when their cysteines are oxidized by ROS [68]. Since ROS promote the signalling cascades at multiple steps, this means that ROS/H₂O₂ has a considerable inducing influence on signal pathways (Fig. 4.6) [69]. We noted that when PDGF acts on its receptors, there is that transient generation of H₂O₂, which aids the activation of Src kinases and PI3K [24]. Note also that if ATP is released to act on P2X7 receptors of macrophages, it induces in them the genes for an oxidative stress response, and the outcome is activation of caspase 1, which accounts for the secretion of IL-1 β and IL-18 (Fig. 12.3) [70].

ROS formation in cells could pose a serious hazard. Therefore, although it is a digression, one may wish to consider the consequences of levels of oxidative stress. Low-level oxidative stress does lead to the generation of antioxidant enzymes. Greater oxidative stress will activate NF- κ B and therefore is a component of the inflammatory response. Finally, high levels of oxidative stress will lead to activation of JNK and p38 kinases, and their activities will often lead to apoptosis of cells.

Fortunately, p53 is able to lower formation of ROS in cells, so protecting from oxidative stress-induced DNA damage. It operates through TIGAR, TP53 induced glycolysis and apoptosis regulator [71]. Thioredoxin reduces mitochondrial oxygen stress [72].

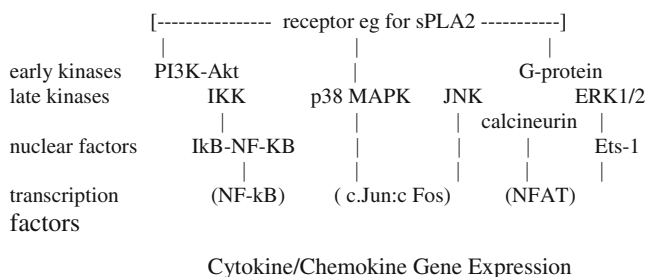


Fig. 4.6 Schema of signal pathways for cytokine/chemokine production

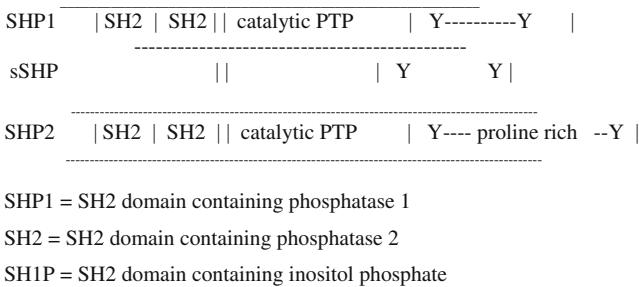


Fig. 4.7 Domain structure of SHP1/SHP2 phosphatases. SHP1/SHP2 come up in many contexts; thus, SHP2(PTPN11) attenuates epidermal growth factor (*EGF*)-dependent PI3 kinase (*PI3K*) activation. Activation of SHP2 and its association with Grb2 binding protein (Fig. 4.1) is critical for sustained downstream activation of extracellular signal-related kinase (*ERK*). Commonly SHP1(PTPN6) dephosphorylates a vital component of the actin cytoskeleton. The *essential structure* is two N-terminal Src-homology domain 2 (*SH2*) domains, a PTP catalytic domain and two C-terminal tyrosines (Y) as shown. 145-kDa Src homology inositol polyphosphate 5-phosphatase 1 (*SHIP1*) and 150-kDa SHIP2 hydrolyse phosphatidyl-inositol (*PtdIns*) 3,4,5P3 to PI 3,4P2

4.3 Transcription Factors and DNA-Binding Domains

As Fig. 4.3 indicates, specific TFs are induced or activated to effect downstream programs by regulating expression of target genes [5]. TFs and gene regulatory proteins bind to DNA via interaction of amino acids of their protein with the nucleotides of the DNA. Many cell-type-specific TFs behave as autoregulatory factors in the activation of their own genes. When it became possible to clone genes, the TFs that they encode could then be identified from the DNA binding domain (Fig. 4.8). Both the DNA binding and transactivation function of a TF may be regulated positively or negatively by phosphorylation. Thus, c-Jun is phosphorylated at five sites. Three are phosphorylated by a kinase CKII, causing inhibition of DNA binding. Two sites in the N-terminal transactivation domain are phosphorylated by the c-Jun N-terminal kinases (JNKs) (Fig. 4.3).

Gene regulation is imposed by gene promoter and regulatory DNA sequences. A gene *promoter* is sited near the gene and just upstream of the transcription site, where RNA polymerase II and the TFs assemble (Fig. 4.8). A gene *enhancer* can be up to 10 kb upstream or downstream of the gene. Likewise, a *silencer* can be upstream or downstream.

The DNA binding proteins are of four types. A *homeodomain protein* consists of three linked α -helices in an α -helix- β -turn- α -helix motif, and within helix-3 a 60-amino acid homeodomain binds to DNA segments 5'-A-T-T-A-3'. A *leucine zipper protein* consists of an α -helix that has a region in which every seventh amino acid is leucine. It has the effect of lining up all leucine residues on one side of the α -helix. Those leucines then allow formation of leucine zipper protein homodimers, like Jun-Jun or heterodimers like Fos-Jun. Both Fos and Jun proteins bind to

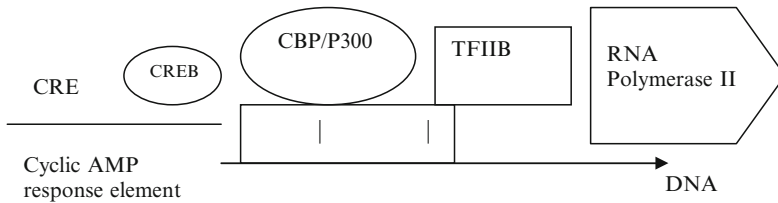


Fig. 4.8 Cofactor cAMP response element-binding protein (*CREB*) binding protein (*CBP*)/P300 bridging of transcription factors (*TFs*) to the transcription machinery. Ca^{2+} acting on Cam kinases II/IV activates CREBs and so early response genes like *c-fos*. CREB affects cell growth and proliferation. CREB-cAMP response element (*CRE*) activation via prostaglandin E2 (*PGE2*) $\rightarrow$ protein kinase A is used for the expression of *FcγRIIA* receptors to defend against pathogens (Sect. 10.3). CREB enables expression of target genes like interleukins 2 and 6 (*IL-2*, *IL-6*) and proliferating cell nuclear antigen (*PCNA*) [80b]. There is also a cAMP response element modulator (*CREM*), which is a gene that encodes activating and repressor isoforms of CREs

DNA sequences known as AP-1 sites, so they mediate gene induction following phorbol ester treatment. A *zinc finger protein* contains repeats in which two pairs of cysteine and histidine residues coordinate a zinc atom. This results in a finger-like structure. The tips of the fingers make contact with the major groove of the DNA, with alternate fingers binding on opposite sides of the helix. Fourth, a *helix-loop-helix protein* consists of a short α -helix connected by a loop to a longer α -helix. Again, there is homodimer or heterodimer formation of the proteins. The myc protein product of the *c-Myc* oncogene is classified as a helix-loop-helix. It actually has a leucine zipper and a helix-loop-helix.

As shown in Fig. 4.8, TFs have to activate RNA polymerase II by means of binding cofactors like cAMP response element-binding protein (*CREB*)-binding protein (*CBP*) and the related P300 [73–76]. Lipophilic hormone receptors (steroids, thyroxine, retinoids, and vitamin D) interact directly with *CBP/P300*. *CBP* [77, 78] is actually a histone acetylase (*HAT*) that acetylates histones H3 and H4 but not H2A, H2B. Transcriptional activators direct *HAT* [77] to the nucleosomes. *P300/CBP* *HAT* activity promulgates the action of many genes. Later removal of acetyl groups occurs by means of the histone deacetylase (*HDAC*). That and methylation of DNA stabilize the chromatin and inhibits transcription.

Amplification or repression of TF activity is by coactivators or corepressors, respectively. Cofactors do not bind to the DNA. Class I cofactors have enzymatic activity that results in alterations in DNA tertiary structure or modification of histones. Class II cofactors lack enzymatic activity but interact with RNA polymerase II (Fig. 4.8) to amplify the activity of a TF. TFs are implicated in development of haemopoietic cells [79]. cAMP stimulates protein kinase A, and thereby CREB phosphorylation, to control immature dendritic cells so that they secrete IL-10 but not TNF α and IL-12. CREB plays a central role as regulator of differentiation and proliferation in developing thymocytes [80]. Indeed, CREB affects cellular growth and proliferation generally and plays a specific role in certain tumours.

In this text there are brief references to several transcription factors that bind at promoter sites upstream of cytokine genes (Table 4.2). Hence, one has to define what are NFAT factor, AP-1 complexes, Rel/NF- κ B, octamer, and Ets factors. Look at the outline of the IL-2 gene promoter in T lymphocytes (Fig.13. 5) to see AP-1 and NFATc. Oct-1 and Oct-2 are also expressed in all T cells.

The NFAT TFs, NFAT 1–4, control several lymphokine promoters during T cell activation. They all have a Rel similarity domain (RSD), so that they have a DNA binding domain of 300 amino acids that is similar to the Rel DNA binding domain of NF- κ B (Chap. 11). Calcineurin, the phosphatase PP2B, stimulates nuclear retention of NFAT by dephosphorylation of serine residues. Sustained signalling by NFAT promotes the development of Th1 lymphocytes [81]. NFATc2 is synthesized constitutively in T cells, whilst NFATc1/ α A is induced strongly following T cell receptor and coreceptor stimulation, and it is maintained by positive autoregulation [82, 83].

AP-1 complexes consist of Jun, Fos, ATF, Maf homo- or heterodimers that bind to palindromic sites of consensus sequence GGGATTTCCC that occur in the promoters of IL-2, IL-6, TNF α , and IFN γ genes. The TRE through which AP-1 acts was defined originally by its binding of the proinflammatory tetradecanoylphorbol-13-acetate. The different AP-1 dimers control transactivation or suppression of various genes for cell proliferation, cell differentiation, cell apoptosis or transformation. Generally, MAPKs ERK1/2 regulate *Fos*/AD-1 (activated protein 1) activity, whilst JNK kinases regulate *Jun*/AP-1 activity.

Nuclear factor-kappa B generates cytokines and chemokines required for inflammation (Chap. 11). It is important for protection of immune cells against apoptosis (Chap. 8 and Table 11.3). Immune cells that are mobilised to fight an invading pathogen are being stimulated to proliferate and survive. Yet, when the infection is cleared, the now-effete cells will die by apoptosis. NF- κ B controls life-and-death decisions [81], in particular by exerting control over the JNK cascade that can cause apoptosis.

An Octamer consensus site ATGCAAAT occurs near many transcriptional start sites. Oct-1 is constitutively expressed, and Oct-2 is induced in T cells and pre-Bs.

Ets TFs bind to the core sequence GGA. Ets TFs are involved in cell differentiation and growth and in cell migration. Ets-1 reduces expression of IL-2 and in fact is highly expressed in resting T cells. When T cell receptor stimulation induces protein kinase C (PKC) and so Ca²⁺ influx, Ets-1 decreases rapidly.

4.3.1 More About Histone Acetylation

The nucleosomes of the chromosomes are arranged as octamers, with four paired core histones: H2A, H2B, H3 and H4 proteins. The histone octamer grips a 120- to 160-base pair (bp) DNA segment that is wound around. The amino-terminal tails that protrude outwards from the nucleosome surfaces are targets for acetylation, methylation, phosphorylation and sumoylation [84]; glycosylation or adenosine diphosphate (ADP) ribosylation; or finally ubiquitination. Residue modifications are used to control the transcription of genes. Thus, the coactivator proteins (Fig. 4.8)

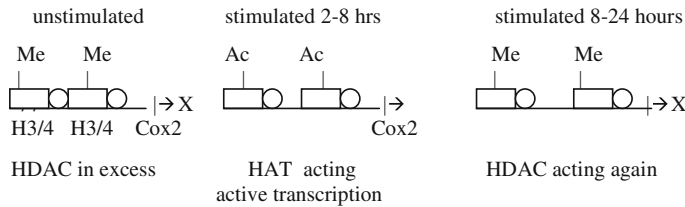


Fig. 4.9 Lipopolysaccharide stimulation of the cyclooxygenase 2 (*Cox-2*) promoter in macrophages. Histone deacetylase (*HDAC*) expression prevents excess inflammatory responses by macrophages. X means that transcription is suppressed

like CBP have intrinsic HAT activity that is required for the formation of an open chromatin structure, so that there can be transcription by the RNA polymerase II [85, 86]. Acetylation affects the four lysines on the N-terminal of histone 4. Acetylation of the histone tails disaggregates the nucleosomes. There are also 1–17 HDACs that will close the structure again. Other changes to histones are also used, as when IL-1 induces coactivator-mediated H3Lys4 methylation.

When LPS induces the acetylation of histones and opens the structure of chromatin, a variety of possibilities arise. Thus, Rel (of NF- κ B) then has access to the promoters for IL-6 and for IL-12, and there is transcription. Consider the induction of Cox-2 in macrophages as in Fig. 4.9.

When epithelial cells are stimulated by IL-1, there is increased binding of NF- κ B to DNA (Chap. 11), increased HAT activity and thus histone acetylation, and so increased inflammatory gene transcription. Interestingly, ROS (H_2O_2) increase HAT activity.

Accordingly, there can be increased release of IL-8 from cells [87]. Histone acetylation is associated with gene expression in CD8 cytotoxic T lymphocyte responses [88]. Glucocorticoid-mediated transrepression in inflammation is partly explicable by its suppression of CBP-induced histone acetylation [89].

Accordingly, acetylation of histones by CBP/P300 leads to unwinding of the DNA. In asthma, there is reduction of HDAC but an increase of HAT that will account for the increased airway inflammatory response [90]. Underexpression of HDAC, potentiating histone acetylation, results in induction of GM-CSF in the airway epithelium (Fig. 13.11).

4.4 Formation of Enhanceosomes on Gene Promoters and Enhancers

4.4.1 Cytokine Genes

To activate a gene, there will be cooperative activation of the gene promoter by TFs that bind to individual sites within it [91]. Relevant examples are the cooperative binding of NFAT and AP-1 proteins (Fig. 13.5) to promoters of the cytokines

IL-2/3/4 and GM-CSF. Similarly, NF- κ B family member Rel-A and C/EBP (enhancer bending protein) (Fig. 4.10) bind to adjacent binding sites on IL-8 and granulocyte colony-stimulating factor (G-CSF) promoters. Recruitment of coactivators and modifiers of chromatin structure are needed for the IL-8 promoter at which CBP/P300 is recruited to the promoter by interaction with assembled TFs AP-1, CREB, C/EBP, and NF- κ B.

Glucocorticoid (GC) represses IL-8 transcription. It interferes with the ability of the kinase p-Tefb to phosphorylate Ser² of RNA polymerase II, preventing its association with the IL-8 promoter [92]. On endothelium, NO prevents IL-8 gene expression by inhibiting the binding of NF- κ B to the DNA.

The genes for TNF α and lymphotoxin are within a linkage group within the MHC (major histocompatibility complex) locus on mouse chromosome 17. The TNF α promoter forms 2–3 kb of DNA between the site of LT β and the transcriptional start site of the TNF α gene. As shown in Fig. 4.11, for TNF α that will be produced by monocyte macrophages. TF complexes assemble on the promoter [93], and there they recruit CBP/P300 with its HAT activity.

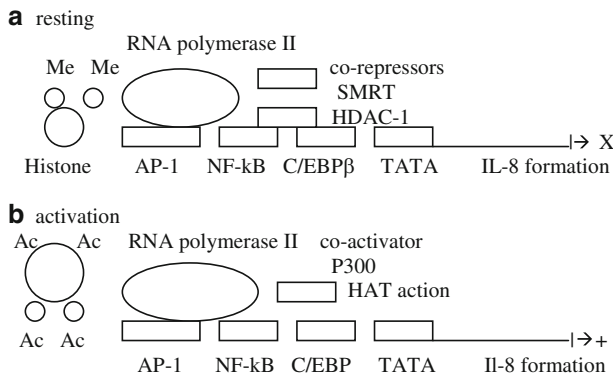


Fig. 4.10 Control over the production of interleukin 8 (*IL-8*). (a) When histone deacetylase (*HDAC*) predominates, there is no *IL-8* formation (X); (b) histone acetylase (*HAT*) results in activation

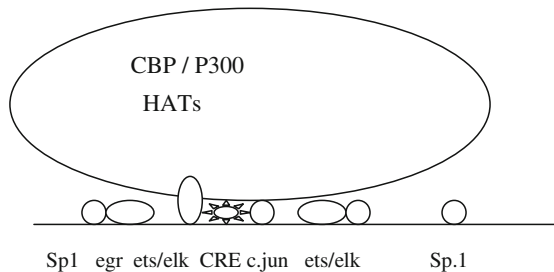


Fig. 4.11 Enhanceosome on the tumor necrosis factor- α (*TNF α*) promoter

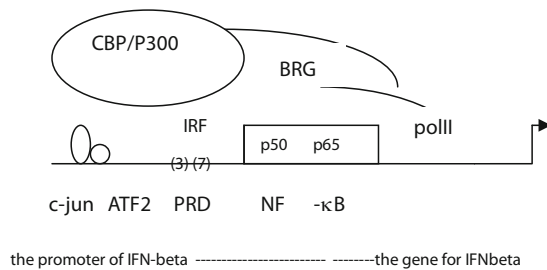
4.4.2 The *IFN* β Enhanceosome

The DNA binding protein NF- κ B (p50/p65) is discussed in Chap. 11. Near the *IFN* β promoter, which is normally covered by a nucleosome, there is an enhancer that, following viral infection, directs the assembly of an “enhanceosome” that contains NF- κ B, the activating TF ATF2 in a heterodimer with c-Jun, the *IFN* regulatory factors IRF3/IRF7 (Fig. 4.12) and the high-mobility group protein HMG-1(Y). Once these TFs are assembled, they recruit CBP/P300 as coactivator, and to enable RNA polymerase II to do its work, the chromatin-remodelling complex (CRC) is bound. A CRC uses energy from ATP hydrolysis to disrupt histone-DNA contacts. The best defined of the CRCs are BRG (Brahma-related gene) complexes [94].

SW1/SNF (switch/sniff) is a complex that regulates the position and structure of nucleosomes using energy of ATP hydrolysis. It can interact with a variety of activators or repressors to cause gene activation or repression. There are more than ten components of the SW1/SNF but BRG is the adenosine triphosphatase (ATPase) subunit. BRG is required for a subset of *IFN* γ responsive genes, for the *IFN* β or - α genes, and likewise for expression of IL-6-induced target genes. In fact, BRG helps the recruitment of STATs (signal transducers and activators of transcription) to the DNA (Sect. 12.9). So, in respect of IL-6-inducible genes, BRG-1 is required for STAT3 recruitment. BRG-1 ATPase also associates with the promoter for the chemokine Rantes [95], and one can expound on these lines. Using small interfering RNA, it was shown that BRG-1 is involved in transcription in the promoter region of erythropoietin, as triggered by hypoxia, and likewise in hypoxia-dependent activation of vascular endothelial growth factor (VEGF). BRG-1 is also required for recruitment of SP-1, AP-2, and polymerase II to the metalloproteinase MMP2 promoter (cf. Fig. 4.4).

Tat is a critical viral transactivator for human HIV expression. Note that Tat (transactivator regulation of transcription) activation of the HIV promoter requires the SW1/SNF complex in cooperation with coactivator P300.

Fig. 4.12 Activation of the interferon- β (*IFN* β) promoter. *PRD* positive regulatory domain for IRF3/IRF7 (cf. Fig. 14.4), *BRG* Brahma-related gene (also called Brahma-associated factor [*BAF*], which is the chromatin remodeling complex [*CRC*])



4.4.2.1 Brahma-Associated Factor Complexes Prime for IFN α Activation

BRG/BAF is also required for the basal and induced expression of IFN α response genes. As will become clear in Fig. 14.4, IFN α activates TFs Stat1 and Stat2 that form ISGF3 (IFN-stimulated gene factor 3), which translocates to the nucleus and binds there to IFN-stimulated response elements (ISREs). Since there is a nucleosome at the promoter site of the IFN-induced transmembrane protein 1 (IFITM1) gene, BRG has to remodel the nucleosomes to allow low-level histone acetylation. Following treatment with IFN α , ISGF3 can rapidly access the ISRE that has been partially exposed by BRG [94]. In fact, BRG has a dual role in IFN α -mediated signalling. First, it prepares chromatin of IFN α -responsive genes in anticipation of signalling, and thereafter it models the chromatin further as signalling occurs [94].

4.4.2.2 BRG Expression and the Induction of MHC Class II Transactivator

Antigen-presenting cells (APCs) present processed neoantigen in association with MHC class I or II molecules to recipient T lymphocytes. CD4 T helper cells receive processed antigen along with class II MHC molecules, whilst CD8 cytotoxic T lymphocytes receive antigen bound to MHC class I molecules. In brief, internalized proteins in phagocytic APCs are degraded enzymatically within the cell's endosomes and lysosomes to generate peptides, most of which bind to MHC glycoproteins produced from the Golgi apparatus.

The MHC class II transactivator (CIITA) is the controller of the coordinated expression of MHC class II genes [96, 97]. This is activated by IFN γ through Jak1/2 and formation of a Stat1 dimer. Actually, BRG regulates the CIITA promoter in response to IFN γ -mediated signalling [98]. The MHC class II enhanceosome recruits factors for chromatin remodelling: CBP and P300, BRG-1, and a methyl-transferase CARM1. pCREB/reg factor X/nuclear factor Y serve as a scaffold for CIITA [99]. CIITA has intrinsic HAT activity. Furthermore, CIITA is able to repress expression of the IL-4 gene [100].

4.4.3 Chromatin Remodelling and Epigenetics

“Epigenetic changes” in histones control the accessibility of *cis*-regulatory elements i.e. promoters, enhancers, insulators, silencers to TF regulators and the RNA polymerase machinery. Programs of gene expression are controlled by potentially reversible epigenetic processes such as (a) DNA methylation [101], (b) methyl CpG-binding proteins, (c) modification of the histones (HAT/HDAC as in Figs. 4.9, 4.10), (d) TF action, and (e) chromatin structure. For example, chromatin-remodelling events are important for the expression of the MHCII genes and the associated transactivator CIITA [102a]. When there is “endotoxin tolerance” as a result of repeated exposure to endotoxin, the gene for TNF α is silenced because epigenetic change results in repression at its promoter [102b].

“Heterochromatin” (i.e., silent chromatin) necessitates histone deacetylation and histone methylation at H3K9 or H3K27. There are chromatin-binding proteins with chromodomains for methylated histones and bromodomains for acetylated histones and conversely SANT-domain-containing proteins that bind to unmodified histone tails [103]. Demethylation is required for expression of the IL-2 gene.

Accessibility of DNA is also regulated by CRCs that use energy from ATP to remove and assemble nucleosomes to slide them along the DNA strand or to create loops of DNA on the surfaces of nucleosomes [104]. Such chromatin remodellers include SWI/SNF, which merges two nucleosomes into a new looser structure, and the smaller ISWI imitation switch, which will slide nucleosomes relative to the DNA strand.

Clearly, chromatin remodelling to allow access of TFs to a gene locus is a general phenomenon. TGF β in cooperation with IL-6 induces histone acetylation at the IL-17 promoter. IL-17 T helper cells are thought to be protagonists of autoimmune diseases.

4.4.4 Control of IL-4 and IL-13 Production (Th2 Cytokines)

In cell-mediated immunity (CMI), the T lymphocytes that help the activation of APCs are the Th1 CD4 helper T cells and CD8 cytolytic T cells since both of these types will secrete IFN γ , which is a macrophage-activating factor (MAF) (Chap. 12). Conversely, Th2 CD4 helper T cells that produce IL-4 reduce macrophage activation so that CMI does not become a damaging tissue response. IL-4 can induce anti-inflammatory macrophages. Cellular sources of IL-4 include mast cells, basophils, eosinophils, natural killer T (NKT) cells and already differentiated Th2 cells.

IL-4 is required for the formation of Th2 lymphocytes (Chap. 13). IL-4 acts through Stat6 to induce the GATA promoter, which then undergoes autoactivation. GATA3 TF augments enhancer activity near to the IL-5 and IL-13 promoters in the conserved noncoding region CNS-1. Here, near to the IL-5/IL-13 and IL-4 genomic regions [86], there are Th2-specific DNase hypersensitive sites (HSs) [105]. Essentially, the Th2 cytokine genes are induced by p38 MAPK-mediated phosphorylation of GATA3 [105]. There will have to be hyperacetylation of histone H3, lysine 9, and hypermethylation of histone H3, lysine 4 [106]. Concurrent CD28 costimulation for the T cell receptors activates NF- κ B that supports Stat6 activation and so the whole process. NFAT proteins bind to the IL-4, IL-5 and IL-13 promoters.

Notch plays its part in IL-4 expression via RBP-J κ binding sites in a conserved downstream enhancer CNS-2 [107]. Notch enhances IL-4 production in part by increasing the expression of the transcription factor GATA3.

There are many accessory points. Thus, IL-2 signals transmitted through STAT5 stabilize IL-4 expression. IL-2 works via SOCS3, which is an inhibitor of IL-12-induced Stat4 activation. JNK leads to phosphorylation of Itch with upregulation of its E3 ligase ubiquitination activity, so that junB is proteolysed, and there is modulation of IL-4 production. It also turns out that Src homology inositol polyphosphate 5-phosphatase (SHIP) phosphatase acts to repress differentiation of Th2 lymphocytes.

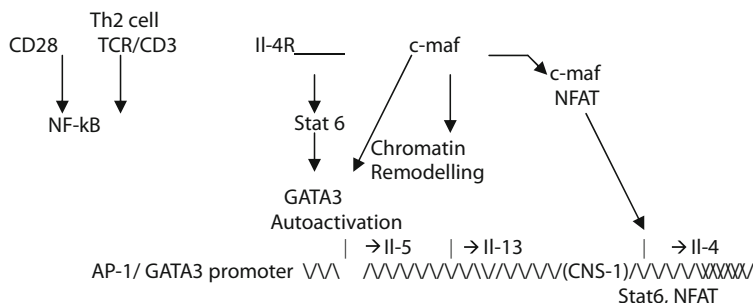


Fig. 4.13 GATA3 promotes interleukins (IL) 5, 13 and 4. *CNS-1* conserved noncoding region 1 in the DNA. At the Th2 locus, the cytokine promoters are looped at the central region. The chromatin has to be unwound, and GATA then transactivates the IL-5 and IL-13 promoters, and nuclear factor of activated T cells (*NFAT*) and *c-maf* drive the IL-4 promoter. Protein kinase A (*PKA*) regulates GATA3-dependent activation of IL-5 gene in Th2 cells [108]. Interaction between GATA proteins and activator protein 1 (*AP-1*) promotes IL-13 transcription in mast cells [104]. Furthermore, IL-1 is required for the formation of Th2 cytokines, and IL-6 by inducing *c-maf* promotes Th2 cells. IL-10 supports *c-maf* actions. This same *c-maf* likewise induces the GM-CSF promoter.

Hypersensitive sites (HSS1–3) with relatively open chromatin are described in the intergenic region between IL-4 and IL-13. The HSS1/2 are Th2 cell specific [109], and HSS3 occurs in all CD4 cells. *CNS-1* (Fig. 4.13) is about 10 kb upstream of the IL-4 ATG start codon and corresponds to Flavell's HSS2/3 DNA HSs. The HS1 that is 1.6 kb upstream of IL-13 was shown to bind GATA3, CBP/P300, and the RNA polymerase II. So, in Th2 lymphocytes there is (a) DNase1 hypersensitivity, (b) H3 hyperacetylation, and (c) DNA demethylation. In fact, DNA demethylation correlates with the activation and transcriptional competence of cytokine genes [110]. DNA demethylation in the locus control region plays a role in rapid increase of IL-4 expression in Th2 conditions. GATA3 induces DNase1 hypersensitivity and targeted hyperacetylation in the IL-4 locus. GATA3 induces chromatin remodelling activity. GATA3 is the “pioneer factor” that opens the locus to epigenetic remodelling machinery.

A locus central region (LCR) is where TF complexes come close to a promoter and activate a gene by “looping out” the intervening chromosomal stretch. This means that the opened chromatin complex potentiates the expression of Th2 cytokine genes [110]. The *Rad50* gene is on the looped-out piece of chromatin.

4.4.5 The Anti-inflammatory Action of PPAR γ s and Retinoids

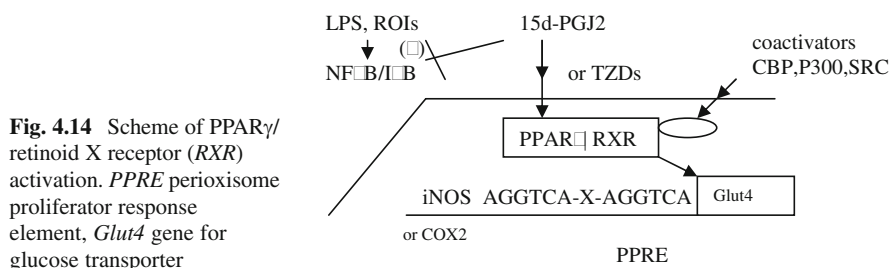
In their metabolic role, PPAR γ aids the cellular assimilation of lipids via anabolic pathways [111], and PPAR α control liver and skeletal muscle lipid metabolism [111]. PPAR α is also anti-inflammatory [112] and antiproliferative, and PPAR α prevents the proatherogenic effect of cholesterol accumulation in macrophages

[113]. Agonists of PPAR α increase expression of eNOS and inhibit adhesion molecule expression; they reduce plasma IL-6 and decrease Cox-2 in vascular smooth muscle by their inhibition of NF- κ B. Importantly, PPAR α is expressed more in CD4 T cells of males than in females. Since PPAR α represses NF- κ B and c-jun and its removal results in higher IFN γ and TNF α production, its action could explain why females are more prone to autoimmune diseases [114].

Peroxisome proliferator activated receptor- γ agonists like the antidiabetic thiazolidine-diones or the prostaglandin 15-PGJ2 stop the release of proinflammatory cytokines [113, 115], activation of Cox-2 and the formation of NO by interaction with nuclear receptors as in Fig. 4.14. Normally, PPAR γ is inactive, being bound either as a homodimer to another PPAR γ or to retinoid X receptor (RXR). Activation allows the recruitment of coactivators like CBP/P300 or SRC steroid receptor coactivator. Retinoids potentiate the PPAR γ in myeloid cells [116]. PPAR γ , by provoking synthesis of retinoids in dendritic cells, enables the expression of CD1d molecules that cooperate with iNKT cells [117]. Actually, PPARs, together with glucocorticoid receptors (GRs) and liver X receptors (LXR α s), are known as *nuclear receptors*, and they repress inflammatory responses [118]. They work as monomers or dimers (homo- or heterodimers) with the RXR. Alternatively, they can act by “transrepression” to inhibit TFs AP-1 or NF- κ B. Indeed, PPAR γ inhibits dendritic cells.

The cyclopentenone prostaglandin 15d-PGJ2, which is a product of Cox-2, or thiazolidinedione (TZD) drugs inhibit the action of NF- κ B but stimulate PPAR γ receptors, so that some genes are activated, such as heat shock proteins or Glut 4, whilst others like iNOS or Cox-2 are inactivated. The way in which PPAR ligands negate inflammatory cytokine expression is complicated. New data show that sumoylation of PPAR γ leads to the retention of repressor complexes on the promoters of inflammatory genes [119]. This prevents heterodimerization of PPAR γ with RXR. So, for example, iNOS is not activated by NF- κ B, but instead sumoylated PPAR γ binds to a multisubunit repressor complex containing NCoR (Fig. 11.4).

Vitamin A retinoic acid receptors RARs - α -, - β -, and - γ are similar. Indeed, RAR γ , which is activated by all-*trans* retinoic acid, forms RAR γ /RXR heterodimers that



recruit coactivators for target genes. Vitamin A engages RAR γ to help synthesis of IL-6, IL-12, and TNF α by macrophages, and it aids IFN γ formation and production of CD8 T lymphocytes [120]. RAR α is activated when T cells produce IL-2 [121]. RAR α is involved in G-CSF-induced neutrophil differentiation or GM-CSF-induced monocyte development [122].

4.4.6 Estrogen Receptors

Estrogen receptors (ERs) are relevant to the breast cancer section. The ER is a typical example of a steroid receptor. ERs not only are cytoplasmic but also appear within the nuclei of cells, where ERs exist as inactive complexes with heat shock proteins and immunophilins. Each ER is a zinc finger-binding protein that, after activation, loses its cytoplasmic receptor-associated proteins and migrates as a TF to bind to the ERE (estrogen response element) of the DNA. EREs are present in the regulatory regions of estrogen target genes [123] (Fig. 4.15). As has been emphasized, coregulators affect the local histone acetylation and deacetylation [124].

When the ER β receptor is stimulated, receptor complexes interact with the ERE at the promoter region of target genes or with the antioxidant response element. So, eNOS can be stimulated, and manganese superoxide dismutase (SOD) formed. The TNF α gene is inhibited.

ERs interact with several coregulatory proteins [124] that are intercalated between the activated receptor and the gene transcription machinery catalysed by RNA polymerase II. Associated proteins that might activate or repress the transcription-initiation complex are assembled at a TATA box, a DNA sequence that is rich in T-A base pairs. TATA is the promoter for RNA polymerase II. The dimerized ERs display a hydrophobic surface that is recognised by coactivators such as the p160 (160-kDa steroid receptor coactivator protein) and the CBP (P300 CREB binding protein). The ERE (Fig. 4.15) is a palindrome of PuGGTCA motifs separated by 3 bp.

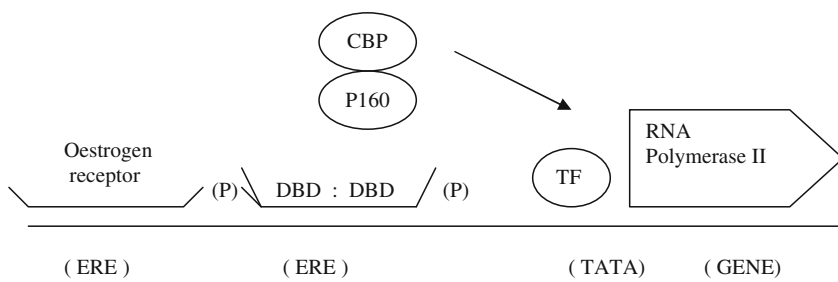


Fig. 4.15 Estrogen receptor complex promoting transcription. *ERE* estrogen response element (as homo- or heterodimers), *DBD* DNA binding domain of the dimerized zinc finger estrogen receptor

4.4.7 Corticosteroid Receptors

The glucocorticoid response element (GRE) is similar to the ERE. Isoforms of the GR are expressed at different ratios in various cell types [125] since the human GR mRNA has alternative splice variants. Two GR isoforms (α and β) were described originally, and the nuclear GR β has a dominant-negative effect on GR α via the formation of GR α /GR β heterodimers [126]. So, along with the GC resistance that characterizes rheumatoid arthritis or septic shock, there are elevated values of GR β [127]. Actually, neutrophils are resistant to GCs because they have high levels of the inactive β -isoform of the receptor. The GR isoforms account for transcriptional specificity in various tissues [128, 129]. Macrophage inhibitory factor (MIF) increases sensitivity of cells to GCs by its ability to cause phosphorylation of p38 MAPK.

Anti-inflammatory effects of the GCs occur by delayed genomic and rapid non-genomic actions. Corticosteroids stop proliferation of T lymphocytes [130], for CD28 costimulation is blocked and IL-2 formation is halted [131]. GCs prevent release of cytokines from lymphocytes and macrophages. They cause apoptosis of thymocytes. GCs induce the synthesis of I κ B α , and this inhibitor then complexes NF- κ B and so prevents its activity [132]. GCs inhibit production of prostaglandins by three delayed genomic mechanisms: (a) there is induction and activation of annexin 1, (b) there is repression of transcription of Cox-2, and (c) there is induction of MPK-1, which inactivates JNK kinase. In fact, GCs can repress the action of ERK, p38 MAPK, and JNK [132].

NF- κ B can induce histone acetylation and other histone modifications in a temporal manner. Yet, GCs modulate CBP-induced histone acetylation (Fig. 4.16) [133]. GCs use HDAC2 to block NF- κ B transcription, for example, when used in chronic obstructive airway disease [134]. Recruitment of HDAC2 reverses the increase in gene transcription induced by NF- κ B (Fig. 11.4). Unfortunately, in obstructive airway disease of the lungs increased oxidative and nitrosative stress will impair HDAC2. So, airway obstruction is typically refractory to corticosteroids.

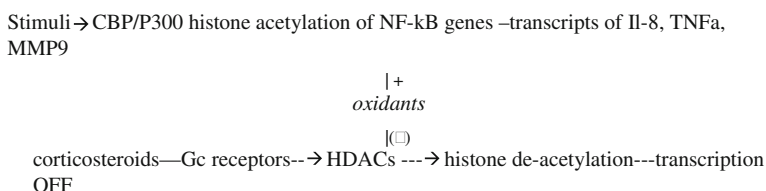


Fig. 4.16 Histone acetylation helping nuclear factor kappa B (NF- κ B) and glucocorticoid-induced deacetylation. Stimuli $\rightarrow$ CREB-binding protein (CBP)/P300 histone acetylation of NF- κ B genes—transcripts of interleukin 8 (IL-8), tumor necrosis factor- α (TNF α), matrix metalloproteinase 9 (MMP9), GCR glucocorticoid receptor

Considerable detail is now available on how the GC/cGCR complexes [127] modulate the activities of AP-1, NF- κ B and NFAT so that there is repression of inflammatory and immune genes. The cortisol-GR complex directly interacts with NF- κ B to block its transcriptional activity. GCs are anti-inflammatory by other means, including IL-10 facilitation. They displace key signalling proteins from membrane lipid rafts of T lymphocytes. There is a decrease of saturated fatty acids with consequent increase of membrane fluidity. Insofar as the GCs like dexamethasone induce the expression of MKP-1, they thwart the action of TNF α and IL-1 β in conditions like rheumatoid arthritis [135, 136]. MKP-1 can restrain p38 MAPK kinase. In any case, in macrophages GCs can directly inhibit the p38 MAPK [137]. Since GCs elevate the activity of MKP-1 in endothelial cells [138], the result is inactivation of TNF α and inhibition of the expression of E-selectins.

Dexamethasone inhibits TNF α and IL-6 less in monocytes compared with T lymphocytes because in monocytes there is less available MKP-1 [139]. However, GCs do induce a subset of monocytes that have an anti-inflammatory phenotype [140].

GCs also exhibit some rapid nongenomic actions like the inhibition of cytosolic phospholipase A2 (Fig. 6.2b), so that arachidonic acid will not be available to form the eicosanoids. There is a plasma membrane form of GR. In macrophages, it inhibits phagocytosis and superoxide anion formation. In T cells, plasma membrane GR displaces Lck and Fyn kinases so that T cell receptor signalling is suppressed.

It is actually therapeutic doses of GCs that suppress the macrophages or pathophysiological levels. Low doses of GCs enhance macrophage function and formation of chemokines and cytokines. Basal levels of GCs serve to keep the immune system inactive.

4.5 DNA Methylation and Gene Control (Epigenetics)

DNA methylation on cytosine residues stabilizes chromatin and inhibits transcription of genes. Normal methylation affects only cytosine bases that are followed by guanine, so called CpG methylation, which occurs in CpG-rich areas affecting 1% of the genome [141, 142]. Following methylation, acetyl groups are removed from the histones. Arginine methylation of histones in the N-terminal tails occurs among sites for lysine methylation, lysine acetylation and serine phosphorylation [143]. Hence, repressive chromatin configurations are created, making genes inaccessible, as with histone arginine methyltransferase-induced methylation of specific lysine residues (e.g. the lysine 9 of histone H3 and Arg-2, -17 and -26). The methyl CpG-binding proteins stop recruitment of HDAC, so that there is only condensed chromatin. In addition, methyl CpG-binding proteins sequester essential TFs, thwarting their action. Furthermore, there can be methylation of the transcriptional coactivators like CBP and P300 (Fig. 4.8), methylation of DNA binding TFs like the Stat1 and methylation that regulates the transcription elongation machinery [143]. It all adds up.

The innate immune system senses unusual molecules like nucleic acids, which can form part of host-associated molecular patterns (HAMPs). In bacterial DNA

there are unmethylated CpG motifs that activate the immune response. However, in mammalian DNA such motifs are methylated and rendered innocuous (Fig. 4.17).

Mutation, altered gene expression, hypomethylation and sometimes hypermethylation all contribute to cancer [144]. In fact, a generalised decrease in genomic 5-methyl-cytosine concentrations occurs as mammalian cells age, and this gradual loss of DNA methylation can result in aberrant gene activation. The decrease of 5-methyl-cytosine occurs at the same time as there is localized hypomethylation of CpG islands at gene promoters. Many carcinogens interfere with DNA methylation and so allow oncogene activation. SMYD3 encodes a histone H3 lysine methyl-transferase that aids the proliferation of cancer cells [145]. It operates in breast cancer. Hypermethylation commonly inactivates tumour suppressor genes like von Hippel-Lindau in renal cancer, CDKN2B in haematological malignancies and MLH1 and APC in colon cancer.

Interestingly, memory CD4 T lymphocytes produce large amounts of IL-2 after demethylation occurs in the promoter-enhancer region of the IL-2 gene [146].

The IL-2 gene undergoes DNA demethylation in response to activation. Conversely, methylation of a specific subset of CpG represses IL-2 transcription. The DNA methyl-transferase 1 (DNMT1) silences the Th2 cytokines IL-4/5/13/10 in CD8 T cells. As CD4 T cells differentiate into Th2 lymphocytes, DNA methylation is slowly lost, and thus those cytokines can then be produced [147]. Expression of IL-4/IL-13 and IL-5 not only results from DNA demethylation but also favourable histone modifications in the IL-4/IL-13 locus, as recounted in relation to Fig. 4.13.

We shall find that there is augmented expression of IFN γ (Fig. 13.10) after treatment with DNA methylation inhibitors. In fact, there are epigenetic modifications around the IFN γ gene like DNA methylation, histone H3/H4 acetylation and histone H3K4-Me methylation. However, in DNMT^{null} T cells IFN γ is enhanced five to ten times following cell activation. Furthermore, there are DNase HSs in the introns of the mouse IFN γ gene that show increased accessibility during differentiation of Th1 lymphocytes.

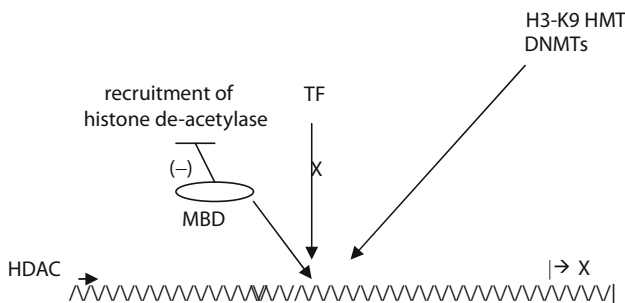


Fig. 4.17 Methylated binding proteins stop transcription. Methyl CpG binding proteins bind to methylated DNA to repress transcription. CpG island methylation attracts methyl-binding proteins (MBDs). Paradoxically, cancer cells acquire aberrant locus-specific hypermethylation in normally unmethylated CpG islands. HMT histone methyltransferase, DNMT DNA methyltransferase, HDAC histone deacetylase, MBD methyl binding protein

4.5.1 *c-Myc and Cell Proliferation*

c-Myc is a helix-loop-helix leucine zipper TF (Table 4.2). c-Myc stops cell differentiation and instead drives cell growth and unrestricted cell proliferation. When cell proliferation is required, there is activation of c-Myc via the MAPKs. In quiescent fibroblasts, Myc is degraded rapidly by ubiquitin (Ub)-mediated proteolysis. Following the addition of serum, Myc is stabilized.

Withdrawal of mitogens or cell differentiation stimuli leads to loss of Myc expression, but instead there are max-mad dimers. Mnt, a Max-interacting protein, often mediates repression of c-Myc.

The Myc gene is subject to complex transcriptional and posttranscriptional regulation.

Myc controls the decision of cells to divide [148] for it can repress growth arrest genes like p15 and p21 and GADD45 [149]. At the same time, it induces genes involved in cell cycle control (Chap. 17) like cyclins D1, D2, A and E, Id2, cdk4 and cdc25A phosphatase.

As a TF, Myc binds to specific DNA sequences called E-boxes, as a heterodimer complex with partner Max [150] (Fig. 4.18). However, Mad protein hetero-dimers block its transcription. Myc controls cell numbers but not cell size [151]. Myc itself functions as a repressor when bound to Miz-1 [152] (Fig. 4.18).

4.5.2 *Forkhead Transcription Factors (Foxos)*

Forkhead TFs (Foxos) regulate cell stress, cell cycle and cell death pathways. They regulate cell longevity and suppression of tumours. The Foxos can (a) block cell proliferation, (b) induce cell apoptosis and (c) offset oxidative stress by induction of manganese SOD. The Foxos will inhibit c-Myc-induced genes. To do this, Foxos bind directly to DNA, but they can also interact with transcription binding partners like P300/CREB or the steroid receptor coactivator protein. Indeed, Foxos induce cell cycle arrest genes like the inhibitor p27^{kip.1} and GADD45, proapoptotic factor Bim and death genes. Since Foxos stop cell proliferation, when IL-2 and -3 promote proliferation and survival of T lymphocytes, they have to use phosphorylation mediated

Table 4.1 Serine-threonine kinases in signal transduction

Cyclic adenosine monophosphate (cAMP)-dependent protein kinase (i.e. protein kinase A [PKA])
Protein kinase C (PKC), protein kinase B (PKB/Akt)
Cyclic guanosine monophosphate (cGMP)-dependent protein kinase
Ca ²⁺ -calmodulin-dependent protein kinases
S6 kinase for S6 ribosomal protein
MAPKs (mitogen-activated protein kinases, e.g. Raf, MEK, extracellular signal-related kinase [ERK])
DAPK (death-associated protein kinase), leading to apoptosis

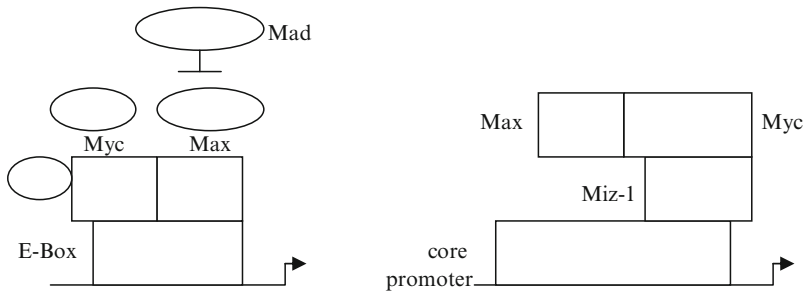


Fig. 4.18 Transcriptional complexes formed by Myc. c-Myc, L-Myc, and N-Myc dimerize with the related protein Max to induce the D-type cyclins. The Myc-Max heterodimer binds to the E-box CAC(G/A)TG motif. Myc can then interact with the TATA-binding protein, leading to transcriptional activation. c-Myc inhibits expression of cyclin-dependent protein kinase (*CDK*) inhibitors p21^{cip.1} and p15^{Ink4b} since it prevents Miz1 from activating the p21 and p15 promoters. C-Myc likewise inhibits p15 and p21 induction by transforming growth factor- β (*TGF β*) through direct binding to Smads2/3. Hence, c-Myc opposes cycle arrest by *TGF β*

Table 4.2 Examples of transcription factors

Homeodomain

Oct-1, which regulates histone gene H2B and thymidine kinase

Oct-2, which regulates immunoglobulin genes

Pit-1, which regulates growth hormone

Leucine zipper protein

C/EBP (CCAAT/enhancer binding protein) regulates albumin, α 1-antitrypsin genes, also a regulator of granulopoiesis

CREB (cyclic AMP response element-binding protein) (Figs. 1.1 and 4.8)

Fos and Jun proteins that form AP-1 (activator protein 1) complexes

Zinc finger proteins

Transcription factor IIA engages RNA polymerase II to its promoter

Sp-1 engages RNA polymerase II to the gene promoter

Glucocorticoid, estrogen, progesterone, vitamin D receptors

Helix-loop-helix

Myc proteins regulate genes in the cell cycle

MyoD protein for muscle genes

Scf-Tal for the earliest step in haemopoiesis

E2A or HEB for rearrangements of T cell receptor loci

Foxos (forkhead transcription factors)

by PI3K $\rightarrow$ protein kinase B (PKB, Akt) [153] to inactivate Foxo 3a. Likewise, PI3K-PKB signalling has to inactivate Foxo1 and Foxo3A when there is B cell receptor-triggered proliferation of B cells. At different stages in the cell cycle, antiapoptotic PI3K-PKB is inverse to the proapoptotic Foxos. More than 100 Foxos regulate development. Foxos regulate cell cycle progression and promote resistance to oxidative stress. They also mediate the effects of insulin on metabolism [154]. With regard to immunology [155, 156], note that (a) FoxN1 regulates development of thymic epithelial cells. (b) Foxp 3 identifies regulatory T cells induced by *TGF β* [157], but

it is not expressed by those Treg cells that secrete IL-10 [158]. Estrogen can induce Foxp 3 expression, which helps when Treg cells are required in pregnancy. Furthermore, TGF β through activation of Smads (Chap. 14) induces the expression of Foxp 3. Foxp 3-binding sites are identified in the promoters of the genes for TNF α , GM-CSF and IL- 4. (c) In Foxjl^{-/-} mice, NF- κ B is greatly increased, resulting in overproduction of Th1 cell cytokines. In fact, Foxjl can directly induce the expression of I κ B, resulting in suppression of NF- κ B activation (Chap. 11). Accordingly, Fox3a modulates T helper cell activation by inhibiting NF- κ B [159].

4.6 Differential Gene Expression Patterns

Using oligonucleotide microarrays, one can now examine gene expression profiles for any cell type, in particular in the peripheral blood mononuclear cells. A study compared the response to LPS of adult blood monocytes with those taken from the umbilical cord [160, 161]. How LPS stimulates macrophages is studied in this way [162]. An obvious application is the study and classification of lymphomas. Hypermutable genes that cause DNA strand breaks were found to characterise diffuse large B cell lymphomas [163].

Genomic microarray technology for the study of how TFs affect gene targets comes in several technical forms [164]. Clearly, one can identify the genes regulated by each factor during the sequential stages of development. Thus, T-bet is required for the development of Th1 cells. The role of Max in the regulation of Myc target promoters (Fig. 4.18) has been elucidated [165].

4.7 A Note about Ubiquitination

Unwanted cell surface glycoproteins, activated receptors and cytosolic and nuclear proteins are all removed by a planned process of ubiquitination (ubiquitylation) by the cell Ub ligase system [166, 167]. First, enzyme E1 activates Ub; second, active Ub is transferred to an E2-conjugating enzyme; third, E3 Ub ligase attaches Ub to lysine residues on the protein that is targeted for degradation. Thus, when oxygen levels are adequate in cells, the hypoxia-inducible factor 1 α (HIF1 α) transcriptional activator undergoes prolyl hydroxylation and then ubiquitination by E3 enzymes. Protein ubiquitination will influence the ability of a target protein to interact with other proteins. *Monoubiquitination* is used as a signal for receptor internalisation, vesicle sorting, DNA repair and gene silencing. *Polyubiquitination* results from attachment of a chain of Ub molecules to one or more Lys residues [159, 160]. Ubiquitination affects protein processing or translation, protein-protein interactions and proteasome-mediated degradation of proteins. S-Nitrosylation inhibits proteins like the p50 of NF- κ B, IKK β , Ask-1, and JNK1 in macrophages. As a consequence, such proteins can be ubiquitinated and undergo proteasomal degradation. In general, Ub has a major role in modification of protein functions.

Furthermore, ubiquitination plays a pivotal role in the regulation of gene transcription by its modification of histones and TFs [168]. Thus, JNK via phosphorylation-dependent activation of E3 ligase Itch affects the turnover of TFs c-Jun and JunB. Forms of Ub ligase control cell cycle checkpoints [169]. Proliferating cell nuclear antigen (PCNA) is a protein that assembles as a trimer ring, which encircles DNA and thus recruits DNA polymerases during DNA replication and postreplicative DNA repair. It is modified by Ub chains. As a result of DNA damage, there is sequential DNA sumoylation [85, 169a] and ubiquitination [166, 167] (Table 4.3).

Study of the major deubiquitinase for histone H2A has revealed that H2A deubiquitination is critically involved in control of the progression of the cell cycle and in gene expression [169b].

Ubiquitination plays major roles in the control of immune regulation [170]. Ubiquitination is used to control G protein subunits and MAPKs. Dendritic cells regulate exposure of MHC class II molecules on their surfaces by oligoubiquitination. There is *stimulatory ubiquitination* when there is activation of IKK/NF- κ B (Chap. 11) [171]. In Chap. 11, it is described how K63 ubiquitination of Traf2 on the TNF receptor path or of Traf6 on the IL-1 receptor/Toll-like receptor 4 (TLR4) pathways leads to phosphorylation of the IKK complex of the NF- κ B. This is supported by the phosphorylation of I κ B α , which leads to its K48 polyubiquitination and subsequent proteasomal degradation. There is *inhibitory ubiquitination* when c-Cbl protein induces degradation of the p85 unit of PI3K, so that T lymphocytes are held in check [172]. The Ub ligase Cbl promotes multiple monoubiquitination of activated receptor tyrosine kinases, ensuring their movement into lysosomes. A20 is an Ub-modifying enzyme that dampens signals through TNF-RI destined to activate NF- κ B [173]. A20 can remove K63-linked polyubiquitin chains from TRAF6, RIP and IKK γ respectively (Figs. 12.2–12.5), and it also promotes K48 polyubiquitination of RIP in the TNF signal pathway, thereby targeting it for proteasomal degradation [174]. A20 likewise dampens signals to NF- κ B when TLRs engage with microorganisms. A20 thus imparts anti-inflammatory effects [175], and A20 is antiapoptotic (Fig. 8.2). Then, in macrophages the Ub-like protein MNSF β has been shown to regulate the ERK cascade and TNF α production [176]. MEKK1 was depicted in Fig. 4.3. This MAPKK is also an Ub ligase that regulates both ERK1/2 and c-Jun amino-terminal kinase. In fact, MEKK1 regulates the AP-1 repertoire [24] (Fig. 4.4). In MEKK1^{-/-} cells, Jun B mRNA is increased, demonstrating that MEKK1 suppresses Jun mRNA expression [177].

Table 4.3 Modifications of histone lysines affecting transcription

Histone modification	Enzyme	Opposed by	Transcriptional effect
Acetylation	HATs	HDACs	Activation
Methylation	HMTs		Repression/activation
Ubiquitylation	Proteases		Activation
Sumoylation	Proteases		Repression

HAT histone acetylase, *HDAC* histone deacetylase, *HMT* histone methyltransferase

4.7.1 Sumoylation and Gene Repression

Sumoylation, via small ubiquitin-related modifier (SUMO), is an enzyme process akin to ubiquitination [85]. It also can regulate transcriptional repression, but unlike ubiquitination, sumoylation does not target protein substrates for degradation. SUMO conjugation regulates protein-protein interaction, protein-DNA interaction and enzymatic activity. The target lysine on histones is substrate for multiple modifying enzymes. The amino tail of histone H4 has five lysines, all of which may be candidates for sumoylation. So, there may be decreased transcriptional activity of Elk-1 (**Fig. 4.3**), Sp3, c-Myb, c-Jun, etc. Elk-1, acting via SRE, mediates cell cycle entry and, by the use of Mcl-1, it is antiapoptotic. SUMO is known to recruit HDAC2. PPAR γ represses the transcriptional activation of inflammatory response genes like iNOS in macrophages (Fig. 4.14) by using a sumoylation pathway [178].

4.8 Notch Signalling in Haemopoiesis

Notch family transmembrane receptors help the development of thymocytes and T cells, whereas Id helps the formation of NK cells. The ligands for the Notch receptors are similar transmembrane proteins on adjacent cells (often dendritic cells or thymic epithelium) with names like Delta and Jagged. There are four Notch receptors (Notch 1–4) and five ligands, named Jagged-1 and Jagged-2 and Delta-1, -3 and -4 [179, 180]. The Notch receptor has a large extracellular domain formed of EGF repeats that interact with EGF repeats of the ligands. The cytosolic domain has ankyrin-like repeats and a PEST domain (glutamine, serine and threonine). Ligand binding releases the NICD notch intracellular domain, which translocates into nuclei and regulates gene expression by interaction with the DNA binding protein RBP-Jk (also called CSL), converting it from a repressor to a coactivator. The RBP-Jk binds to the promoters of cellular genes. Notch activation involves a large membrane multisubunit protease, γ -secretase, to which inhibitors are being developed.

Notch is expressed on lymphoid, myeloid and erythroid precursors. Notch limits the number of cells that adopt a particular fate. It leaves some progenitors uncommitted and thus able to follow alternative fates [180]. Notch is antiapoptotic since generally it induces NF- κ B activity [181]. Hence, Notch is implicated in haematological malignancies [182]. Notch interferes with the scaffold JNK-interacting protein 1 to inhibit the JNK signal pathway [183]. Thymic epithelial cells express Notch ligands Jagged-1, -2 and D11-1. Notch directs stem cells into double-positive thymocytes, and it promotes T cell development, but not that of B or NK cells. A Jagged-Notch 1 signal has been shown to induce GATA3 [184a] and so Th2 cells, whilst Delta4-Notch3 signals induce IFN γ and so Th1 cells [184b]. Activated T cells that express Notch are not liable to apoptosis [185] since Akt is supported and Bcl-Xl. Notch 4 is involved in regulatory T cells [186].

Notch ligand Delta-1 inhibits differentiation of monocytes into macrophages, but it allows monocyte differentiation into dendritic cells [187]. DCs upregulate

Jagged or Delta-like ligands in response to different stimuli. Notch-2 directs formation of marginal zone B cells but suppresses the formation of follicular B cells [188a]. In CD8 T cells Notch 2, by interaction with CREB, creates a complex on the promoter of granzyme B that determines T cell cytotoxicity [188b].

Notch signals inhibit development of erythroid and megakaryocytic cells by suppressing GATA1 activity through the induction of HES1 [189]. In the development of mammary tissues and in 50% carcinomas, Numb signalling opposes Notch signalling [190]. Loss of the negative regulation by Numb over Notch is relevant to breast carcinogenesis (Chap. 18).

4.9 Hypoxia-Inducing Factor

HIF is a TF [191]. The β -unit HIF1 β is present constantly in cell nuclei. When oxygen is available, its α -unit HIF1 α is degraded rapidly since it is targeted for ubiquitination by von Hippel-Lindau protein (Fig. 4.19). When cells are short of oxygen, HIF1 α is stabilized since the degrading enzyme prolyl-4-hydroxylase requires molecular oxygen [191, 192]. Furthermore, to activate HIF1, there must be release of mitochondrial ROS, which trigger p38 MAPK [193].

HIF1 α regulates at least 30 genes like (a) erythropoietin for red cell formation and organ anabolism; (b) Glut-1 transporter and enzymes of glycolysis; (c) iNOS, whose product NO produces vasodilatation and neutralizes superoxide anions; and (d) VEGF, which is implicated in tissue vascularization and that of tumours. HIF1 regulates genes that govern vascular tone like endothelin, adrenomedullin, haemoxygenase 1 and nitric oxide synthetase (NOS2) and α 1-adrenergic receptor.

HIF1 overexpression is observed in many cancers [194]. The interior of a tumour becomes progressively hypoxic. A combination of decreased oxygen consumption by oxidative phosphorylation and increased ROS/H₂O₂ generation is probable [195]. A dominant-negative HIF1 α reduces tumorigenicity of pancreatic cancer cells through the suppression of glucose metabolism [196]. A growing number of additional nonhypoxic agents also stimulate HIF, such as superoxide anions, hydrogen peroxide, glycolytic intermediates, Cox-2, prostaglandin E2 (PGE2), TNF α and the growth factors PDGF, EGF and IGF-1. Even in normoxia HIF1 α regulates the

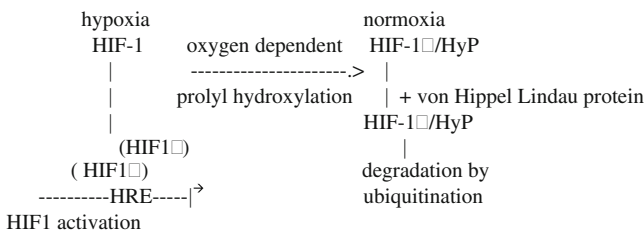


Fig. 4.19 Operation of hypoxia-inducing factor 1 α /1 β (HIF1 α /HIF1 β)

bactericidal capacity of phagocytes by inducing release of antimicrobial peptides, granule proteases, TNF α and NO [197]. In the context of inflammation or spread of tumours, HIF induces expression of adhesion molecules [198]. Thus, leucocytes in a hypoxic environment show HIF1-dependent β 2-integrin-mediated adhesion [199]. As stressed, movement of HIF into cell nuclei occurs also under normoxic conditions when there is stimulation by (a) ROS or RNS, (b) cytokines and (c) growth factors like IGF-1. VEGF is known as an inducer of angiogenesis, and it restores blood flow and normal metabolism in tissues affected by degenerations such as Alzheimer's or motor neurone disease.

Whenever there is local tissue hypoxia, HIFs help by inducing vasodilatation and direct support for glycolysis. At the same time, adenosine (derived from ATP/ADP) acts on A2A receptors on cells, which are now "sensors of tissue damage". Adenosine elevates cAMP in phagocytes and immune cells, and their responses are dampened [200]. Indeed, adenosine inhibits NF- κ B [201] (cf. Chap. 6, Sect. 3). Adenosine also induces HIF1 α in macrophages [202].

The joints of persons with rheumatoid arthritis are subjected to periodic hypoxia. Hypoxia of the rheumatoid synovium induces HIF1s [203], so there is formation of VEGF and chemokine SDF-1 (CXCL12). Essentially SDF-1 attracts lymphocytes to the synovium, and it can attract neutrophils, monocyte-macrophages and dendritic cells (Sect. 12.16).

Von Hippel-Lindau protein inhibits hypoxia-inducible genes, like those for cell growth (TGF α), glucose uptake (Glut1) and angiogenesis (VEGF). When Von Hippel-Lindau protein is lost by mutation, these proteins are overexpressed, creating a microenvironment favourable to epithelial cell proliferation. Defects in the Von Hippel-Lindau gene are responsible for many cases of sporadic clear cell renal cell carcinoma [204].

4.10 Vascular Endothelial Growth Factor

VEGF is released by hypoxia (i.e. via HIF1) [205]. Cell signalling by VEGF receptors (VEGFRs) is quite standard [205], but complexity arises from the VEGF isoforms and the fact that there are two receptors, VEGFR1 and VEGFR2 [206, 207]. The initial dimerization of VEGFRs leads to (a) activation of phospholipase C, so that PIP3 is converted to IP3 and diacylglycerol (DAG), and the latter activates PKC (**Fig. 2.1**). PKC, acting on sphingosine kinase, then activates the cascade Ras-Raf-MEK-ERK so that there is gene transcription that will help cell proliferation [207]. In turn, hypoxic activation of PI3K and thence Rho $\rightarrow$ Rock activates c-Myc, which also helps to induce VEGF [206]. (b) There will be activation of PI3K so that PKB (Akt) is activated (**Fig. 2.8**) and thereby endothelial NOS, so that NO $^{\cdot}$ can increase vascular permeability. (c) PI3K activates Rac GTPase, leading to reorganization of actin filaments, which help cell migration (cf. **Figs. 9.6–9.8**). (d) ERK activation results also in activation of cytosolic phospholipase A2 (cPLA2) (**Fig. 6.2**), so that there is formation of prostacyclin, which aids integrity of the vasculature. By

its activation of big MAPK (i.e. big mitogen-activated protein kinase 1 [BMK1] or ERK5), VEGF helps the survival of endothelial cells [208].

Above all, VEGF is known for its ability to promote angiogenesis. VEGF plays an important role in maintaining the integrity of the (renal) microcirculation. Too much VEGF is pertinent to the problem of diabetic proliferative retinopathy, which destroys vision. In fact, VEGF promotes antiapoptosis of endothelial cells (Chap. 8) via the upregulation of bcl-1 and agent A1 [203]. VEGF up-regulates inhibitor of apoptotic proteins (IAPs; Chap. 8). In endothelial cells, VEGFRs work together with VE cadherin receptors to cause activation of PI3K (survival factor) and thence NF- κ B (Chap. 11), so creating Gas-1 gene product, which is antiapoptotic [209]. VEGF via VEGFR2 promotes cell proliferation, and via VEGFR1 and p38 MAPK, it promotes vascular permeability. Details of angiogenic signalling pathways are in ref [210].

VEGF aids the migration of monocytes and macrophages. Often, VEGF activates integrin α V β 3 (cf. Fig. 9.11) in a PI3K- and PKB/Akt-dependent manner, resulting in enhanced cell adhesion and migration [211]. One will deduce that tumour cells will be affected likewise. Indeed, in breast cancer (Chap. 18) epithelial-mesenchymal cell transition leads to autocrine action of VEGF that promotes the integrin α 6 β 4 [212].

It is intriguing how monocytes are recruited into tumours (Chap. 18) and differentiate into macrophages, which accumulate in avascular and necrotic areas where there is hypoxia [213]. They are probably attracted by cell debris from necrotic cells, such as HMGB1 high-mobility factor that is TNF α -like [214]. Tumours release VEGF, and this is chemotactic for monocytes and macrophages. VEGF promotes formation of new blood vessels (angiogenesis) within tumours, and it helps growth of tumour cells and their metastasis [210]. Interestingly, MKP-1 is upregulated by hypoxia, and the dephosphorylation of phosphorylated MAPK by MKP-1 could explain how migration is inhibited, so that macrophages then remain at such locations.

References

1. Edelman AM, Blumenthal DA, Krebs EG. Protein serine/threonine kinases. *Annu Rev Biochem* 1987;56:567–613
2. Flockhart DA, Corbin JD. Regulatory mechanisms in the control of protein kinases. *CRC Crit Rev Biochem* 1982;12:133–186
3. Hancock JT. Protein phosphorylation, kinases and phosphatases, pp. 57–92 in *Cell Signalling*, 1999, Longman, Edinburgh
4. Garrington TP, Jolman GL. Organisation and regulation of MAP kinase signaling pathways. *Curr Opin Cell Biol* 1999;11:211–218
5. Lu Z, Xu S. ERK1/2 MAP kinases in cell survival and apoptosis. *IUBMB Life* 2006;58(11):621–631
6. Latchmann DS. Eukaryotic transcription factors. *Biochem J* 1990;270:281–289
7. Brivanlou AH, Darnell JE. Signal transduction and the control of gene expression. *Science* 2002;295:813–818
8. Firestein GS, Manning AM. Signal transduction and transcription factors in rheumatic disease. *Arthritis Rheum* 1999;42:609–662

9. Fantl WJ, Escobedo JA, Martin GA et al.. Distinct phosphorylation on a growth factor receptor bind to molecules that mediate differing signal pathways. *Cell* 1992;69:413–423
10. Oued S, Yarden Y. Molecular ticket to enter cells. *Nature* 2002;416:133–136
11. Martin EP, Mohnhey RP, Dunn S et al. Intersectin regulates EGF receptor endocytosis, ubiquitylation and signaling. *Mol Pharmacol* 2006;70:1643–1653
12. Wang P-Y, Wang J, Anderson RGW. OSBP is cholesterol-regulated scaffolding protein in control of ERK1/2 activation. *Science* 2005;307:1472–1476
13. Blumer KJ, Johnson GL. Diversity in function and regulation of MAP kinase pathways. *Trends Biochem Sci* 1994;19:236–240
14. Sasagawa S, Ozaki Y-I, Fujita K, Kuroda S. Prediction and validation of the distinct dynamics of transient and sustained ERK activation. *Nat Cell Biol* 2005;7:365–374
15. Whitmarsh AJ, Davis RJ. Transcription factor AP-1 regulation by mitogen activated protein kinase signal transduction pathways. *J Mol Med* 1996;74:589–607
16. Murphy LO, Small S, Chen RH et al. Molecular interpretation of ERK signal duration by immediate early gene products. *Nat Cell Biol* 2002;4:556–564
17. Murphy LO, Blenis J. MAPK signal specificity. *Trends Biochem Sci* 2006;31:268–275
18. Zhuang S, Schnellmann RG. A death-promoting role for extracellular signal regulated kinase. *J Pharmacol Exp Ther* 2006;319:991–997
19. Kholodenko BN. Untangling the signalling wires. *Nat Cell Biol* 2007;9(3):247–249
20. Kyriakis JM, Avruch J. Protein kinase cascades activated by stress and inflammatory cytokines. *BioEssays* 1996;18:567–579
21. Kolch W. The regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. *Biochem J* 2000;351:289–305
22. Kolch W. Coordinating ERK/MAPkinase signaling through scaffolds and inhibitors. *Nat Rev Mol Cell Biol* 2005;6:827–837
23. Arcaro A, Aubert M, Espinosa del Hierro ME et al. Critical role for lipid raft associated Src kinases in activation of PI3K-Akt signaling *Cell Signal* 2007;19:1081–1092
24. Morrison DK, Davis RJ. Regulation of MAP kinase signaling modules by scaffold proteins in mammals. *Annu Rev Cell Dev Biol* 2003;19:91–118
25. Zama T, Aoki R, Kaminoto T et al. Scaffold role of MAP kinase phosphatase SKRP1 for the JNK signaling pathway. *J Biol Chem* 2002;277:23919–23926
26. Fusello AM, Mandik-Nayak L, Shih F et al. The MAPK scaffold KSR is involved in ERK activation by stress and cytokines in induction of arthritis. *J Immunol* 2006;177:6152–6158
27. Brown MD, Sacks DB. IQGAP1 in cell signaling. *Trends Cell Biol* 2006;16:16242–16249
28. Cooper CE, Patel RP, Brookes PS, Darley-Usmar VM. Nanotransducers in cellular redox signalling: modification of thiols by reactive oxygen and nitrogen species. *Trends Biochem Sci* 2002;27:489–492
29. Bae YS, Sung J-Y, Lim CS, Kim YJ et al. PDGF induced hydrogen peroxide production requires the action of phosphatidylinositol 3-kinase. *J Biol Chem* 2000; 275:10527–10531
30. Valko M, Leibfritz D, Moncol J et al. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007;39:44–84
31. Chiarugi P, Cirri P. Redox regulation of protein tyrosine phosphatases during receptor tyrosine kinase signal transduction. *Trends Biochem Sci* 2003;28:509–513
32. Stamler JS, Lamas S, Fang FC. Nitrosylation: the prototypic redox based signaling mechanism. *Cell* 2001;106:675–683
33. Marshall HE, Hess DT, Stamler JS. S-Nitrosylation: physiological regulation of NF- κ B. *Proc Natl Acad Sci U S A* 2004;101:8841–8842
34. Davis RJ. Signal transduction by JNK group of MAP kinases. *Cell* 2000;103:239–252
35. Karin M, Gallagher E. Jun kinases. *IUBMB Life* 2005;57(4/5):283–295
36. Lina A. JNK signaling: braking the brake of apoptosis. *BioEssays* 2003;25:17–25
37. Pantano C, Reynaert NL et al. Redox sensitive kinases of the NF- κ B signaling pathway. *Antioxid Redox Signal* 2006;8(9–10):1791–1806
38. Kanda H, Miura M. Regulatory roles of JNK in programmed cell death. *J Biochem* 2004;136:1–6

39. Sunayama J, Tsuruta F, Masuyama N, Gotoh Y. JNK antagonizes Akt mediated survival signals by phosphorylating 14-3-3. *J Cell Biol* 2005;170:295–304
40. Lu Z, Serghides L, Patel SN et al. Disruption of JNK2 decreases the cytokine response to *Plasmodium falciparum* and protects in cerebral malaria. *J Immunol* 2006;177: 6344–6352
41. Tao J, Gao Y, Li MO et al. JNK2 negatively regulates CD8⁺ T cell effector function and anti-tumour immune response. *Eur J Immunol* 2007;37:818–829
42. Vincenti MP, Brinckerhoff CE. The potential of signal transduction inhibitors for treatment of arthritis. *J Clin Invest* 2001;108:181–183
43. Kumar S, Boehm J, Lee JC. p38 MAP kinases: key signalling molecules as therapeutic targets for inflammatory diseases. *Nat Rev Drug Discov* 2003;2:717–726
44. (a) Varghese J, Chattopadhyaya S, Sevin A. Inhibition of p38 kinase reveals a TNF α mediated caspase dependent apoptotic death pathway. *J Immunol* 2001;166:6570–6577; (b) Han J, Sun P. The pathways to tumor suppression via route p38. *Trends Biochem Sci* 2007;32: 364–371
45. Shibuya H, Yamaguchi H, Shirakabe K. TAB1: an activator of TAK1 in TGF β signal transduction. *Science* 1996;272:1179–1182
46. Saccani S, Pantano S, Natoli G. p38 dependent marking of inflammatory genes for increased NF κ B recruitment. *Nat Immun* 2002;3:69–75
47. Dean JL, Sully G, Clark AR, Saklatvala J. The involvement of AU-rich element binding proteins in p38 MAPkinase mediated mRNA stabilisation. *Cell Signal* 2004;16:1113–1121
48. Westra J, Limburg PC, de Boer P, van Rijswijk MH. Effects of RWJ 67657, a p38 MAP kinase inhibitor on the production of inflammatory mediators by rheumatoid synovial fibroblasts. *Ann Rheum Dis* 2004;63:1453–1459
49. van den Blink B, Juffermans NP, Ten Have T et al. p38 MAPK inhibition increases cytokine release by macrophages. *J Immunol* 2000;163:538–544
50. Puig-Kroger A, Rellosio M, Fernandez-Capetillo O et al. ERK signaling pathway negatively regulates the phenotypic and functional maturation of monocyte derived human dendritic cells. *Blood* 2001;98:2175–2182
51. Cora DC, Kaur J, Forster M et al. Role of p38 MAP kinase in chemokine induced emigration and chemotaxis in vivo. *J Immunol* 2001;167:6552–6558
52. Hall DJ, Bates ME, Guar L et al. The role of p38 MAPK in rhinovirus induced MCP-1 production by monocytic lineage cells. *J Immunol* 2005;174:8056–8063
53. Woo C-H, Lim J-H, Kim J-H. Lipopolysaccharide induces MMP9 expression via a mitochondrial ROS-p38 kinase activator protein-1 pathway in Raw 264.7 cells. *J Immunol* 2004 173:6973–6980
54. Rudd CE. MAPK p38: alternative and nonstressful in T cells. *Nat Immun* 2005;6:368–370.
55. Dodeller F, Skapenko A, Kalden JR et al. The p38 MAP kinase regulates effector functions of primary human CD4 T cells. *Eur J Immunol* 2005;35:3631–3642
56. Jirmanova L, Jankovic D, Fornace AJ, Ashwell JD. GADD45 α regulates p38 dependent DC cytokine production and Th1 differentiation. *J Immunol* 2007;178:4153–4158
57. Whitmarsh AJ, Davis RJ. A central control for cell growth. *Nature* 2000;403:255–256
58. Roovers K, Assoian RK. Integrating the MAP kinase signal into the G1 phase cell cycle machinery. *BioEssays* 2000;22:818–826
59. Lang R, Hammer M, Mages J. Dual specificity MAPK phosphatases in control of the inflammatory response. *J Immunol* 2006;177:7497–7504
60. Camps M, Nichols A, Arkinstall S. Dual specificity phosphatases: a gene family for control of MAP kinase function. *FASEB J* 1999;14:6–16
61. Pouyssegur J, Lenormand P. Fidelity and spatio-temporal control in MAPK signalling. *Eur J Biochem* 2003;270:3291–3299
62. Dand N, Peter M. Scaffold proteins in MAPkinase signaling. *BioEssays* 2006;28:146–156
63. Abraham SM, Clark AR. Dual specificity phosphatase-1: a critical regulator of immune responses. *Biochem Soc Trans* 2006;34(6):1018–1023
64. Xu Q, Konta T, Nakayama K et al. Cellular defence against H2O2 induced apoptosis via MAPkinase MKP-1 pathway. *Free Rad Biol Med* 2004;36:985–993

65. Tanoue T, Moriguchi T, Nishida E. Molecular cloning and characterization of a novel dual specificity phosphatase MKP-5. *J Biol Chem* 1999;274:19949–19956
66. Shanley TP, Vasi N, Denenberg A, Wang HR. The serine threonine phosphatase PP2A endogenous regulator of inflammatory cell signaling. *J Immunol* 2001;166:966–972
67. van Kriegsheim A, Pitt A, Grindlay GJ et al. Regulation of the Raf-MEK-ERK pathway by protein phosphatase 5. *Nat Cell Biol* 2006;8:1011–1016
68. Chiarugi P, Cirri P. Redox regulation of protein tyrosine phosphatases during receptor tyrosine kinase signal transduction. *Trends Biochem Sci* 2003;28:509–512
69. Fuh KC, Meneshian A, Patel CB, Takiar V, Bulkley CB. Signal transduction by reactive oxygen species. Alternative paradigms of signaling specificity *Surgery* 2002;131:601–612
70. Cruz CM, Rinna A, Forman HJ. ATP activates ROS dependent secretion of proinflammatory cytokines in macrophages. *J Biol Chem* 2007;282:2871–2879
71. Bensaad K, Tsuruta A, Selak MA. TIGAR regulator of glycolysis. *Cell* 2006;126:107–120
72. Patwari P, Lee RT. Thioredoxins, mitochondria and hypertension. *Am J Pathol* 2007;170:805–808
73. Calkhavan CF, Geert AB. Multiple steps in the regulation of transcription factor level and activity. *Biochem J* 1996;317:329–342
74. Levine M, Tjian R. Transcription regulation and animal diversity. *Nature* 2003;424:148–151
75. Shaywitz AJ, Greenberg ME. CREB: a stimulus induced transcription factor activated by diverse extracellular signals. *Annu Rev Biochem* 1999;68:821–861
76. Chakravarti S, LaMorte VJ, Nelson MC et al. Role of CBP/P300 in nuclear receptor signalling. *Nature* 1996;383:99–103
77. Utley RT, Ikeda K, Grant PA et al. Transcriptional activators direct acetyltransferase complexes to nucleosomes. *Nature* 1998;398:29–30
78. Jenuwein T, Allis CD. Translating the histone code. *Science* 2001;293:1074–1080
79. Kyriakis JM. Life or death decisions. *Nature* 2002;414:265–266
80. (a) Grady GC, Mason SM, Stephen J et al.. CREB protein plays a central role in mediating proliferation and differentiation downstream of the preTCR complex in developing thymocytes. *J Immunol* 2004;173:1802–1810; (b) Mayr B, Montminy M. CREB. *Nat Rev Mol Cell Biol* 2001;2:599–609
81. Porter CM, Clipstone NA. Sustained NFAT signaling promotes a Th.1 like pattern of gene expression in primary murine CD4 T cells. *J Immunol* 2002;168:4936–4945
82. Serfling E, Chuvpilo S, Liu J et al. NFATc1 autoregulation: a crucial step for cell-fate determination. *Trend Immunol* 2006;27:461–468
83. Macian F. NFAT proteins; key regulators of T cell development and function. *Nat Rev Immunol* 2005;5:472–484
84. Orkin SH.. 2001, Transcription factors that regulate lineage decisions, *The Molecular Basis of Blood Diseases*, Saunders, St. Louis, pp. 80–102.
85. Johnson ES. Protein modification by Sumo. *Annu Rev Biochem* 2004;73:355–382
86. Kalkhoven E. CBP and p300 histone acetyltransferases for different occasions. *Biochem Pharmacol* 2004;68:1145–1155
87. Tomita M, Barnes PJ, Adcock IM. The effect of oxidative stress on histone acetylation and interleukin 8 release. *Biochem Biophys Res Commun* 2003;301:572–577
88. Fann M, Godlove JM, Catalfamer M et al. Histone acetylation associated with gene expression in the rapid memory CD8 T cell response. *Blood* 2006;108:3363–3370
89. Kagoshima M, Wilche T, Ito K et al. Glucocorticoid mediated trans-repression is regulated by histone acetylation and DNA methylation. *Eur J Pharm* 2001;429:327–334
90. Cosio BG, Mann B, Ito K et al. Histone acetylase and deacetylase activity in alveolar macrophages and blood monocytes in asthma. *Am J Resp Crit Care Med* 2004;170:141–147
91. Holloway AF, Rao S, Shannon MF. Regulation of cytokine gene transcription in the immune system. *Mol Immunol* 2001;38:567–580
92. Luecke HF, Yamamoto KR. The glucocorticoid receptor blocks P-TEFb recruitment by NFkB to effect promoter specific transcriptional request. *Genes Dev* 2005;19(9):1116–1127
93. Falvo JV, Ugialoro AM, Brinkron BM et al. Stimulus specific assembly of enhancer complexes on the TNFa gene promoter. *Mol Cell Biol* 2000;20:2239–2247

94. Chi TA. BAF-centred view of immune system. *Nat Rev Immunol* 2004;4:965–977
95. Ayn Y-T, Huang B, McPherson L et al. Dynamics of chromatin remodeling regulate late expression of chemokine Rantes. *Mol Cell Biol* 2007;27:253–266
96. Accolla RS, DeLerma Barbaro A, Mazza S et al. The MHC class II transactivator. *Trends Immunol* 2001;22:560–563
97. Wright KL, Ting JP. Epigenetic regulation of MHCII and CIITA genes. *Trends Immunol* 2006;27:405–412
98. Pattenden SG, Klose R, Karakov G, Bremner R. Interferon-gamma induced chromatin remodelling at the CIITA locus is BRG1 dependent. *EMBO J* 2002;21:1978–1986
99. Lochamy J, Rogers EM, Boss JM. CREB and pCREB interact with RFX5 and CIITA to regulate MHC class II genes. *Mol Immunol* 2007;44:837–847
100. Gourley TA, Roys S, Lukacs NW et al. A novel role for the CIITA is the repression of IL-4 production. *Immunity* 1999;1:377–386
101. Wilson CB, Makar KW, Shryreva M, Fitzpatrick DR. DNA methylation and the expanding epigenetics of T cell lineage commitment. *Semin Immunol* 2005;17:105–119
102. (a) Wright KL, Ting JP-Y. Epigenetic regulation of MHCII and CIITA genes. *Trends Immunol* 2006;27:405–412; (b) Gazzar M, Yoza BK, Hu JY-G et al. Epigenetic silencing of TNF α during endotoxin tolerance. *J Biol Chem* 2007;282:26857–26864
103. Bottomley MJ. Structures of proteins that create or recognize histone modifications. *EMBO Rep* 2004;5:464–469
104. Peterson CL, Workman JL. Promoter targeting and chromatin remodeling by the SWI/SNF complex. *Curr Opin Genet Dev* 2000;10:187–192
105. Maneechotesuwan K, Xin Y, Ito K et al. Regulation of Th2 cytokine genes by p38 MAPkinase mediated phosphorylation of GATA3. *J Immunol* 2007;178:2491–2498
106. Kaneko T, Hosokawa H, Yamashita M. Chronatin remodelling at the Th2 cytokine gene loci in human type 2 helper T cells. *Mol Immunol* 2007;44:2249–2256
107. Aneel KM, Djuretic I, Tanasa B, Rao A. Regulation of Th2 differentiation and IL-4 locus accessibility. *Annu Rev Immunol* 2006;24:607–656
108. Klein-Hessling S, Jha MK, Santer-Nonan B et al. Protein kinase A regulates GATA3 dependent activation of IL-5 gene expression in Th.2 cells. *J Immunol* 2003;170:2956–2961
109. Masuda A, Yoshikai Y, Kume H, Matsuguchi T. The interaction between GATA proteins and AP-1 promotes the transcription of IL-13 in mast cells. *J Immunol* 2004;173:5564–5573
110. Lee GR, Kim ST, Spilianakis CG, Fields PE, Flavell RA. T helper cell differentiation: regulation by *cis* elements and epigenetics. *Immunity* 2006;24:369–379
111. Semple RK, Chatterjee VKK, O’Rahilly S. PPAR γ and human metabolic disease. *J Clin Invest* 2006;116:581–589
112. Cuzzocrea S, Mazzon E, DiPaola R et al. Role of PPAR α in regulation of acute inflammation. *J Leukoc Biol* 2006;79:999–1010
113. Lefebvre P, Chinetti G, Fruchart J-C, Staels B. Sorting out the roles of PPAR α in energy metabolism and vascular homeostasis. *J Clin Invest* 2006;116:571–579
114. Dunn SE, Ousman SS, Sobel RA et al. PPAR α expression in T cells mediates gender differences in development of autoimmunity. *J Exp Med* 2007;204:321–330
115. Oates JC, Reilly CM, Crosby MB, Gilkeson GS. Peroxisome proliferator-activated receptor agonists. *Arthritis Rheum* 2002;46:598–607
116. Szanto S, Nagy L. Retinoids potentiate PPAR γ action in differentiation, gene expression and lipid metabolic processes in developing myeloid cells. *Mol Pharm* 2005;67:1935–1943
117. Szatmari I, Pap A, Ruhl R et al. PPAR γ controls CD1d expression by turning on retinoid synthesis in human dendritic cells. *J Exp Med* 2006;203:2351–2362
118. Rizzo G, Fiorucci S. PPARs and other nuclear receptors in inflammation. *Curr Opin Pharmacol* 2006;6:421–427
119. (a) Bailey ST, Ghosh S. PPARting ways with inflammation. *Nat Immunol* 2005;6:966–967; (b) Pascual GA. Sumoylation dependent pathway mediates transrepression of inflammatory response genes by PPAR- γ . *Nature* 2005;437:759–763

120. Dzhagalov I, Chambon P, He Y-WQ. Regulation of CD8 T lymphocyte function and macrophage cytokine production by retinoic acid receptor γ . *J Immunol* 2007; 178:2113–2121
121. Ishaq M, Fan M, Natarjan V. Accumulation of RXR during activation of cycling T lymphocytes. *J Immunol* 2000;165:4217–4222
122. Taschner S, Koesters C, Blatzer B. Downregulation of RXR α expression is essential for neutrophil development. *Blood* 2007;109:971–979
123. Gruber CJ, Tschugguel W, Schneeberger C, Huber JC. Production and action of estrogens. *N Engl J Med* 2002;346:340–352
124. (a) Kumar R, Wang RA, Barnes CJ. Coregulators and chromatin remodelling in transcriptional control. *Mol Carcinog* 2004; 41:221–230; (b) Cuoro A, Tatomer D, Tee M-K. Selective estrogen receptor β agonists repress transcription of proinflammatory genes. *J Immunol* 2008;180:630–636
125. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids. *N Engl J Med* 2005;353:1711–1723
126. Ito K, Chung KF, Adcock IM. Update on glucocorticoid action and resistance. *J Allergy Clin Immunol* 2006;117:522–543
127. Goecke A, Guerrero J. Glucocorticoid receptor- β in acute and chronic inflammatory conditions. *Immunobiology* 2006;211:85–96
128. Almauri WY, Melemedjian OK. Molecular mechanisms of glucocorticoid antiproliferative effects: antagonism of transcription factor activity by glucocorticoid receptor. *J Leukocyte Biol* 2002;71:9–15
129. Lu NZ, Cidlowski JA. Glucocorticoid receptor isoforms generate transcription specificity. *Trend Cell Biol* 2006;16(6):301–307
130. Fessler BJ, Paliogianni F, Hama M et al. Glucocorticoids modulate CD28 mediated pathways for IL-2 production in T cells. *Transplant* 1996;62:1113–1117
131. Scheinman RI et al. Role of transcriptional activation of I κ B α in mediation of immunosuppression by glucocorticoids. *Science* 1995;270:283–286
132. Barnes PJ, Adcock IM. How do corticosteroids work in asthma? *Ann Int Med* 2003;139:359–370
133. Ito K, Barnes PJ, Adcock IM. GR recruitment of histone deacetylase 2 inhibits IL-1 β -induced histone 4 acetylation. *Mol Cell Biol* 2000;20:6891–6903
134. Barnes PJ. Transcription factors in airway diseases. *Lab Invest* 2006;86:867–872
135. Toh M-L, Yang Y, Leech M, Santos L, Morand EF. Expression of MAKp phosphatase 1: negative regulator of MAPkinases in rheumatoid arthritis. *Arthritis Rheum* 2004;50:3118–3128
136. Abraham SM, Lawrence T, Kleiman A et al. Anti-inflammatory effects of dexamethasone are dependent on induction of DUSP-1. *J Exp Med* 2006;203:1883–1889
137. Bhattacharyya S, Brown DE, Brewer JA et al. Macrophage glucocorticoid receptors regulate TLR4 mediated inflammatory responses by selective inhibition of p38 MAPkinase. *Blood* 2007;109:4313–4319
138. Furst R, Schneider T, Eilken JHM et al. MAPkinase phosphatase-1 represents a novel anti-inflammatory target of glucocorticoids in endothelium. *FASEB J* 2007;21:74–80
139. Li L-B, Leung DYM, Hall CF, Goleva W. Divergent expression and function of glucocorticoid receptor β in human monocytes and T cells. *J Leukoc Biol* 2006;79:818–827
140. Ehrchen J, Steinmuller L, Barczyk K et al. Glucocorticoids induce differentiation of an anti-inflammatory subtype of human monocytes. *Blood* 2007;109:1265–1274
141. Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 2004;429:457–463
142. Jaenish R, Bird A. Epigenetic regulation of gene expression. *Nat Genet* 2003;33(Suppl):245–254
143. Lee DY, Teyssier C, Strahl BD, Stallcup MR. Role of protein methylation in the regulation of transcription. *Endocr Rev* 2005;26(2):147–170
144. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med*. 2003;349:2042–2054
145. Hamamoto R, Furukawa Y, Morita M et al. SMYD3 encodes a histone methyltransferase involved in the proliferation of cancer cells. *Nat Cell Biol* 2004;6:731–740

146. Bruniquel D, Schwartz RH. Selective stable demethylation of the IL-2 gene enhances transcription by an active process. *Nat Immunol* 2003;4:235–240
147. Makar KW, Wilson CB. DNA methylation is a non-redundant repressor of the Th2 effector program. *J Immunol* 2004;173:4402–4406
148. Facchini LM, Penn LZ. The molecular role of Myc in growth and transformation FASEB J 1998;12:633–635
149. Gartel AL, Shehorsk K. Mechanisms of c-myc mediated transcriptional repression of growth arrest genes. *Exp Cell Res* 2003;283:17–21
150. La Thangue NB. Chromatin control: E2F/Myc. *Science* 2002;296:1034–1035
151. Trumpff A, Refaete Y, Oskarranch T. C-myc regulates mammalian body size by controlling cell numbers but not cell size. *Nature* 2001;414:768–773
152. Patel JH, McMahon SB. Bcl-2 is a downstream effector of Miz-1 essential for blocking c-Myc induced apoptosis. *J Biol Chem* 2007;282:5–13
153. Fabre S, Lang V, Harriaque J et al. Stable activation of PI-3kinase in the T cell immune synapse stimulates Akt signaling. *J Immunol* 2005;174:4161–4171
154. Barthel A, Schmoll D, Untermann TG. FoxO proteins in insulin action and metabolism. *Trends Endocrin Metab* 2005;16:183–190
155. Birkenkamp KU, Coffey PJ. Foxo transcription factors as regulators of immune homeostasis. *J Immunol* 2003;171:1623–1629
156. Coffey PJ, Burgering BMT. Forkhead-box transcription factors and their role in the immune system. *Nat Rev Immunol* 2004;4:889–899
157. Fontini MC, Becker C, Monteleone G et al. TGF β induces a regulatory phenotype through Foxp3 induction and downregulation of Smad 7. *J Immunol* 2004;172:5149–5153
158. Vieira PL, Christensen JR, Minase S et al. IL-10 secreting regulatory T cells do not express Foxp3 but have comparable regulatory function to naturally occurring CD4+CD25+ regulatory T cells. *J Immunol* 2004;172:5986–5993
159. Lin L, Hran JD, Peng SL. Regulation of NF κ B, T helper cell activation and autoinflammation by the Forkhead transcription factor Foxo3a. *Immunity* 2004;21:203–213
160. Jiang H, van de Ven C, Satwani P, Baxi L, Cairo MS. Differential gene expression patterns by oligonucleotide microarray of basal versus LPS activated monocytes. *J Immunol* 2004;172:5870–5879
161. Weeraratna AT, Nagel JE, Mello, Coelho V, Taub DD. Gene expression profiling. *J Clin Immunol* 2004;24:213–224
162. Nau GJ, Richmond JFL, Schlessinger A et al. Human macrophage activation programs induced by bacterial pathogens. *Proc Natl Acad Sci U S A* 2002;99:1503–1508
163. Psqualucci L, Neumeister P, Goossens T et al. Hypermutation of multiple protooncogenes in B cell diffuse large cell lymphomas. *Nature* 2001;412:341–346
164. Weinmann AS. Novel ChIP based strategies to uncover transcription factor target genes in the immune system. *Nat Rev Immunol* 2004;4:381–386
165. Mao DY et al. Analysis of Myc bound loci identified by CpG island arrays shows that Max is essential for Myc dependent repression. *Curr Biol* 2003;13:882–886
166. Haglund K, Dikic I. Ubiquitylation and cell signaling. *EMBO J* 2005;24:3353–3359
167. Sun L, Chen ZJ. The novel function of ubiquitination in signaling. *Curr Opin Cell Biol* 2004;16:119–126
168. Muratani M, Tansey WP. How the ubiquitin-proteasome system controls transcription. *Nat Rev Mol Cell Biol* 2003;4:192–201
169. (a) Gutierrez GJ, Ronai Z. Ubiquitin and Sumo systems in the regulation of mitotic checkpoints. *Trend Biochem Sci* 2006;31:324–332; (b) Joo H-Y, Zhai L, Yang C et al. Regulation of cell cycle progression and gene expression by H2A deubiquitination. *Nature* 2007;449:1068–1072
170. Liu Y-C, Penninger J, Karin M. Immunity by ubiquitylation: a reversible process of modification. *Nat Immunol* 2005;5:941–952
171. Ben-Nariah Y. Regulatory functions of ubiquitination in the immune system. *Nat Immunol* 2002;3:20–26
172. Liu Y-C. Ubiquitin ligases and the immune response. *Annu Rev Immunol* 2004;22:81–127

173. Beyaert R, Heyninck K, van Huffel S. A20 binding proteins as cellular inhibitors of NF- κ B dependent gene expression and apoptosis. *Biochem Pharmacol* 2000;60:1143–1151
174. Li H, Kobayashi M, Blenska M, You Y, Lin X. Ubiquitination of RIP is required for TNF α induced NF κ B activation. *J Biol Chem* 2006;281:13636–13643
175. Silverman N, Fitzgerald K. Dubbing down innate immunity. *Nat Immunol* 2004;5:1010–1012
176. Nakamura M, Yamaguchi S. The ubiquitin-like protein MNSF β regulates the ERK-MAPkinase cascade. *J Biol Chem* 2006;281:16861–16869
177. Cuevas BD, Uhlik MT, Garrington TP, Johnson GL. MEKK1 regulates the AP-1 dimer repertoire via Jun transcription and Fra-2 protein stability. *Oncogene* 2005;24:801–809
178. Pascual G, Fong AL, Ogawa S et al. A sumoylation dependent pathway mediates transrepression of inflammatory response genes by PPAR γ . *Nature* 2005;437:759–762
179. Karner FN, Murdoch B, Gallacher L et al. The Notch ligand Jagged-1 represents a growth factor of human hematopoietic stem cells. *J Exp Med* 2000;192:1365–1375
180. Kojika S, Griffin JD. Notch receptors and hematopoiesis. *Exp Hematol* 2001;29:1041–1052
181. Cheng P, Zlobin A, Volgina V et al. Notch-1 regulates NF κ B activity in hemopoietic progenitor cells. *J Immunol* 2001;167:4458–4467
182. Leong KG, Karsan A. Recent insight into Notch signaling in tumorigenesis. *Blood* 2006;107:2223–2233
183. Kim JW, Kim MJ, Kim KJ et al. Notch interferes with the scaffold function of JNK-interacting protein1 to inhibit the JNK signaling pathway. *Proc Natl Acad Sci U S A* 2005;102:14308–14313
184. (a) Fang TC et al. Notch directly regulates Gata3 expression during T helper 2 cell differentiation. *Immunity* 2007;27:100–110; (b) Amsen D, Blander JM, Lee GR et al. Instruction of distinct CD4 T helper cell fates by different Notch ligands on antigen presenting cells. *Cell* 2004;117:515–526
185. Bheeshmachar G, Purushotaman D, Sade H et al. Notch signaling in the cytokine-dependent survival of activated T cells. *J Immunol* 2006;177:5041–5050
186. McKenzie GJ, Young LL, Bried E et al. Notch signaling in regulation of peripheral T cell function *Semin. Cell Dev Biol* 2003;14:127–134
187. Weijzen S, Velders MP, Elms AG et al. Notch ligand Jagged-1 is able to induce maturation of monocyte derived human dendritic cells. *J Immunol* 2002;169:4273–4278
- 188a. He Y, Pear WS. Notch signaling in B cells. *Semin Cell Dev Biol* 2003;14:135–142
- 188b. Maekawa Y, Minato Y, Ishifune C et al. Notch 2 integrates signaling by the transcription factors RBP-J and CREB1 to promote T cell cytotoxicity. *Nature Immunol.* 2008;9:1140–1147
189. Ishiko E, Matsumura I, Ezoe S et al. Notch signals inhibit development of erythroid/ megakaryocytic cells by suppressing GATA-1 activity through the induction of HES1. *J Biol Chem* 2005;280:4929–4939
190. Pece S, Serresi M, Santolini E et al. Loss of negative regulation by Numb over Notch is relevant to human breast carcinogenesis. *J Cell Biol* 2004;167:215–222
191. Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. *Nat Med* 2003;9:677–684
192. Schofield CJ, Ratcliffe PJ. Oxygen sensing by HIF hydroxylases. *Nat Rev Mol Cell Biol* 2004;5:343–354
193. Emerling BM, Platanaias LC, Black E et al. Mitochondrial reactive oxygen species activation of p38 MAPkinase is required for hypoxia signaling. *Mol Cell Biol* 2005;25:4853–4862
194. Brahimi-Horn C, Berra E, Pouyssegur J. Hypoxia: the tumor's way to progression along the angiogenic pathway. *Trends Cell Biol* 2001;11:S32–S36
195. Lopez-Lazaro M. HIF1 α : hypoxia inducible factor or dysoxia inducible factor. *FASEB J* 2006;20:828–832
196. Chen J, Zhao S, Nakada K et al. Dominant-negative HIF1 α reduces tumorigenicity of pancreatic cancer cells. *Am J Pathol* 2003;162:1283–1291

197. Peyssonnaud C, Datta V, Cramer T, Doedens A et al. HIF-1 α expression regulates the bactericidal capacity of phagocytes. *J Clin Invest* 2005;115:1806–1815
198. Koiko T, Kimura N, Miyazaki K et al. Hypoxia induces adhesion molecules on cancer cells. *Proc Natl Acad Sci U S A* 2004;101:8132–8137
199. Kong T, Eltzschig HK, Karhausen J et al. Leucocyte adhesion during hypoxia is mediated by HIF-1 dependent induction of β 2-integrin gene expression. *Proc Natl Acad Sci U S A* 2004;101:10440–10445
200. Khowry J, Ibla JC, Neish AS, Colgan SP. Anti-inflammatory adaptation to hypoxia through adenosine-mediated cullin-1 deneddylation. *J Clin Invest* 2007;117:703–711
201. Sitkovsky MV, Lukashev S, Apasov A et al. Physiological control of inflammatory tissue damage by HIFs and adenosine A2A receptors. *Annu Rev Immunol* 2004;22:657–682
202. De Ponti C, Carnini R, Alchera E et al. Adenosine A2a receptor mediated normoxic induction of hypoxia inducing factor.1. *J Leukoc Biol* 2007;82:392–402
203. Hitchon C, Wong K, Mag G et al. Hypoxia induced production of stromal cell derived factor 1(CXCL12) and VEGF by synovial fibroblasts. *Arthritis Rheum* 2002;46:2587–2597
204. Carroll VA, Ashcroft M. Role of hypoxia inducing factor-1alpha versus HIF2 α in regulation of HIF target genes in response to hypoxia, IGF-1 or loss of Hoppel-Lindau function. *Cancer Res* 2006;66:6264–6270
205. Forsythe JA, Jiang BH, Iyer NV et al. Activation of VEGF gene transcription by hypoxia inducible factor 1. *Mol Cell Biol* 1996;16:4604–4613
206. Olsson A-K, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling-in control of vascular function. *Nat Rev Mol Cell Biol* 2006;7:359–370
207. Takahashi H, Shibuya M. VEGF/VEGF receptor system and its role under physiological and pathological conditions. *Clin Sci* 2005;109:227–241
208. Olson EN. Undermining the endothelium by ablation of MAPK-MEF2 signaling. *J Clin Invest* 2004;113:1110–1112
209. Spagnuolo R, Corada M, Orseigo F et al. Gas.1 is induced by VE-cadherin and VEGF and inhibits cell apoptosis. *Blood* 2004;103:3305–3312
210. Bicknell R, Harris AL. Novel angiogenic signaling pathways and vascular targets. *Annu Rev Pharmacol Toxicol* 2004;44:219–238
211. Byzova TV, Goldman CK, Pampori N et al. A mechanism for modulation of cellular responses to VEGF: activation of the integrins. *Mol Cell* 2000;6:851–860
212. Mercurio AM, Backelder RE, Bates RC, Chung J. Autocrine signaling in carcinoma:VEGF and α 6 β 4 integrin. *Semin Cancer Biol* 2004;14:115–122
213. Murdoch C, Giannoudis A, Lewis CE. Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. *Blood* 2004;104:2224–2234
214. Dumitriu IE, Baruah P, Manfredi AA et al. HMGB1:guiding immunity from within. *Trends Immunol* 2005;26:381–386

Chapter 5

Cell Proliferation

Abstract Ras is a key guanosine triphosphatase (GTPase) on the MAPK pathway that may lead to cell proliferation. The text is concerned with how Ras operates and is utilised. Attention then turns to the receptor protein tyrosine kinases and how, for example, they are involved in control of smooth muscle cells.

5.1 Ras as a GTP-Binding Protein and GTPase

Ras proteins are members of the small guanosine triphosphatase (GTPase) superfamily (cf Fig. 5.1b), and they function akin to the heterotrimer G proteins since they are active in the guanosine triphosphate (GTP)-bound state and inactive in the guanosine diphosphate (GDP)-bound state [1, 2]. The activation state of Ras is self-limited by the intrinsic GTPase activity of the protein. The tyrosine kinases of growth factor receptors connect to the Ras proteins [3] and thence to the mitogen-activating protein kinase (MAPK) cascades that lead to the induction of genes for growth of cells, differentiation and proliferation. An acylation cycle (lipid modification) regulates the trafficking of Ras isoforms. Although one might have the impression that prenylation attaches Ras to the plasma membrane, prenylation initially targets Ras proteins to the endoplasmic reticulum (ER), where there are processing enzymes [4]. K-Ras is then sent to the plasma membrane. N-Ras and H-Ras are processed further on the Golgi and then are sent via vesicular transport to the plasma membrane. A significant pool of Ras GTPase is on the Golgi. H-Ras becomes associated with lipid rafts, whilst K-Ras does not. Mutations of Ras or B-Raf of the MAPK cascade often lead to malignancy.

Guanine nucleotide exchange factors (GEFs) activate Ras (Fig. 5.1a,b) by stimulating GDP-GTP exchange since Ras family proteins have low intrinsic GTPase activity. The protein Sos (Son of Sevenless) (Fig. 4.2) is a GEF. Conversely a GAP (GTPase activating protein) converts the GTP to GDP [5]. The GAP ensures that signalling proceeds for a brief period only.

In response to extracellular signals, there is modulation of Ras GTPase by any of three types of regulatory protein: activation by a GEF or inhibition by a GAP or

Ras guanine nucleotide releasing protein 1 (GRP1), is an important GEF and is activated by diacylglycerol (DAG) and calcium ions. In lymphocytes, the sequence DAG $\rightarrow$ PKC $\rightarrow$ Ras GRP is responsible for Ras activation on the Golgi [9]. This path differs from the Grb2/Sos-mediated pathway of Ras activation. Ras GRP1 and Ras GRP3 regulate proliferation of B lymphocytes by facilitating B cell receptor $\rightarrow$ Ras signalling. Ras GRP4 determines c-kit-induced development of mast cells. Activation of Ras isoforms either by growth factors or by ligation of the antigen receptors of B or T cells has been studied. Growth factors like interleukin 3 (IL-3) and CSF-1 activate N-Ras and K-Ras. Ligation of the T cell receptor (TCR) or BCR activates H-Ras, N-Ras and K-Ras via Ras-GRP.

Phospholipase C is regulated by Rac and cdc42, and phospholipase D by Rho, Rac and cdc42. Rho and Rac stimulate DAG kinase and likewise PIP 5-kinase, whilst Rac and cdc42 help to activate PI3K. *Endostatin*, an angiogenesis inhibitor, stops migration of endothelial cells. Acting via the surface integrin $\alpha 5 \beta 1$, it activates Rho-GAP so that Rho-GTP is exhausted and so there is disassembly of actin stress fibres [11].

ARF (ADP ribosylation factor) GTP-binding proteins need some explanation [12]. Interaction of GEFs (like Arno) with ARF-GDP induces formation of ARF-GTP. The ARF-GTP interacts with a diverse group of enzymes like vesicle-coating proteins (Fig. 9.1) and lipid-metabolizing enzymes. ARF-GTP promotes formation of PtdIns(4,5)P₂.

5.2 Cell Proliferation in Response to PDGF

When platelet-derived growth factor (PDGF) stimulates its receptor PDGFR, phosphotyrosine kinases (PTKs) that are part of the receptor are activated as a result of *receptor dimerization*. The phosphorylation of tyrosines is a switch that enables binding of proteins that have –SH2 (Src-homology domain 2) domains, such as Src kinase: –SH3 (Src-homology domain 3), SH2, kinase-P. Furthermore, autophosphorylation of the receptor suppresses local phosphatase activity. Thereafter, –SH2 adaptor proteins Shc and Grb2, and then Sos, become connected and lead to the activation of Ras GTPase and thence the MAPK cascade (Fig. 4.3). There is also concurrent activation of vital enzymes like phospholipase C and PI3K [13], which also have –SH2 domains. PI3K actually relocates from the cytosol to the membrane, and activated Ras binds to its catalytic unit (Fig. 2.8) [14].

The PDGFR also activates Jak1 protein tyrosine kinase [15], so that Stats become involved in transcriptional activation (Fig. 5.2). Stat proteins also have an –SH2 domain through which they bind specific P-Tyr sites on the activated receptor (Fig. 5.3). One has to pay some attention to how the various signal molecules and their protein modules (Fig. 5.6) connect with the activated receptor (Fig. 5.2). The PDGFR connects with Src kinases, so that Stat3 is activated and thus c-Myc, thereby accounting for cell proliferation. The PDGFR tyrosine kinases and associated

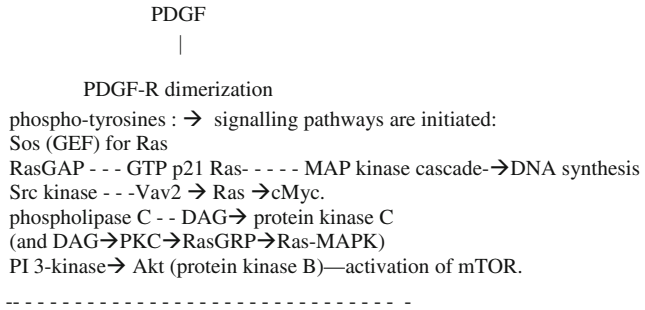


Fig. 5.2 Activation of platelet-derived growth factor (*PDGF*) receptors of fibroblasts (or the fibroblast growth factor receptor *FGFR*)

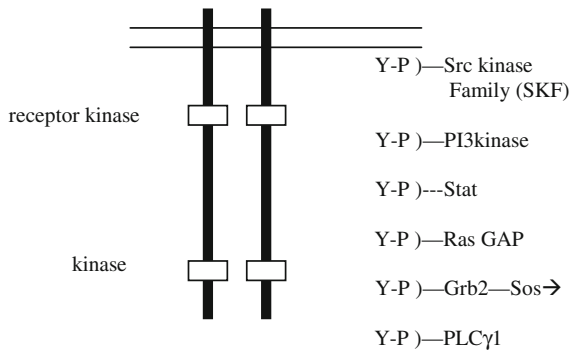


Fig. 5.3 Molecules binding to the platelet-derived growth factor receptor(*PDGFR*). The topic of PDGFR activation is so important that PDGFR kinase inhibitors are in the process of development [17]. Low molecular weight tyrosine phosphatase modulates PDGFR signalling in conjunction with the oxidoreductase glutaredoxin [18]

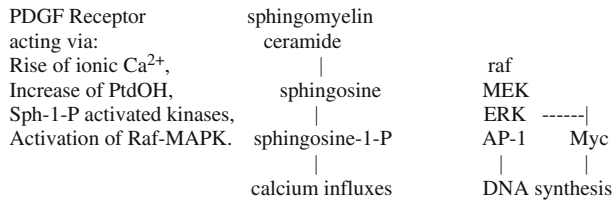


Fig. 5.4 Sphingosine mediates the response to platelet-derived growth factor (*PDGF*)

Src kinases generate hydrogen peroxide, and the oxidative stress that arises promotes downstream signalling [16]. There are now many examples of signalling systems being driven by oxidative stress.

Additional to all this, sphingosine and sphingosine-1-phosphate (Fig. 5.4) contribute to the mitogenic action of PDGF since the receptor SIP1 (SIP1.R) (originally

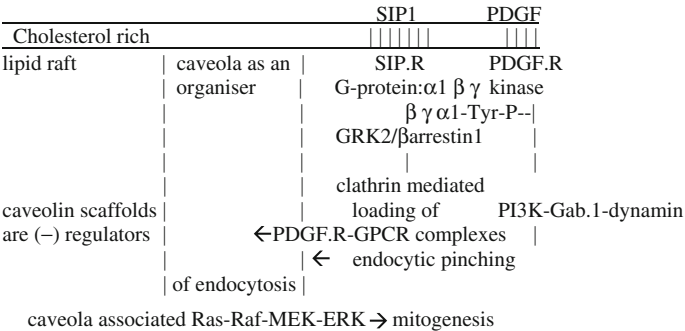


Fig. 5.5 Caveolae and sphingosine-1-phosphate (*SIP1*)/(*PDGF*) signalling, *GRK* G Protein coupled receptor kinase, *Grb1* = *Grb2* associated binder 1

Edg1) is “transactivated” by receptor tyrosine kinase (RTK) agonists by its ligand SIP (sphingosine-1-phosphate) [19].

The PDGFR and the sphingosine-1-phosphate receptor form a *signalling complex* [20]. When SIP activates its receptor, which is a GPCR, the G protein splits into the α1- and βγ-moieties. The βγ-components attract G protein-coupled receptor kinase 2 (GRK2)/β-arrestin-1, which then aids clathrin-mediated *endocytic loading* of the PDGFβR/GPCR signal complexes. Furthermore, the PDGFR stimulates the Gi-dependent tyrosine phosphorylation of Gab1 (Grb2-associated binder 1), which promotes the recruitment of dynamin to a PI3K-Gab.1 complex. Dynamin catalyses the pinching off of the endocytic vesicle containing the PDGFβR/SIPR receptor-signalling complex for relocalisation with and activation of cytoplasmic MEK1 and p42/p44 of the MAPK cascade [21]. This process is illustrated in Fig. 5.5.

Termination of signals from growth factors like epidermal growth factor (EGF) or PDGF occur when their receptors are endocytosed into surface vesicles [22]. The *endophilins*, lipid enzymes that influence membrane curvature, help to regulate the clathrin-coated pit (Fig. 5.5). *Dynamin* is a GTPase that aids clathrin-mediated endocytosis. It has the structure GTPase-PH-Pro-. In endosomes, the effete receptors meet Cbl of the family of E3 ligases, which via its RING domain, causes their ubiquitination.

5.3 Caveolae and Lipid Rafts

Src-like kinases and other signal molecules are abundant in *lipid rafts*, which are membrane segments (<5 nm) rich in cholesterol and sphingomyelin. Often, they are within *caveolae*, the 50- to 100-nm flasklike invaginations rich in cholesterol, which are a focus for signalling molecules like GF receptors, phosphoinositides, the IP3 receptor, GTPases and Ca-ATPase. The β-adrenergic receptor (Fig. 1.1) and

the EGF receptor (EGFR) (Fig. 4.2) signal through caveolae-localised proteins, which include scaffold proteins of 20 amino acids called *caveolins*, which can be negative regulators of signalling. Caveolin is known to be a negative regulator of endothelial nitric oxide synthase (eNOS) formation of nitric oxide, for example, in the lungs. Yet, an important function of Cav1 is to tether caveolae to the actin cytoskeleton through binding to the actin-binding protein filamin. Scaffolding by caveolins is often quite independent of caveolae. Caveolins occur in lymphocytes and neurones that are devoid of caveolae.

Lipid rafts (detergent-resistant membranes) serve as signal platforms to bring surface receptors close to activating kinases and to scaffold proteins and adaptor molecules. G proteins and the annexins are found within lipid rafts. Typical lipid raft designations are the anchoring of GPI proteins by N-terminal glycine myristoylation and cysteine palmitoylation [23]. Lipid rafts enable focussed signalling in lymphocytes by TCRs (Fig. 13.1), the B cell receptors (BCRs) and natural killer (NK) cell receptors NKR-P1. Furthermore, ceramide-enriched rafts mediate the internalization of bacteria, viruses and parasites into host cells, triggering release of cytokines and often apoptosis of the infected cells.

It has been shown that in small cell lung cancer cells specific growth factor receptors like c-kit are associated with lipid rafts. Application of stem cell factor to intact lipid rafts could activate Src family kinases, activating PI3K signalling by means of specific PI3K isoforms [24].

5.4 Protein Modules

Each receptor with its tyrosine kinase activity is a *platform* that connects with other signal proteins. Docking proteins are able to engage by means of defined protein modules [25] (Fig. 5.6).

One observes that RTK phosphorylation recruits SH2 domain proteins [26]. Indeed, two tandem SH2 adaptor proteins will dock with a biphenylated protein like tyrosine Zap70 the chain associated protein of 70 KDa. A PTB is a phosphotyrosine-binding domain ζ . A PTB recognises motifs with the consensus sequence Asn-Pro-X-p-Tyr. Pleckstrin homology (PH) domains bind phosphoinositides, albeit weakly [27]. Proteins with PH domains include β -adrenergic receptor kinase, the

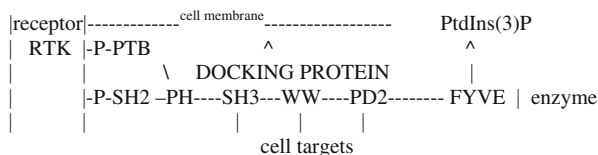


Fig. 5.6 Modules on docking proteins for receptor tyrosine kinases (RTKs)

5.5.1 Janus Gateway Phosphotyrosine Kinases

Jak kinases are cytosolic [38]. JAKs help the transmission of signals from growth factors and cytokines into haemopoietic cells. They contain tandem catalytic domains but do not have –SH2 or –SH3 groups (cf. Fig. 12.7). As seen in Table 12.11, Jak1, Jak2 and Tyk2/Jak3 are expressed widely. The JAKs phosphorylate Stat DNA binding proteins; thus, the signal leads to transcription [39].

5.5.2 Transactivation

Referring again to Fig. 4.2, one may not be surprised to learn that the reality is complicated, and in fact, other cell surface receptors can “transactivate” the EGFRs. In particular, the GPCRs described in Chap. 1 can activate RTKs like the EGFRs [19]. So, a given receptor is activated by a ligand of a heterologous receptor. GPCR stimulation activates a metalloproteinase of the ADAM family so that there is shedding of membrane-bound heparin-binding EGF, which activates its receptor [40].

RTK transactivation by ligands of the GPCRs also occurs by generation of reactive oxygen species (ROS), which inactivate the phosphatases that control the activities of the RTKs (Sect. 4.2.5). All this is to say that there are multiple tracts to the activation of the extracellular signal-related kinase (ERK)/MAPK cascade.

5.6 Abl and Bcr-Abl

The Abelson murine leukemia virus encodes an N-terminal Gag protein from the MuLV and a C-terminal Abl protein of cellular origin. This Abl has tyrosine kinase activity that is critical to Gag-Abl induction of cellular transformation (Fig. 5.8). Abl family kinases actually phosphorylate key proteins that bind to cytoskeletal proteins, explaining how they regulate cell shape and movement [41]. When Cocksackie virus loosens tight junctions between epithelial cells, this occurs because Abl kinase causes Rac-dependent actin fibre rearrangements [42].

TGF β , via the intermediary of PI3K activated Pak2, promotes c-Abl activity in fibroblasts. Abl kinase has other subtle effects. Thus, in T lymphocytes it protects c-Jun from ubiquitination and degradation [43]. Abl phosphorylation of the adapter protein Crk is a negative regulator of p130Cas-Crk signalling [44] (cf. Figs. 9.10, 9.11). Abl-mediated uncoupling of Cas-Crk leads to cell death [44].

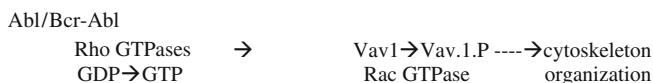


Fig. 5.8 Abl TK (tyrosine kinase) assembles cytoskeletal components

Clinicians know that in chronic myeloid leukaemia (CML) a Bcr-Abl fusion protein (p210) arises from a reciprocal chromosomal translocation. The bcr-abl tyrosine kinase explains all the features of CML [45]. Furthermore, success in the treatment of CML is now being achieved using the RTK inhibitor imatinib mesylate [46, 47].

5.7 Control Over Smooth Muscle Cell Proliferation

PDGF stimulates the proliferation of vascular smooth muscle cells (VSMs) via the MAPK cascade. In those cells in which MAPK also activates cytoplasmic phospholipase A2, releasing arachidonic acid, which is substrate for cyclooxygenase 2 (Cox-2) cyclooxygenase enzyme to form prostaglandin E2 (PGE2), there is natural negative regulation when cyclic adenosine monophosphate (cAMP) elevation stimulates protein kinase A (PKA; Fig. 5.9). This is the situation for VSMs [48], and it is the case for mesangial cells [49].

This system has general relevance for stimulation of PKA type I is utilised to curb antigen-specific proliferation of T lymphocytes and their release of cytokines [50]. The PKA can activate GTPase Rap-1, which, according to cell type, can activate or inhibit ERK signalling.

Note that phosphorylation of Raf1 frees the MAPK cascade from repression by the scaffold protein KSR (kinase suppressor of Ras). Otherwise, Raf1 is activated by binding of protein 14–3–3. The 14–3–3 proteins bind to other proteins and phosphorylate them to influence their transport or their binding to cell organelles [51].

5.8 Angiotensin II and Smooth Muscle Cells

When there is kidney damage and excess of angiotensin II (Ang II) is formed, the patient develops constriction and then proliferation of the smooth muscle cells of the arterioles, so the blood pressure becomes elevated and the arteriolar walls

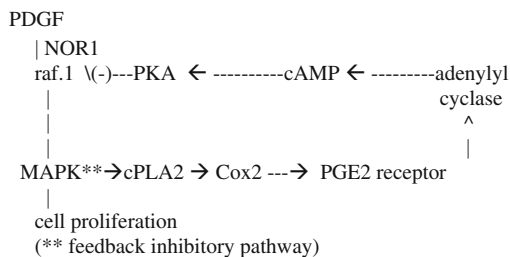


Fig. 5.9 Mitogen-activated protein kinase (MAPK) and vascular smooth muscle cell (VSM) proliferation

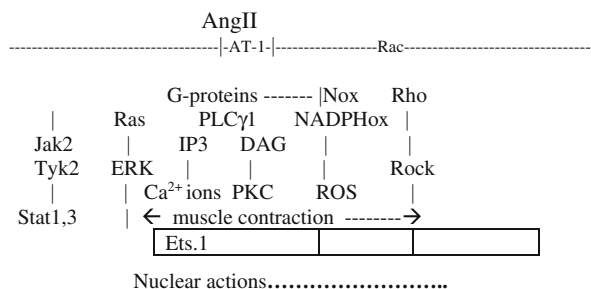


Fig. 5.10 Effects of angiotensin II (*Ang II*) via AT-1 receptors. The various cascades are initiated by G proteins, Src serine-threonine kinases, tyrosine kinases and the Shc-Grb2-extracellular signal-related kinase 2 (*ERK2*) pathway [55]. Ang II activates nonphagocytic *NADP* oxidase (*Nox2*) in muscle cells, creating oxidative stress that helps the signal transduction [56]. AT-1 receptors are drawn by Cav1 as central organiser into caveolae/lipid rafts, and activated phosphorylated c-Abl translocates Rac1 of *Nox2* there, which results in reactive oxygen species (*ROS*)-dependent “transactivation” of the epidermal growth factor receptor (*EGFR*) [57] and its egress from caveolae. As shown by use of the inhibitor SP600125, *JNK* helps Ang II-mediated cell proliferation, and Akt determines vascular smooth muscle cell (*VSM*) cell hypertrophy. The crucial regulator of Ang II-mediated vascular inflammation and remodelling is the Ets-1 transcription factor. The AT-1 receptor activates nonreceptor tyrosine kinases (Src, FAK, JAK2) and receptor tyrosine kinases like the EGFR and platelet-derived growth factor receptor (*PDGFR*)

thicken. Via the Ang II receptors that are designated AT-1 on VSMs, there is activation of phospholipase C, so formation of DAG activates protein kinase C, and production of IP3 leads to elevated intracellular Ca^{2+} , which induces muscle contraction [52]. By means of muscle cell Na^+/H^+ exchangers, Ang II causes an initial intracellular acidification accompanied by the rapid Ca^{2+} ion mobilisation, followed by a sustained alkalinization that aids the vasoconstriction (Fig. 5.10).

Along with activation of the tyrosine kinases of the AT-1 receptors, there is activation of phospholipase D, so hydrolysis of phosphatidylcholine to produce phosphatidic acid (PtdOH) helps the mobilization of Ca^{2+} ions within cells.

AT-1 receptor-induced activation of tyrosine and Src family kinases also leads to activation of the ERK cascade and therefore genes that account for cell growth and synthesis of proteins of the extracellular matrix. Indeed, Ang II also causes EGFR transactivation [53]. Accordingly, there is “vascular remodelling” of the walls of arterioles that results in permanent pathology [45]. Transcription factor Ets-1 helps Ang II effects on vessel walls [54].

One will find that ROS participate in diverse biological processes like signal transduction (Figs. 4.2, 4.3), cell growth, programmed cell death, cell senescence and maintenance of the transformed state [58]. As noted, the way in which signal transduction is potentiated is by ROS-induced reversible inhibition of phosphotyrosine phosphatase [59], with the result that an exaggerated signal is transmitted. Nonphagocytic NADPHox like the vascular oxidases [60] are regulated by vasoactive agents like Ang II and endothelin, by cytokines or by growth factors such as PDGF or TGF β and by mechanical forces like cyclic stretch and laminar shear

stress. Gp91 phox, Nox1 and Nox4 are expressed in endothelial cells, fibroblasts and VSMs [60]. The Nox components are stimulated by src kinases stimulating Rac, by protein kinase C and by phospholipase A2.

Contractility of smooth muscle is controlled by entry of calcium ions, leading to activation of myosin light-chain kinase (MLCK), which phosphorylates the light chain of myosin II (MLC20). Contraction also results from Rho-dependent inhibition of MLC20 dephosphorylation by myosin phosphatase. Rho-A, acting via Rho-kinase, tones up the system, accounting for Ca^{2+} -mediated vasoconstriction [61].

5.9 Endothelin-1 and Its Action

Endothelins are powerful vasoconstrictors that are produced by endothelial cells. Et-1 is the only vasoconstrictor that engenders the compensatory release of PGE2, which aids vasodilatation. Vasoconstriction of VSMs by endothelin-1 (Fig. 5.11) using its ET-A receptors is offset via the ET-B receptors, which release nitric oxide. This NO also reduces adhesiveness of leucocytes to the endothelium [62, 63].

The action of Et-1 on VSMs evokes an immediate response [64], akin to that of Ang II, namely, turnover of phosphoinositides, so that intracellular Ca^{2+} ions rise, and there is activation of the MAPKs. Et-1 stimulates all three MAPK pathways (i.e. ERK1 and ERK2, the p38 MAPK and the JNK paths). So, there is enhanced cyclin-dependent kinase 4 and cyclin D1 expression (Chap. 17). Et-1 directly and indirectly (via PDGF release) stimulates mitogenesis of mesangial cells. Et-1, acting on mesangial cells induces formation of fibronectin and collagen types I and IV; hence, there is deposition of extracellular matrix. In fact, Et-1 and TGF β induce CCN matricellular proteins that act as adaptors between the surfaces of cells and the ECM. These CCN proteins regulate crucial processes like cell differentiation, proliferation, adhesion, ECM production and angiogenesis [65].

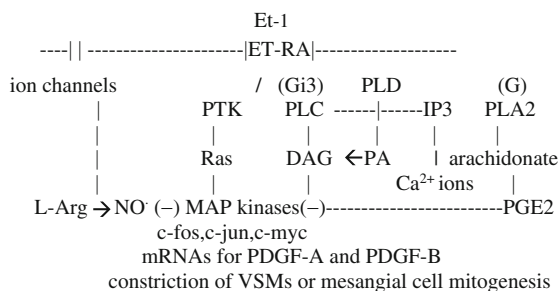


Fig. 5.11 Activation of smooth muscle by endothelin. Et-1 causes vasoconstriction of vascular smooth muscle cells (VSMs) or mitogenesis of mesangial cells, in which platelet-derived growth factor (PDGF) A and B participate. There is control by prostaglandin E2 and nitric oxide (NO)

Endothelins are produced by vascular endothelial cells [64]. Endothelins are produced by macrophages under the influence of T lymphocytes [66]. Their actions within the immune system are only just being elucidated. It is noteworthy that the function and survival of dendritic cells depends on autocrine Et-1 and thus on the expression of endothelin receptors [67]. ET-A receptor blockade reduces the formation of mature DCs, and it decreases IL-12 production and helps DC apoptosis. ET-B receptor blockade has the opposite effect. It is interesting that mast cells help to limit Et-1 induced effects. ET-A-dependent mast cell activation results in diminution of Et-1 levels and of Et-1-induced pathology [68].

References

1. Lowy DR, Willumsen BM. Function and regulation of Ras. *Annu Rev Biochem* 1993;62:851–891
2. Macara IG, Lounsbury KM, Richards SA, McKierman N, Bar-Sagi D. The Ras family of GTPases. *FASEB J* 1996;10:625–630; (b) Hall A (Ed). *GTPases*. Oxford University Press, 2000. (c) Karnoub AE, Weinberg RA. Ras Oncogenes. *Nat Rev Mol Cell Biol* 2008;9:517–531
3. Schlessinger J. How tyrosine kinases activate Ras. *Trends Biochem Sci* 1993;18:273–276
4. Mor A, Philips MR. Compartmentalized Ras/MAPK signaling. *Annu Rev Immunol* 2006;24:771–800
5. Bernardes A, Setteman J. GAP control: regulating the regulators of small GTPases. *Trends Cell Biol* 2004;14:377–385
- 6a. Gupta S, Ramjuan AR, Haiko P et al. Binding of ras to phosphoinositide 3-kinase p110 α is required for ras-driven tumorigenesis in mice. *Cell* 2007;129:957–968
- 6b. Jonssen KP, Abala M, EL Marjou F et al. Mouse models of K-ras initiated carcinogenesis *Biochim Biophys Acta*. 2005;1756:145–154
7. McCormick F. Signal transduction: how receptors turn Ras on. *Nature* 1993;363:15–16
8. Bustelo XR. Regulatory and signaling properties of Vav family. *Mol Cell Biol* 2000;20:1461–1477
9. Bivona TG, Perez de Castro I, Ahearn IM et al. Phospholipase C γ activates Ras on the Golgi apparatus by means of RasGRP1. *Nature* 2003;424:694–698
10. Ingram DA, Zhang L, McCarthy J et al. Lymphoproliferation in mice lacking expression of neurofibromin. *Blood* 2002;100:3656–3662
11. Wickstrom SA, Alitalo K, Keski-Oja J. Endostatin inhibits endothelial cells migration and induces disassembly of the actin cytoskeleton *J Biol Chem* 2003;278:37895–37901
12. Nie Z, Hirsch DS, Randazzo PA. Arf and its many interactors. *Curr Opin Cell Biol* 2003;15:396–404
13. Choudhury GG, Biswas P, Grandaliano G et al. PDGF mediates activation of PI3kinase in human mesangial cells. *Kidney Int* 1994;46:37–47
14. Klinghoffer RA, Duckworth B, Valius M, Cantley L et al. PDGF dependent activation of PI3kinase is regulated by receptor binding of SH2-domain containing proteins which influence Ras activity. *Mol Cell Biol* 1996;16:5905–5914
15. Choudhury GG, Marra F, Kiyomoto H, Abboud HE. PDGF stimulates tyrosine phosphorylation of Jak.1 protein tyrosine kinase in human mesangial cells. *Kidney Int* 1996;49:19–25
16. Lee Y-J, Cho H-Nyun, Soh JW et al. Oxidative stress induced apoptosis is mediated by ERK1/2 phosphorylation. *Exp Cell Res* 2003;291:251–266
17. Levitzki A. PDGF receptor kinase inhibitors. *Cytokine Growth Factor Rev* 2004;15:229–235
18. Konda M, Ihora Y, Murata H et al. Glutaredoxin modulates PDGF-dependent signaling by regulating the redox status of low MW protein tyrosine phosphatase. *J Biol Chem* 2006;281:28518–28528

19. Delcourt N, Bockaert J, Marin P. GPCR-jacking: new concept in GPCR activation. *Trends Pharmacol Sci* 2007;28:602–607
20. Pyne NJ, Waters C, Moughal NA et al. Receptor tyrosine-kinase-GPCR signal complexes. *Biochem Soc Trans* 2003;31:1220–1225
21. Korade-Mirnics Z, Corey SJ. Src kinase mediated signaling in leukocytes. *J Leukoc Biol* 2000;68:603–61318
22. Dikic I. Endocytosis from the surface. *Nature* 2002;416:183–187
23. Dykstra M, Cherukuri A, Pierce SK. Rafts and synapses in the spatial organisation of immune cell signal receptors. *J Leukoc Biol* 2001;70:699–707
24. Shaw AS. Lipid rafts: now you see them, now you don't. *Nat Immun* 2006;7:1139–1142
25. Campbell ID. Modular proteins at the cell surface. *Biochem Soc Trans* 2003;31:1107–1112
26. Pawson T. Protein modules and signalling networks. *Nature* 1995;373:573–580
27. Musacchio A, Gibson T, Rice P et al. The pleckstrin homology domain: a common piece in a patchwork of signaling problems. *Trends Biochem Sci* 1993;18:343–348
28. Fruman DA, Rameh LE, Cantley LC. Phosphoinositide binding domains: embracing 3-phosphate. *Cell* 1999;97:817–820
29. Fantl WJ, Johnson DE, Williams LT. Signalling by receptor tyrosine kinases. *Annu Rev Biochem* 1993;62:453–481
30. Hubbard SR. Structural analysis of receptor tyrosine kinases. *Prog Biophys Mol Biol* 1999;71:343–358
31. Robinson DR, Wu YM, Lin SF. The phosphotyrosine kinase family of the human genome. *Oncogene* 2000;19:5548–5557
32. van der Geer P, Hunter T, Lindberg RA. Receptor protein tyrosine kinases and their signal transduction pathways. *Annu Rev Cell Biol* 1994;10:251–332
33. Sicheri F, Kuriyan J. Structure of Src-family tyrosine kinases. *Curr Opin Struct Biol* 1997;7:777–785
34. Schlesinger J. Cell signaling by receptor tyrosine kinases. *Cell* 2000;103:211–225
35. Zoller KE, MacNeill IA, Brugge JS. Protein tyrosine kinases Syk and Zap70 display distinct requirements for Src family kinases in immune response signal transduction. *J Immunol* 1997;158:1650–1659
36. Finkelstein LD, Schwartzberg PL. Tec kinases: shaping T cell activation through action. *Trends Cell Biol* 2004;14:443–445
37. Saito K, Tolias KF, Saci A, Koon HB. Btk regulates PtdIns 4,5P₂ synthesis: importance for calcium signaling and PI3kinase activity. *Immunity* 2003;19:669–678
38. Ihle JN. The Janus protein tyrosine kinase family and its role in cytokine signaling. *Adv Immunol* 1995;60:1–35
39. Leonard JW, O'Shea JJ. JAKs and STATs: biological implications. *Annu Rev Immunol* 1998;16:293–322
40. Ohtsu H, Dempsey PJ, Eguchi S. ADAMs as mediators of EGF receptor transactivation by G protein coupled receptors. *Am J Physiol Cell Physiol* 2006;291:C1–C10
41. Hernandez SE, Krishnaswari M, Miller AL, Loeske AJ. How do Abl family kinases regulate cell shape and movement? *Trends Cell Biol* 2004;14:36–44
42. Coyne CB, Bergelson JM. Virus induced Abl and Fyn kinase signals permit Cocksackie virus entry through epithelial tight junctions. *Cell* 2006;124:119–131
43. Gao B, Lee S-M, Fang D. The tyrosine kinase c-Abl protects c-jun from ubiquitination mediated degradation in T cells. *J Biol Chem* 2006;281:29711–29718
44. Holcomb M, Rufini A, Barila D, Klemke RL. Deregulation of proteasome function induces Abl mediated cell death by uncoupling Cas and c-Crk. *J Biol Chem* 2006;281:2430–2440
45. Shet AS, Jahagirdar BN, Verfaillie CM. Chronic myelogenous leukaemia. *Leukemia* 2002;16:1402–1411
46. Goldman KM, Melo JV. Chronic myeloid leukaemia: advances. *N Engl J Med* 2003;349:1451–1464
47. Wong S, Witte ON. The BCR:Abl story. *Annu Rev Immunol* 2004;22:247–306

48. Bornfeldt KE, Campbell JS, Koyama H, Argast GM et al. The mitogen activated protein kinase pathway can mediate growth inhibition and proliferation in vascular smooth muscle cells. *J Clin Invest* 1997;100:875–885
49. Chen J, Thomson MA, Walker HJ et al. Differential regulation of mesangial cell mitogens by cAMP phosphodiesterase isozymes 3 and 4. *Am J Physiol Renal* 2004;287:F940–F953
50. Aandahl EM, Moretto WJ, Haslett PA et al. Inhibition of antigen specific T cell proliferation and cytokine production by protein kinase A type I. *J Immunol* 2002;169:802–808
51. Shikano S, Coblitz B, Wu M, Li M. 14–3–3 proteins. *Trends Cell Biol* 06;16:370–375
52. Touyz RM, Schiffrin EL. Signal transduction mechanisms mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. *Physiol Rev* 2000;52:639–672
53. Dzau VJ, Lopez-Illasaca M. Searching for transcriptional regulators of Ang II-induced vascular pathology. *J Clin Invest* 2005;115:2319–2322
54. Lautrette A, Li S, Alili R et al. Angiotensin II and EGF receptor crosstalk in chronic kidney disease. *Nat Med* 2005;11:867–874
55. Sowers JR. Hypertension, angiotensin II and oxidative stress. *N Engl J Med* 2002;346:1999–2001
56. Zhang A, Ding G, Huang S et al. c-Jun NH2-terminal kinase mediation of Ang II induced proliferation of human mesangial cells. *Am J Physiol Renal Physiol* 2005;288:F1118–F1124
57. Ushio-Fukai M, Alexander RW. Caveolin dependent angiotensin type 1 receptor signaling in vascular smooth muscle. *Hypertension* 2006;48:797–803
58. Finkel T. Oxidants signals and oxidative stress. *Curr Opin Cell Biol* 2003;15:247–254
59. Chiarugi P, Cirri P. Redox regulation of protein tyrosine phosphatases during receptor tyrosine kinase signal transduction. *Trends Biochem Sci* 2003;28:509–512
60. Lasseque B, Clempers RE. Vascular NADPH oxidases: specific features, expression and regulation. *Am J Physiol Regul Integr Comp Physiol* 2003;285:R277–R297
61. Loirand G, Guerin P, Pacard P. Rho kinases in cardiovascular physiology and pathophysiology. *Circ Res* 2006;98:322–334
62. Murohara T, Lefer A. Autocrine effects of endothelin-1 on leukocyte endothelial interaction. *Blood* 1996;88:3894–3900
63. Sorokin A, Foschi A, Dunn MJ. Endothelin signalling and regulation of protein kinases in glomerular mesangial cells. *Clin Sci* 2002;103(Suppl 48):132s–136s
64. Kaehler J, Sill B, Koerster R et al. Endothelin-1 mRNA in vascular cell walls is increased by reactive oxygen species. *Clin Sci* 2002;103(Suppl 48):176s–178s
65. Leask A, Abraham DJ. All in the CCN family: essential matricellular signaling modulators. *J Cell Sci* 2006;119(Pt 23):4803–4810
66. Ehrenreich H, Anderson RW, Fox CH et al. Endothelins are produced by human macrophages. *J Exp Med* 1990;172:1741–1748
67. Guruli G, Pflug BR, Pecher S et al. Function and survival of dendritic cells depends on Et-1 and Et-receptor autocrine loops. *Blood* 2004;14:2107–2115
68. Mawer M, Wedemeyer J, Metz M et al. Mast cells promote homeostasis by limiting endothelin-1 induced toxicity. *Nature* 2004;432:512–516.

Chapter 6

Mast Cell Activation and the Role of Eicosanoids

Abstract Mast cells trigger allergic and other immune reactions. Details of their signal transduction pathways leading to discharge of cellular granules are given. Control is exerted also by eicosanoids generated by activation of cell phospholipase A2, by cyclic adenosine monophosphate (AMP) and by nitric oxide.

6.1 Signal Transduction in Mast Cells

Research on rodents has provided much of the information on how the FcεRI high-affinity receptors for immunoglobulin E (IgE) on mast cells become activated in allergic reactions. Human mast cells were cultured relatively recently [1]. Mouse bone marrow-derived mast cells and the rat basophil leukaemia cell line RBL 2H3 have been examined [2]. Activation of mast cells leads to release of granule mediators like (a) histamine and tryptase; (b) phospholipid-derived molecules like platelet-activating factor (PAF), prostaglandin (PG) D2, and leukotriene C4 and (c) cytokines and chemokines.

The FcεRI receptor has subunits α , β and a disulphide linked γ - γ homodimer (Fig. 6.1). On human mast cells and basophils, the receptor constituents are α , β , γ 2 but on macrophages are α , γ 2. Thus, signals can arise without the β -unit, but really the β is a signal amplifier. The FcεRI receptor has no intrinsic tyrosine kinase activity, but it is linked to a lipoxygenase that produces reactive oxygen species (ROS) [3], and the subsequent receptor phosphorylations are driven by an oxidative process. First, the p56 Lyn phosphotyrosine kinase (PTK) phosphorylates ITAMs of the β -, γ -chains, and then Syk (spleen tyrosine kinase) PTK binds to the γ -unit and further activates Lyn (Fig. 6.1), which is within lipid rafts. So, there is activation as in Fig. 6.1 of (a) phospholipases C γ 1/C γ 2 and D1/D2, leading to Ca²⁺ influx; (b) activation of Ras-Raf, driving the mitogen-activated protein kinases (MAPKs); (c) Rac1 activation, causing Jun N-terminal kinase (JNK) activation and (d) formation of sphingosine-1-phosphate directing MAPK activation and cell degranulation [4]. JNK activation is necessary [5], arising first via protein kinase C (PKC)-dependent activation of Vav and therefore Rac1, and then p21-activated kinase (PAK) activation of MEKK1, and thereby JNK/SAPK (stress-activated protein kinase) [6]. Second, there is direct PKC-mediated activation of JNK.

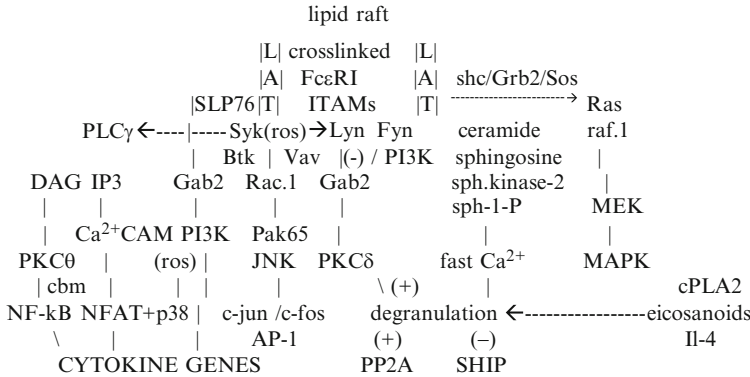


Fig. 6.1 Signal pathways for FcεRI stimulated mast cells. (*ros*) reactive oxygen species. Lyn and Fyn are important proximal kinases. Mast cells have FcεRI receptors for allergic immunity, and Toll-like receptor (*TLRs*), FcγRs and complement receptors that aid recognition of bacteria [16]. The Linker of Activated T cells (*LAT*) adaptors support phospholipases γ1 and γ2. There is a fast Ca²⁺ influx and a phospholipase Cγ (*PLC*γ)-induced slow Ca²⁺ wave [17]. Lyn associated with the β-chain has both (+) and (–) effects [9]. Fyn and the phosphorylation of p38 mitogen-activated protein kinase (*MAPK*) aid cytokine release [18]. NTAL overexpression suppresses early FcεRI activation events [19]. Fyn, via the complementary path, leads to degranulation [20]. NTAL via interaction with Lyn could help set the threshold for degranulation [19]. Indeed, knockdown of the gene encoding NTAL leads to impaired mast cell degranulation

Cytokine and chemokine gene expression by mast cells is elicited via their Toll receptors (Fig. 12.1). So, there is (a) IP3 activation of calcineurin and thus of NFAT (nuclear factor of activated T cells), (b) PKC-induced activation of nuclear factor kappa B (NF-κB) (Fig. 11.1a), (c) activation via Linker of Activated T cells (*LAT*), to Son of Sevenless (*Sos*), then Ras and MAP kinases, and thus c-fos. *LAT* adaptor protein is the focus of a molecular complex that is linked to Vav guanine-nucleotide exchange factor (*GEF*). Adaptor protein ADAP (*SLAP130*) also participates. The combined activity of bcl-10/*Malt-1* activation of NF-κB [7] and activation of p38 *MAPK* [8] leads to the production of interleukin 6 (*IL-6*) and *IL-4* by mast cells. They are also able to produce interleukin 10 (*IL-10*) [8b].

It was emphasised that Lyn initiates Ca²⁺ influx but does not always cause degranulation, whereas the Fyn PTK complementary pathway leads to phosphorylation of Gab2 (*Grb2*-associated binder 2) and activation of PI3 kinase (*PI3K*) (p110) [9] and so cell degranulation and cytokine production. The alternative path does not use adaptors *LAT* and *SLP76* but instead *NTAL* [10]. PKC and calcium flux leading to activation of phospholipase D are important for degranulation. Actually, Lyn does positively regulate degranulation, cytokine production and cell survival, but when there is high-intensity stimulation, its action is negative [11]. This is traditional biofeedback. Gab2 is a pleckstrin homology (*PH*) module protein. At the receptor level, Gab2 forms a point of interdependence between Fyn activation of PKCδ and Lyn modulation [12]. Then, there is the fact that *Hck* also positively regulates activation [13] and most cell degranulation [8b].

Note in Fig. 6.1 how Lyn might inhibit Gab2, so it inhibits PKC δ and thereby granule discharge. Lyn actually regulates SHIP(Src homology inositol polyphosphate 5-phosphatase) phosphatase activity, so opposing PI3K by hydrolysing PI(3,4,5)P₃, with the result that there can be poor Ca²⁺ influx, inhibition of degranulation and inhibition of chemokine and cytokine production.

Even without the presence of allergen, high levels of IgE increase the surface expression of Fc ϵ RI. However, the scaffold molecule CIN85 (Cbl-interacting protein) works in concert with ubiquitin ligase Cbl to promote clathrin-mediated receptor endocytosis [14] and destruction by ubiquitination. Hence, cbl-b is a negative regulator of inflammatory cytokine production by mast cells [15], as it is in T cells or the macrophages. Negative regulators have been reviewed recently [8b, 8c].

There have to be negative controls via ITIMs. Csk-binding protein in the lipid rafts inhibits the activation of Fc ϵ RI receptors. SHIP suppresses pathways to NF- κ B activation by reducing PI3K generated PtdIns(3,4,5)P₃ levels. The Fc ϵ RI connects via PI3K δ to Akt and is reinforced by PI3K γ from the A3R adenosine receptor and G protein-coupled receptor (GPCR). SHIP downregulates degranulation. However, activation of PTKs and PI3K production of PtdIns(3,4,5)P₃ can circumvent the downregulation by SHIP.

In Fig. 6.1, there are six activation pathways. Starting with Syk protein tyrosine kinase activation of phospholipase C (PLC), there is the anticipated formation of IP₃ and diacylglycerol (DAG). IP₃ releases Ca²⁺ ions, which bind as Ca²⁺-calmodulin, which increases the activity of calcineurin, otherwise known as the serine-threonine phosphatase PP2B. The CN-phosphatase activates NFAT, which moves into nuclei and activates the promoter upstream of the IL-2 gene. See also how DAG activates PKC, which can activate NF- κ B and thereby cytokine genes. Via Ras, there is activation of MAPK, leading to transcription factor Elk-1. Through Rac1 and PAK, the stress JNK/SAPK pathway activates c-jun. Activator protein 1 (AP-1) includes members of the c-jun and fos families. AP-1 is a pivotal transcription factor [21] that is stimulated by various extracellular signals, including cytokines, growth factors and tumour promoters. There is also sphingomyelin hydrolysis to form ceramide, and sphingosine-1-P (S1P), which activates the kinase Raf, which phosphorylates and activates the MAP cascade. S1P is required for mast cell calcium influx, PKC activation and cytokine production and degranulation [22, 23].

Just as PKC θ $\rightarrow$ Carma/Bcl-10/Malt-1 (cbm) is involved in T cell activation (Fig. 13.6), so Fc ϵ R receptors acting via bcl-10 will activate NF- κ B and account for cytokine production. The bcl-10 supports mast cell degranulation [19]. Hck activates mast cells by opposing the inhibitory action of Lyn [8b, 13].

6.2 The Formation of Eicosanoids

MAPK activates the cytosolic phospholipase A₂ (cPLA₂), so that arachidonate is released from position 2 of cell membrane phospholipids and that arachidonate (C_{20:4}) is used to form the eicosanoids [24].

6.2.1 The Formation of Prostaglandin E2

The *constitutive* enzyme cyclooxygenase 1 (Cox-1), which is expressed in the endoplasmic reticulum and nuclear envelope of cells, works in conjunction with cytosolic prostaglandin E synthetase (cPGES) at the nuclear membrane to convert AA → PGG2 → PGH2 → PGE2. Cox-1 is expressed constitutively in most tissues. It generates prostaglandins like PGE2 that control many normal physiological processes, like renal blood flow.

Correspondingly, arachidonic acid released by cPLA2 (Fig. 6.2) is converted by inducible Cox-2 enzyme working in conjunction with mPGES-1 to PGG2 → PGH2 → PGE2 [26]. Glutathione is a cofactor for this reaction. Cox-2 is transiently induced by proinflammatory stimuli, growth factors, cytokines and even tumour promoters, accounting for increased PGE2 formation for tissue injury and repair.

As an example, consider that p38 MAPK and extracellular signal-related kinase (ERK) 1/2 together upregulate microsomal PGES, and p38 MAPK upregulates Cox-2 enzyme in human osteoarthritic cartilage [27]. It is all driven by proinflammatory cytokine IL-1.

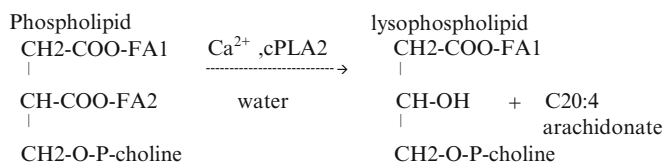


Fig. 6.2a Release of arachidonate from phosphatidylcholine. (a) Arachidonate is usually at position 2 of the phospholipids. (b) Cytosolic phospholipase A2 (*cPLA2*) is an important enzyme determinant of inflammatory mediators [25]. (c) When an acetyl group is added at -2 of lysophospholipid, the new compound is platelet-activating factor (*PAF*), that is, 1-alkyl-2-acetyl sn glycerol-3-phosphorylcholine. (d) Whereas ceramide-1-phosphate activates *cPLA2*, sphingosine-1-P will activate cyclooxygenase 2 (*Cox-2*) (cf. Fig. 11.3). (e) Annexin 1 binds to cell membranes and inhibits *cPLA2*. It is induced by glucocorticoids. Annexin 1 also inhibits inducible nitric oxide synthase (*iNOS*) in macrophages and *Cox-2*. It can negatively regulate expression of interleukin 6

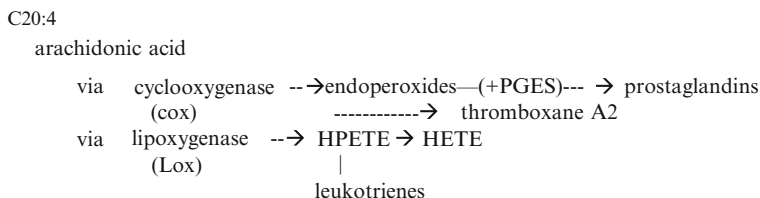


Fig. 6.2b Conversion of arachidonic acid to eicosanoids. *PGES* = prostaglandin E synthetase. Arachidonic acid (AA) via cyclooxygenase (*Cox*) enzyme and *PGES* is transformed into prostaglandin E2 (*PGE2*). Prostaglandins *PGE2*, *PGD2* and leukotrienes *LTB4*, *LTC4* regulate the cells that determine innate versus adaptive immunity

6.3 Mast Cell Degranulation

When mast cells are activated by allergen that binds to serum IgE attached to their FcεR receptors, they release cytokines and eicosanoids and their secretory granules (Fig. 6.3). Sphingosine-1-phosphate activation of MAPK promotes degranulation [22], as does the fast transient Ca^{2+} influx that activates phospholipase D. The positive influence of Hck helps by inactivating Lyn [13] and so does Jun-B [28]. Signals from Kit ligand synergise with antigen activation of FcεRI for mast cell degranulation. Kit helps to mobilise Ca^{2+} ions [28b, 29]. PKC and calmodulin kinase II (CaMKII) are implicated in granule discharge, as are phosphatases like PP2A. In the basophil, a related cell, IL-3 has been shown to release granzyme B [30].

One can assume that SNAPS and SNAREs (Fig. 9.9) are involved in passage of granules through mast cell membranes, akin to the situation in platelets. The cholesterol-rich lipid raft domains contain SNARE proteins STX3/4. Fusion and dissociation of the SNARE complex require recruitment of soluble *N*-ethylmaleimide sensitive factor (NSF) and its attachment to SNAP23 in a process that utilises ATP. NSF is activated by dephosphorylation by phosphatase PTP-MEG2 [31]. SNAP23 phosphorylation presages degranulation. Release of granules is arrested by raising intracellular cyclic adenosine monophosphate (cAMP), as by the action of adrenalin acting on $\alpha 2$ -adrenergic receptors, by means of histamine acting on its type 2 receptors or via the E1/E2 prostaglandins. In most other cell types, cAMP helps granule discharge. Either cAMP promotes the action of protein kinase A (PKA) or cAMP directs Ca^{2+} triggered exocytosis via cAMP-EPAC GEF. $\text{cAMP} \rightarrow \text{EPAC} \rightarrow \text{Rap1} \rightarrow \text{PLC}\epsilon \rightarrow \text{IP}_3 + \text{DAG}$. Nitric oxide inhibits mast cell activation [32]. Human mast cells display multiple EP receptors for PGE2 [33]. PGE2 elevates mast cell cAMP and thereby suppresses FcεRI-driven eicosanoid production and the release of tumour necrosis factor- α (TNF α).

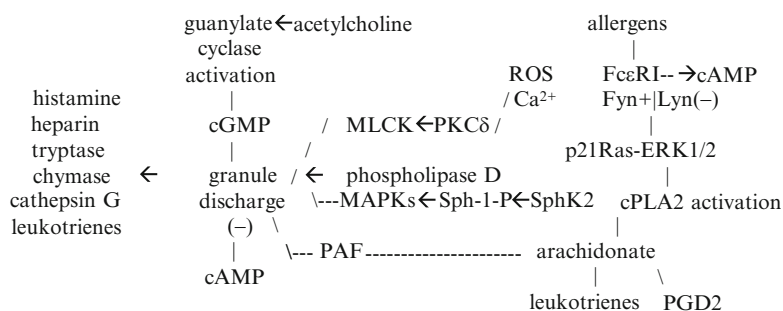


Fig. 6.3 Mast cell degranulation. Protein kinase C functions downstream of the FcεRI receptors and Ca^{2+} ion influx to mediate degranulation. Its action is reactive oxygen species (ROS) promoted [34]. PI3 kinase inhibitors like wortmannin inhibit granule discharge. A seventh pathway (not in Fig. 6.1 for lack of space) involves PI3 kinase activation of Rac/Rho guanosine triphosphatases (GTPases), leading to actin rearrangements for degranulation. Rab-guanine-nucleotide exchange factor 1 (GEF1; akin to neurofibromin) has been shown to bind Ras to negatively regulate it [35]

6.4 Cyclic AMP Downregulates Activation of Immune Cells

When intracellular cAMP is elevated in immune or inflammatory cells (i.e. within polymorphonuclear leucocytes [PMNs], macrophages, lymphocytes), there is down-regulation of their activation [36] and inhibition of ROS formation. Growth factor-induced cell proliferation is suppressed. The agents that do this are those that stimulate adenylyl cyclase or are phosphodiesterase (PDE) inhibitors. The former include prostaglandins PGE1, PGE2 or PGI2 and adenosine acting via its A2 receptors. The PDE inhibitors are theophylline, pentoxifylline and lisophylline [37], or there are selective agents like rolipram. A2 receptors for adenosine via G α protein stimulate adenylyl cyclase, forming the cAMP that elevates PKA, which inhibits PKC. In neutrophils, this means that FMLP can no longer lead to activation of phospholipase D (Fig. 10.1), thereby forming phosphatidic acid to stimulate NADPH oxidase [38]. Likewise, PGE2 inhibits FMLP stimulation of the phospholipase D pathway in human neutrophils [39]. So, one can use PDE4 inhibitors or adenosine A2a agonists to subdue neutrophils (cf. Sect 10.1.1) [40]. Cyclic AMP, by its induction of PKA, keeps T lymphocytes quiescent [41].

When cAMP forms in some cell types, it induces ICER (inducible cyclic AMP element repressor), which will inhibit formation of IL-2. Hence, cell proliferation is stopped. This occurs when histamine is acting on H2 receptors [42]. It also occurs when Treg cells are suppressing T effector lymphocytes [43].

6.5 Cyclic AMP and Cell Proliferation

Cyclic AMP is often implicated in cell proliferation [44]. During mitogenesis, cAMP will activate the MAPK cascade. cAMP activates PKA, which activates Ras $\rightarrow$ MAPKs. Indeed, PKA activates Rap1, and so B-raf, which activates the MAPK cascade [45]. PKA also activates transcription factor CREB (cAMP response element-binding protein), which binds to the cAMP response element (CRE) of the DNA (Fig. 4.8). CREB is also activated by Ca²⁺-calmodulin.

6.6 The β c Cytokine Receptors and CREB Control of Growth

The cytokines like IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF), which make myeloid cells grow, are important for haemopoiesis. These cytokines use the β c receptor unit, and so Jak2, and they induce phosphatidylcholine-specific PLC (PC-PLC), and the DAG that is produced activates PKC ϵ . Either directly or indirectly, PKC ϵ leads to the phosphorylation of CREB that binds to nuclear CRE [46]. This is akin to the action of cAMP in inducing cell growth. Furthermore, CREB induces bcl-2 antiapoptotic protein; thus, cell survival is helped.

6.7 Other Modulators of Mast Cell Degranulation

Nitric oxide inhibits IgE-dependent production of cytokines like IL-4, IL-6 and TNF α . NO stops the phosphorylation of PLC γ 1, and it abrogates DNA binding activity of c-fos and c-jun [47].

CD200 via the CD200R inhibits the activation of myeloid cells. CD200R lacks an ITIM; instead, there is engagement of the adaptor proteins Dok1 (p62) and Dok2 (p56). First, Doks attract SHIP phosphatase; second, they associate with Ras-GAP, which then negates Ras and thus stops activation of ERK, p38 MAPK and JNK [48]. Hence, cytokine release and mast cell degranulation are thwarted.

References

1. Reischl IG, Coward WR, Church MK. Molecular consequences of human mast cell activation following IgE high affinity IgE receptor (IgE-Fc ϵ RI) interaction. *Biochem Pharmacol* 1999;58:1841–1850
2. Tkaczyk C, Gilfillan AM. Fc ϵ RI dependent signaling pathways in human mast cells. *Clin Immunol* 2001;99:198–210
3. Swindle EJ, Coleman JW, DeLeo F, Metcalfe DD. Fc ϵ RI and Fc γ Receptor production of reactive oxygen species by mast cells is lipoxygenase dependent and cyclooxygenase dependent and NADPH oxidase independent. *J Immunol* 2007;179:7059–7071
4. Prieschl EE, Csonga R, Novotny V. The balance between sphingosine and sphingosine-1-phosphate is decisive for mast cell activation after Fc ϵ RI triggering. *J Exp Med* 1999;190:1–8
5. Kawakami Y, Hartman SE, Holland PM et al. Multiple signaling pathways for activation of JNK in mast cells. *J Immunol* 1998;161:1795–1802
6. Kimata M, Inagaki N, Kato T et al. Role of MAP kinase pathways for mediator release from human cultured mast cells. *Biochem Pharmacol* 2000;60:589–594
7. Chen Y, Pappu BP, Zeng H et al. Bcl-10 is essential for Fc ϵ R mediated degranulation and Il-6 production in mast cells. *J Immunol* 2007;178:49–57
- 8a. Boudreau RTM, Hoskin DW, Lin T-J. Phosphatase inhibition potentiates Il-6 production by mast cells in response to Fc ϵ RI-mediated activation: involvement of p38 MAPK. *J Leukoc Biol* 2004;76:1075–1081
- 8b. Kalesnikoff J, Galli SJ. New developments in mast cell biology. *Nat Immunol* 2008; 9:1215–1223
- 8c. Kim M-S, Radinger M, Gilfillan AM. The multiple roles of phosphoinositide 3-kinase in mast cell biology. *Trend Immunol* 2008; 29:493–501
9. Ali K, Bilancio A, Thomas M et al. Essential role for the p110 δ phosphoinositide-3-kinase in the allergic response. *Nature* 2004;431:1007–1011
10. Kambayashi T, Koretzky GA. Proximal signaling events in Fc ϵ RI mediated mast cell activation. *J Allergy Clin Immunol* 2007;119:544–552
11. Xiao W, Nishimoto H, Hong H et al. Positive and negative regulation of mast cell activation by Lyn via the Fc ϵ RI. *J Immunol* 2005;175:6885–6892
12. Frossi B, DeCarli M, Pucillo C. The mast cell: an antenna of the microenvironment that directs the immune response. *J Leukoc Biol* 2004;75:579–584
13. Hong H, Kitauro J, Xiao W et al. The Src family kinase Hck regulates mast cell activation by suppressing inhibitory kinase Lyn. *Blood* 2007;110:2511–2519
14. Molfetta R, Belleudi F, Peruizzi G et al. CIN85 regulates the ligand dependent endocytosis of the IgE receptor. *J Immunol* 2005;175:4208–4216

15. Gustin SE, Thien C, Langdon WY. Cbl-b is a negative regulator of inflammatory cytokines produced by IgE activated mast cells. *J Immunol* 2006;177:5980–5989
16. Marshall JS. Mast cell responses to pathogens. *Nat Rev Immunol* 2004;4:787–799
17. Melendez AJ, Khaw AK. Dichotomy of calcium signals triggered by different phospholipids pathways in antigen stimulation of human mast cells. *J Biol Chem* 2001;277:17255–17262
18. Draberova L, Shaik GM, Volna P. Regulation of calcium signaling in mast cells. *J Immunol* 2007;179:5169–5180
19. Kraft S, Kinet J-P. New developments in FcεRI regulation, function and inhibition. *Nat Rev Immunol* 2007;7:365–378
20. Frossi B, Rivera J, Hirsch E, Pucillo C. Selective activation of Fyn/PI3K and p38 MAPK regulates Il-4 production in BMMC. *J Immunol* 2007;178:2549–2555
21. Angel P, Karim M. The role of Jun, Fos and AP-1 complex in cell proliferation and transformation. *Biochim Biophys Acta* 1991;1072:129–137
22. Jolly PS, Bektas M, Olivera A et al. Transactivation of sphingosine-1-phosphate receptors by FcεRI triggering is required for normal mast cell degranulation and chemotaxis. *J Exp Med* 2004;199:959–970
23. Olivera A, Mizugushi K, Tikhonova A et al. The sphingosine kinase-sphingosine-1-P axis is a determinant of mast cell function and anaphylaxis. *Immunity* 2007;26:287–297
24. Marcus AJ. Eicosanoids as bioregulators in clinical medicine. *Am J Med* 1985;78:805–810
25. Capper EA, Marshall LA. Mammalian phospholipases A2: mediators of inflammation, proliferation and apoptosis. *Progr Lipid Res* 2001;40:167–197
26. Park JY, Pillinger MH, Abramson SB. Prostaglandin E2 synthesis and secretion. *Clin Immunol* 2006;119:229–240
27. Masuko-Hango K, Berenbaum F, Humbert L et al. Upregulation of microsomal prostaglandin E synthase-1 in human osteoarthritic cartilage. *Arthr Rheum* 2004;50:2829–2838
- 28a. Textor B, Licht A, JunB for IgE mediated degranulation. *J Immunol* 2007;179:6873–6880
- 28b. Metcalfe DD. Mast cells and mastocytosis. *Blood* 2008;112:946–956
29. Hundley TR, Gilfillan AM, Kaczyn C. Kit and FcεRI mediate unique and convergent signals for release of mediators from human mast cells. *Blood* 2004;104:2410–2417
30. Tschopp CM, Spiegl N, Didichenko S et al. Granzyme B, its induction and release in blood basophils and human asthma. *Blood* 2006;108:2290–2299
31. Huynh H, Bottini N, Williams S et al. Control of vesicle fusion by a tyrosine phosphatase. *Nat Cell Biol* 2004;6:831–839
32. Coleman JW. Nitric oxide, a regulator of mast cell activation. *Clin Exp Immunol* 2002;129:4–10
33. Feng C, Beller EM, Bagga S, Boyce JA. Human mast cells express multiple EP receptors for PGE2 that differentially modulate activation responses. *Blood* 2006;107:3243–3250
34. Cho SH, Wood CH, Yoon SB, Kim JH. Protein kinase C delta functions downstream of calcium mobilization in Fc epsilon RI signaling to degranulation in mast cells. *J Allergy Clin Immunol* 2004;114:1085–1092
35. Tam S-Y, Tsai M, Snouwaert JN et al. Rab GEF1 is a negative regulator of mast cell activation and skin inflammation. *Nat Immunol* 2004;5:844–852
36. Moore RA, Willoughby DA. The role of cAMP regulation in controlling inflammation. *Clin Exp Immunol* 1995;101:387–389
37. Coon ME, Diegel M, Leokinsy N, Klaus SJ. Selective pharmacological inhibition of murine and human Il-12 dependent Th.1 differentiation and Il-12 signaling. *J Immunol* 1999;163:6567–6574
38. Thibault N, Burelout C, Harbour D et al. Occupancy of adenosine A2a receptors: FMLP induced cAMP accumulation in human neutrophils. *J Leukoc Biol* 2002;71:367–377
39. Burelout C, Thibault N, Levasseur S et al. Prostaglandin E2 inhibits the phospholipase D pathway stimulated by FMLP in human neutrophils. *Mol Pharm* 2004;66:293–301
40. Tintinger G, Steel HC, Anderson R. Taming the neutrophil: calcium clearance and influx mechanisms as targets pharmacological control. *Clin Exp Immunol* 2005;141:191–200
41. Williams RO. A-kinase anchor proteins are involved in maintaining resting T cells in an inactive state. *J Immunol* 2002;168:5392–5396

42. Jutel M, Watanabe T, Klunker S et al. Histamine regulates T cell and antibody responses by differential expression of H1/H2 receptors. *Nature* 2001;413:420–425
43. Bodor J, Fehervari Z, Diamond B, Sakaguchi S. ICER mediated transcriptional attenuation of Il-2 and its role in suppression by regulatory T cells. *Eur J Immunol* 2007;37:884–895
44. Dumont JE, Jauniaux JC, Roger PP. The cyclic AMP mediated stimulation of cell proliferation. *Trends Biochem Sci* 1989;14:67–71
45. Stork PJS, Schmitt JM. Crosstalk between cAMP and MAPkinase signaling in regulation of cell proliferation. *Trends Cell Biol* 2002;12:258–265
46. Gubina E, Luo X, Kwon E et al. β c cytokine receptor induced stimulation of CREB phosphorylation requires protein kinase C. *J Immunol* 2001;167:4303–4310
47. Davis BJ, Flanagan BF, Fiffilan AM et al. Nitric oxide inhibits IgE dependent cytokine production and Fos and Jun activation in mast cells. *J Immunol* 2004;173:6914–6920
48. Zhang S, Cherwinski H, Sedgwick JD, Phillips JH. Molecular mechanisms of CD200 inhibition of mast cell activation. *J Immunol* 2004;173:67876–67893.

Chapter 7

Lipid Products and Cell Signaling

Abstract Cell signaling implicates lipid products like phosphatidic acid. Thus, the action of TNF α (transforming growth factor- α cytokine) stems from activation of phospholipase D. Other cell controls arise from ceramide or sphingosine of the sphingomyelin-ceramide cycle. Serum growth factor turns out to be lysophosphatidic acid, whose action is considered.

7.1 Phosphatidic Acid as a Second Messenger

Phospholipase D and its product phosphatidic acid (PtdOH or PA) are involved in the actions of growth factors [1, 2] and the cytokine tumour necrosis factor- α (TNF α) [3] (Table 7.1).

Phospholipase D (PLD) is involved in protein and vesicle transport [5] from the endoplasmic reticulum (ER) to the Golgi, from the Golgi to the cell surface, then from the plasma membrane to endosomes and from endosomes to lysosomes. PA that is derived from PLD stimulates Arf1-mediated PIPK1 (phosphatidylinositol phosphate kinase 1) activation at the Golgi membranes. Accordingly, Arf1 or Arf6 can modulate formation of the actin cytoskeleton or alter phospholipid structure and cell membrane curvature.

7.2 Protein Kinase D

Protein kinase D (PKD) is driven by diacylglycerol (DAG) and hence by protein kinase C (PKC) [5] (Fig. 7.1). (a) DAG induces activation of PKD by PKC-dependent phosphorylation, and (b) DAG regulates PKD localization by binding to its C1 domain. Requisite phosphorylation on its serines 744 and 748 is also driven by mitogenic peptides [6] like bradykinin, bombesin and endothelin and by thrombin and lysophosphatidic acid (LPA). Oxidative stress and several G protein-coupled receptors (GPCRs) [7] also help to stimulate PKD. In fact, PKD turns out to be a sensor of oxidative stress [8].

The C1 domain is where PKC binds for its activation (Fig. 7.2).

The function of PKD is cell context dependent. PKD is activated by the four novel PKCs (δ , ϵ , η , θ) or by cPKC α (cytosolic PKC α) . Within cells, PKD distributes to different subcellular compartments and shuttles between them (Table 7.2).

Table 7.1 Actions of phosphatidic acid (PA)

(i) PA activates PIP4 kinase and PIP5 kinase	PA activates phospholipase C	PA induces protein kinase C (PKC) isotypes ϵ and ζ
(ii) PA is involved in trafficking of vesicles		
(iii) PA activates Ras or Raf1 kinase		
(vi) A Rho guanosine triphosphatase (GTPase) activates actin stress fibre assembly		
(iv) It regulates microtubule-associated protein		
(v) PA stimulates the (NADPH) oxidase of phagocytes		

PA stimulates the respiratory burst of phagocytic cells since it stimulates Ser and Tyr phosphorylation of the p47^{phox} component of the NADPH oxidase [4] (Figs 10.1 and 10.2)

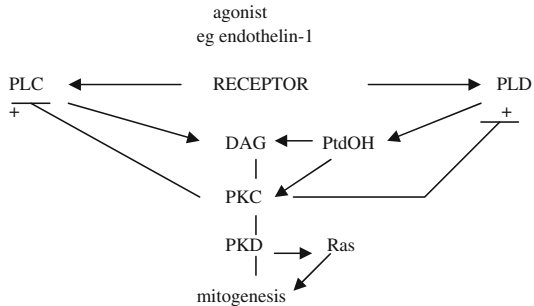


Fig. 7.1 Growth factors stimulating phospholipases C and D. *PA* phosphatidic acid (PtdOH), *PKD* protein kinase D

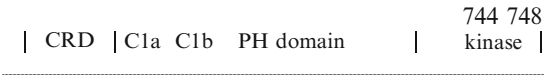


Fig. 7.2 Basic structure of protein kinase D. *CRD* cysteine-rich domain that downregulates the kinase

Table 7.2 The actions of protein kinase D

1. Protein transport within the cell and membrane trafficking
2. Cell motility
3. Cell differentiation/proliferation
4. Activation of T and Blymphocytes; cell survival

The signaling pathways in which PKD participates [9] may seem complex. Table 7.3 gives an indication of how it operates

Table 7.3 Protein kinase D signalling pathways

-
- I. From surface receptors, there is activation of DAG and of PKC and hence of PKD
 - a. PKD upregulates the Ras-Raf-MEK-ERK pathway
 - b. There is downregulation of JNK by the phosphorylation of c-Jun
 - c. There is intranuclear phosphorylation of certain HDACs
 - II. Oxidative stress that leads to PKD activation leads to activation of NF- κ B, which means that there is also production of manganese superoxide dismutase [8]
 - III. At the trans-Golgi, PKD activates transport carriers for membrane trafficking, for example, for the purpose of fibroblast locomotion. Such carriers support Rac-1 leading edge activity in motile cells (Fig.9.7)
-

DAG diacylglycerol, *ERK* extracellular signal-related kinase, *HDAC* histone deacetylase, *JNK* Jun N-terminal kinase, *NF- κ B* nuclear factor kappa B, *PKC* protein kinase C, *PKD* protein kinase D

effects in immunocytes are invariably cell type and stage specific. Ceramide controls the survival of dendritic cells [17].

How these special lipids regulate cell function was originally set out by Hannun [18] and Shayman [19]. The immune effects of ceramide were then reviewed by Ballou [20] and by Hannun [21]. Sphingomyelin is an important component of the lipid rafts that form the “immunological synapse” of T or B lymphocytes. Rapid formation of ceramide helps T cell receptor cross-linking [22]. Ceramide increases the order to the acyl chains in the bilayer, whilst there could be fluidisation by DAG. Ceramide helps the clustering of phagocytic Fc γ RII receptors and their association with lipid rafts [23]. Ceramide converts sphingolipid-enriched microdomains (rafts) into large platforms into which signalling proteins are compartmentalized.

The cytokines that induce the sphingomyelin cycle are IL-1, TNF α , and interferon- γ (IFN γ). Growth factors like platelet-derived growth factor (PDGF) and complement and 1,25-dihydroxy-vitamin D₃ also act like this. Further down, sphingosine kinase, which converts sphingosine to Sph-1-phosphate, is induced in response to growth factors PDGF, EGF, NGF, by activated receptors Fc γ RI or Fc ϵ RI or by the priming agents that act on neutrophils [24]. All these agents stimulate neutral sphingomyelinase (NSMase). Yet, in surface lipid rafts or the endosomes, there is acid sphingomyelinase (ASMase). The difference is apparent in Fig. 7.4.

Studies of TNF receptor (TNFR) internalization into endosomes show that here all the components of the TNFR signalling (Fig. 2.6) come into proximity. ASMase leads to formation of ceramide, which activates caspase 8 and cathepsin D. NSMase leads to caspase 3 activation. So, apoptosis occurs.

In some cells, ceramide will activate Rac-GTPase (guanosine triphosphatase), inducing NADPH oxidase so that superoxide is produced. In fact, ceramide induction of reactive oxygen species (ROS) could be the means of inducing apoptosis in response to TNF α , Fas and ionizing radiation [25].

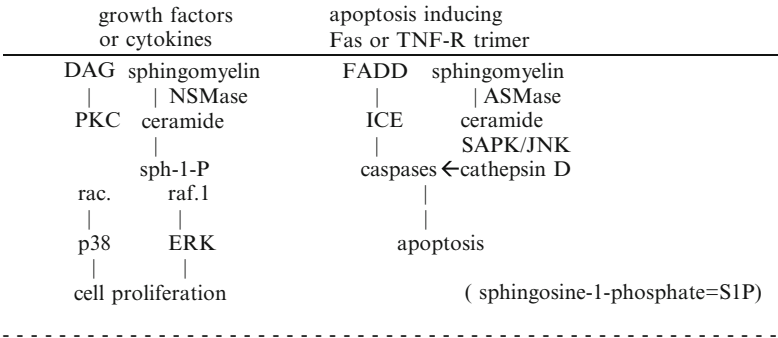


Fig. 7.4 Two forms of sphingomyelin activation

7.4 Sphingomyelin Metabolites/Cell Proliferation/Apoptosis

As seen in Fig. 7.4, in many cells ceramide forms Sph-1-P, which causes cell proliferation and is antiapoptotic. However, in response to Fas or TNF α receptor stimulation, ceramide accounts for apoptosis via the JNK pathway and activation of caspases [26]. So, the rheostat ceramide-sphingosine-1-phosphate determines the fate of a cell [27–29]. The ceramide signal is counterbalanced by the antiapoptotic action of 1,2-DAG and PKC. When ceramide induces apoptosis of endothelial cells, there is intracellular oxidative stress [30]. NSMase is inhibited by intracellular glutathione GSH=glutathione. When TNF α acts on cells, their GSH is lowered, and then NSMase is activated [31]. Hence, ceramide is formed, and apoptosis can occur.

Again, as indicated in Fig. 7.4, ceramide can give rise to ceramide-1-P, which activates c-phospholipase A2. In turn, sphingosine kinase yields sphingosine-1-P (S1P), and that has numerous significant actions. S1P is released from stimulated platelets, and it will act on endothelial cells. It causes chemotaxis of endothelial cells, and it promotes angiogenesis. S1P helps T cells leave the thymus and T cells to cross endothelium during migration [32]. Furthermore, S1P promotes T regulatory cells [33]. The S1P receptor blocker FTY720 reveals how S1P promotes B cell localization in the splenic marginal zone [34].

S1P receptors play a vital role at the vascular barrier [35]. When high molecular weight (1,000-kDa) hyaluronic acid is available on endothelium, Akt of endothelial cells promotes the S1P receptors, and Tiam1 is recruited to lipid rafts, so that there is then Rac1 activation and cytoskeletal restructuring, resulting in endothelial cell cortical actin formation; hence, the integrity of the barrier is boosted. Conversely, as a prelude to vascular permeability, low molecular weight HA (2.5 kDa), acting alongside S1P3 receptors, will activate Src kinases so that there is ROCK (Rhokinase) 1/2 transactivation. Hence, Rho-GEF is recruited to

lipid rafts (not Tiam1), and there is then RhoA activation that leads to cytoskeletal reorganization and a reduction of cell cortical actin, which means vascular permeability [35].

7.5 Lysophosphatidic Acid as Serum Growth Factor

Lysophosphatidic acid (LPA) has greater mitogenic activity than PtdOH. LPA is released from platelets and is then serum growth factor (Fig. 7.5). It is integral to wound healing. It promotes proliferation of fibroblasts and vascular smooth muscle cells. When acting as a chemoattractant for white cells, lysophospholipid receptors for LPA and S1P activate G_i , $G_{12/13}$ and G_q/G_{11} family G proteins [36]. LPA activates a pertussis toxin-insensitive G_i protein, which leads to (a) inhibition of adenylyl cyclase so that cyclic adenosine monophosphate (AMP) is reduced, (b) stimulation of phospholipases C and D and (c) stimulation via phosphotyrosine kinase (PTK) of Ras and Rho [37]. With time, it has become established that serum response factor (SRF) [38] is coupled by ternary complex factors (TCFs) like Elk-SAP1 (stress-activated protein 1) or Net to growth response genes.

LPA acts on immature dendritic cells to promote their trafficking, cytokine release and ability to activate T cells [41]. Interestingly, LPA also helps the proliferation and survival of Hodgkin and Reed-Sternberg cells. Lysophosphatidylcholine (LPC) also has a use. Its a relatively high level in blood serves as a chemoattractant to maintain natural killer (NK) cells in the bloodstream. Both LPA and S1P enhance the adhesion of murine splenic follicular and marginal zone B cells. Their adhesion on ICAM (Intercellular Adhesion Molecule) is mediated via G_{12}/G_{13} and is

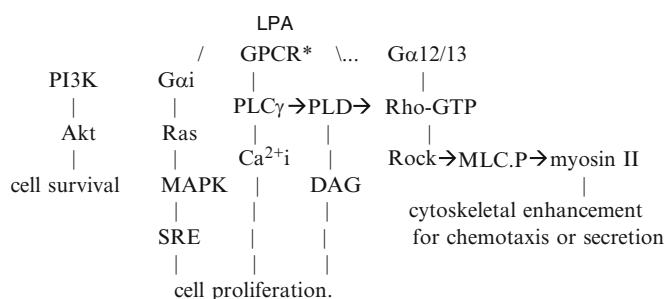


Fig. 7.5 Serum growth factor lysophosphatidic acid (LPA)-mediated signaling. As indicated in Chap. 1, G protein-coupled receptor (GPCRs*) can operate at the nuclear membrane of cells [39]. So, the phospholipase D that is induced is actually intranuclear, and that determines a lipid mediator signalling cascade in the nuclei by which phosphatidylcholine (PC) is converted to phosphatidic acid (PA) and then to diacylglycerol (DAG). Furthermore, forms of lysophosphatidic acid (LPA) can enter nuclei to induce PPAR γ [40], and that process induces neointima formation in arteries

determined by a Rho and Rho-kinase pathway [42]. S1P also enhances T cell proliferation, and actually SIP1 acts on GPCRs at the nuclear membrane to elicit intranuclear phosphorylated ERK1:2 and so the transcription factor p-c-jun [39]. One can anticipate other fascinating discoveries. Thus, LPC liberated by injury within the brain stimulates the microglia so that large amounts of the cytokine IL-1 are then released [43]. The LPA generating enzyme auto taxin (lysophospholipase D) is implicated in lymphocyte egress in the high endothelial venules [44].

7.6 Antineutrophil Cytoplasmic Antibody-Stimulated Neutrophil Adhesion

Antineutrophil cytoplasmic antibodies (ANCA) that cause vasculitis provoke neutrophils to adhere to vascular endothelial cells. This ANCA action on neutrophils involves Fc γ RIIA and Fc γ RIIB receptors that activate DAG kinase, which converts DAG to PtdOH. The latter activates CD11b/CD18 adhesion molecules [45].

References

1. Hodgkin MN, Pettitt TR, Martin A et al. Diacylglycerols and phosphatides: which molecular species are intracellular messengers? *Trends Biochem Sci* 1998;23:200–204
2. Shukla SD, Halenda SP. Phospholipase D in cell signalling and its relationship to phospholipase C. *Life Sci* 1991;48:851–856
3. Sethu S, Mendez-Corao G, Melendez AJ. Phospholipase D1 plays a key role in TNF α signaling. *J Immunol* 2008;180:6027–6034
4. Colsen S, Lambeth JD. Biochemistry and cell biology of phospholipase D in human neutrophils. *Chem Phys Lipids* 1996;80:3–19
5. Van Lint J, Ryky A, Maeda Y, Vantus T et al. Protein kinase D: intracellular traffic regulator on the move. *Trends Cell Biol* 2002;12:193–200
6. Van Lint J, Ryky A, Vantus T, Vardenheede JR. Getting to know protein kinase D. *Int J Biochem Cell Biol* 2003;34:577–581
7. Rozengurt E, Rey O, Waldron RT. Protein kinase D signaling. *J Biol Chem* 2005;280:13205–13208
8. Storz P. Mitochondrial ROS radical detoxification mediated by protein kinase D. *Trends Cell Biol* 2007;17:13–18
9. Wang QJ. PKD at the crossroads of DAG and PKC signaling. *Trends Pharmacol Sci* 2006;27:317–323
10. Pena L, Fuks Z, Kolesnick R. Stress induced apoptosis and the sphingomyelin pathway. *Biochem Pharmacol* 1997;53:613–621
11. Kronke M. Biophysics of ceramide signaling. *Chem Phys Lipids* 1999;101:109–121
12. Falcone S, Perrotta C, DePalma C, Pisconti A et al. Activation of acid sphingomyelinase and its inhibition by the nitric oxide/cGMP pathway: key events in *E. coli* elicited apoptosis of dendritic cells. *J Immunol* 2004;173:4452–4463
13. Brindley DN, Abousalam A, Kikuchi Y, et al. Crosstalk between the bioactive glycerolipids and sphingolipids in signal transduction. *Biochem Cell Biol* 1996;74:469–476
14. Woodcock J. Sphingosine and ceramide in apoptosis. *IUBMB Life* 2006;58:462–466

15. Abboushi N, El-Hed A, El-Assaad W et al. Ceramide inhibits I1-2 production by preventing PKC dependent NFkB activation. *J Immunol* 2004;173:3193–3200
16. Stratford S, Hoehn KL, Liu F, Summers SA. Regulation of insulin action by ceramide. *J Biol Chem* 2004;279:36608–36615
17. Franchi L, Malison F, Tonassini B, Testi R. Ceramide catabolism critically controls survival of human dendritic cells. *J Leukoc Biol* 2006;79:166–172
18. Hannun YA. The sphingomyelin cycle and second messenger function of ceramide. *J Biol Chem* 1994;259:3125–3128
19. Shayman JA. Sphingolipids. *Kidney Int* 2000;58:11–26
20. Ballou R, Laulederkind S, Rosloniec EF, Ragow R. Ceramide signaling and the immune response. *Biochim Biophys Acta* 1996;1301:273–278
21. Hannun YA, Luberto C. Ceramide is the eukaryotic stress response. *Trends Cell Biol* 2000;10:73–80
22. Tonetti L, Veri MC, Bonvini E, D'Adamio L. A role for neutral sphingomyelinase mediated ceramide production in T cell receptor induced apoptosis and MAP kinase signal transduction. *J Exp Med* 2000;189:1581–1589
23. Shakor ABA, Kwiatkowska K, Sobata A. Cell surface ceramide generation precedes and controls FcγRII clustering and phosphorylation in rafts. *J Biol Chem* 2004;279:36778–36787
24. Mackinnon AC, Buchley A, Chilvers ER et al. Sphingosine kinase: a point of convergence in the action of diverse neutrophil priming agents. *J Immunol* 2003;169:6394–6400
25. Kolesnick RN, Haimowitz-Friedman A, Fuks Z. The sphingomyelin signal transduction pathway mediates apoptosis for TNF, Fas and ionising radiation. *Biochem Cell Biol* 1994;72:471–474
26. Kolesnick R. The therapeutic potential of modulating the ceramide/sphingomyelin pathway. *J Clin Invest* 2002;110:3–8
27. Cuvillier O, Pirianov G, Kleuser B et al. Suppression of ceramide mediated programmed cell death by sphingosine-1-phosphate. *Nature* 1996;381:800–803
28. Liu P, Anderson R. Compartmentalized production of ceramide at the cell surface. *J Biol Chem* 1997;279:27179–27185
29. Prieschl EE, Baumruker T. Sphingolipids, second messengers, mediators and raft constituents in signaling. *Immunol Today* 2000;21:555–560
30. Matsunga T, Kotamraju S, Kalivendi SV et al. Ceramide induced intracellular oxidant formation and apoptosis in endothelial cells. *J Biol Chem* 2004;279:28614–28624
31. Liu B, Andrieu-Abodie N, Levande T et al. Glutathione regulation of neutral sphingomyelinase in TNFα induced cell death. *J Biol Chem* 1998;273:11313–11320
32. Rosen H, Goetzl EJ. Sphingosine-1-phosphate and its receptors. *Nat Rev Immunol* 2005;5:560–570
33. Wang W, Graeler MH, Goetzl EJ. Physiological sphingosine-1-phosphate requirement for optimum activity of mouse CD4+ regulatory T cells. *FASEB J* 2004;18:1043–1045
34. Cinamon G, Matkoubian M, Lesneski MJ. Sphingosine-1-phosphate receptor-1 promotes B cell localization in the splenic marginal zone. *Nat Immunol* 2004;5:713–720
35. Singleton PA, Dudek SM, Ma S-F, Garcia JGN. Transactivation of sphingosine-1-phosphate receptors for vascular barrier regeneration. *J Biol Chem* 2006;281:34381–34393
36. Anliker B, Chun J. Lysophospholipid G protein coupled receptors. *J Biol Chem* 2004;279:20555–20558
37. Goetzl EJ, An S. Diversity of cellular receptors and functions for LPA and sphingosine-1-phosphate. *FASEB J* 1998;12:1589–1598
38. Posern G, Treisman R. Serum response factor. *Trends Cell Biol* 2006;16(11):588–596
39. Goetzl EJ. Diverse pathways for nuclear signaling by GPCRs and their ligands. *FASEB J* 2007;21:638–642
40. Zhang C, Baker DL, Yasuda S et al. Lysophosphatidic acid induces neointima formation through PPARγ activation. *J Exp Med* 2004;199:763–774
41. Panther E, Idzko N, Corinti S et al. The influence of lysophosphatidic acid on human dendritic cells. *J Immunol* 2002;169:4129–4135.

42. Rieken S, Herroeder S, Sassmann A et al. Lysophospholipids control integrin dependent adhesion in splenic B cells. *J Biol Chem* 2006;281:36985–36992
43. Stock C, Schilling T, Schwab A, Eder C. Lysophosphatidylcholine stimulates IL-1 β release from microglia via a P2X7 receptor. *J Immunol* 2006;177:8560–8568
44. Nakasaki T, Tanaka T, Okudarna S et al. Involvement of the lysophosphatidic acid generating enzyme autotaxin in lymphocyte-endothelial cell interactions. *Am J Pathol* 2008; 173:1566–1576
45. Williams JM, Pettitt TR, Powell W, Grove J, Savage C, Wakelam MJO. ANCA stimulated neutrophil adhesion depends on phosphatidic acid formation. *J Am Soc Nephrol* 2007; 18:1112–1120

Chapter 8

Programmed Cell Death: Apoptosis

Abstract Apoptosis or programmed cell death is highly topical for immunologists. How it operates to eliminate unwanted damaged cells but to preserve others is really complex. There is focus on mitochondrial function and on caspase (cathepsin-like) enzymes.

8.1 Apoptosis Mechanisms

An adult human produces 10^{11-12} cells each day. The body removes damaged, senescent or abnormal cells that could hamper organ functioning or develop into a tumour. Such cells wither by *apoptosis*, which does not provoke inflammation. Apoptosis of a cell is active and programmed; thus, energy is utilized as adenosine triphosphate (ATP). Apoptotic cells appear with surface blebbing and condensation of their chromatin at the nuclear envelope. High-power microscopy reveals a nucleosomal ladder pattern of their DNA, which reflects endonuclease activity. Apoptotic cells are phagocytosed by adjacent cells, especially by macrophages, without release of oxygen radicals or proteolytic enzymes [1]. Such effete cell phagocytosis is initiated by exposure of phosphatidylserine (PS) and molecules like C-reactive protein (CRP), C3b, and gas-6 on the apoptotic cells and Fc γ R, α V β 3 integrin, the collectin C1qR, CR3, and c-mer on the phagocytes [2]. Blood protein S helps phagocyte interaction with PS. Conversely, if there is *cell necrosis*, resulting from ischaemia, heat shock proteins gp96 and hsp70 are liberated, and they cause inflammation [3a, 3b]. This contrasts with silent removal of apoptotic cells. Necrosis results from rapid dramatic loss of control of energy stores. Necrosis instigated via tumour necrosis factor- α (TNF α) or Fas results from mitochondrial production of reactive oxygen species (ROS). Cells that are developing necrosis swell and show early membrane damage and nuclear lysis without condensation. When there is cell necrosis, the operative factor is inhibition of the caspases.

Interestingly, apoptotic and necrotic cells induce distinct mitogen-activated protein kinase (MAPK) modules in macrophages. Apoptotic cells inhibit macrophage ERK1/2 (extracellular signal-related kinase 1/2) but induce p38 and Jun N-terminal

kinase (JNK), whilst necrotic cells induce Mφ ERK1/2. Apoptotic cells block nuclear factor kappa B (NF-κB) activation in dendritic cells (DCs) [4], and that. anti- inflammatory action is mediated by nitric oxide [46]. Attenuation of pro-inflammatory cytokine formation also results from PRARY sumoylation [cf Fig. 4.14] stepping removal of NCoR (nuclear receptor compressor) from the TNFα promoter [4c]. Apoptotic cells can induce a dramatic immunosuppressive effect!

Many cells have death domain receptors like the Fas receptor or the TNF receptor (TNFR) [6–8], and their activation by the *extrinsic* pathway causes induction of caspase 8 (Table 8.1). Conversely, via the *intrinsic* pathway toxins, agents that deplete ATP or damage the DNA cause mitochondrial permeability, releasing cytochrome C [9–11], which activates caspase 9; in turn, this activates caspase 3, which releases DNA endonucleases (Fig. 8.1). For that to occur, caspase 9 must be bound

Table 8.1 Processes that lead to apoptosis of T lymphocytes

(a)	Withdrawal of trophic factors; loss of Bcl-2
(b)	Oxidative stress to membrane and intracellular thiols
(c)	Nitric oxide from macrophages
(d)	FasL triggering Fas(CD95) or tumour necrosis factor-α (TNFα) acting on TNFR1 receptors
(e)	Activation of intracellular interleukin-1β-converting enzyme (ICE), interleukin 1β (IL-1β) converting enzyme
(f)	Exposure to galectin 1, a physiological lectin
(g)	Granzymes/perforins from killer CD8 cytotoxic T lymphocytes (CTLs) or natural killer (NK) cells
(h)	Damage by ionising radiation or anticancer drugs or toxins
(i)	The action of corticosteroids, used therapeutically

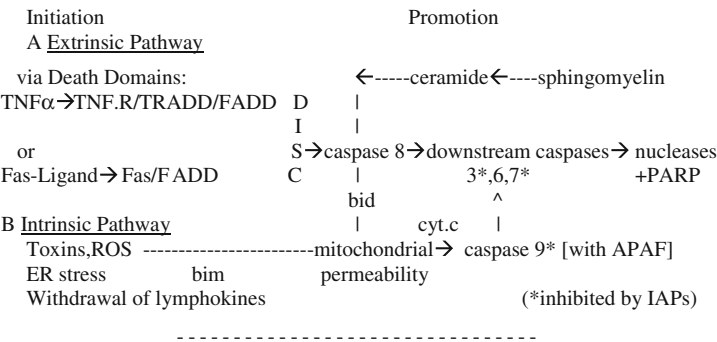


Fig. 8.1 Scheme for induction of apoptosis. To be effective the Fas receptors (*FasRs*) or tumour necrosis factor receptors (*TNFRs*) must be within membrane lipid rafts. *FADD* Fas-associated death domain; *TRADD* TNFR1-associated death domain protein; *DISC* death-inducing signalling complex; *Apaf1* apoptotic protease-activating factor; *PARP* poly(ADP-ribose)polymerase upstream caspases 8, 9, 10 downstream caspases 3, 6, 7; *bim* bcl-2 interacting mediator of cell death. bim-induced apoptosis eliminates self-reactive thymocytes. Other proapoptotic factors are Bax, Bad, Bak, Bik, Bid, AIF and Apaf1. In endoplasmic reticulum (*ER*), stress p53 via Puma interacts with Bak and Bax, inducing mitochondrial leak [5]. Antiapoptotic factors are bcl-2, bcl-Xl and inhibitors of apoptosis proteins (*IAPs*)

to Apaf1 (apoptotic protease-activating factor) [12]. In addition, mitochondrial Smac/Diablo [13] inhibits inhibitor of apoptotic proteins (IAPs), aiding the activation of caspase 9. *Apoptosomes* incorporating caspases 9 or 8 trigger downstream effector caspases 3, 6, 7 and so the endonucleases.

Concurrently, there is loss of reduced glutathione via cell membrane transporters. Excessive production of oxygen radicals by the mitochondria is implicated [14, 15]. Whenever there is DNA damage, activation of the enzyme poly(ADP-ribose) polymerase (PARP), which normally repairs single-strand DNA breaks, will cause release of apoptosis-inducing factor (AIF) from mitochondria. PARP is phosphorylated by ERK. Activated PARP [16] uses NAD^+ and transfers adenosine diphosphate (ADP)-ribose units to target nuclear proteins, accounting for poly(ADP)-ribosylated proteins in affected tissues. The endogenous inhibitors of PARP are KIF of the kinesin family and sirtuin [17].

Calcium ions play their part. Thus, released cytochrome C diffuses to the endoplasmic reticulum (ER) and binds there to InsP3 receptors, enhancing release of Ca^{2+} . Increased intracellular Ca^{2+} results in uptake of calcium by the mitochondria, and thus more cytochrome C is released to form apoptosomes that activate caspases and calpain [18]. Conversely, nitric oxide (NO) can protect against apoptosis (a) because cyclic guanosine monophosphate (cGMP) decreases Ca^{2+}_i and (b) NO $\cdot$ inhibits caspases and prevents bcl-2 (B cell lymphoma 2 integral membrane protein of the outer mitochondrial membrane) degradation and cytochrome C release [19].

The intracellular proteases that effect apoptosis are the numbered caspases [20]. A *caspase* has a cysteine residue in the active site and specificity for cleavage at aspartic acid residues. Caspase 1 is interleukin (IL) 1 β -converting enzyme (ICE). Caspase 2 causes release of cytochrome C from mitochondria, and it cleaves Bid, which offsets Bcl-2 antiapoptotic protein. The upstream caspases are 8, 9 and 10 (Fig. 8.1), and the downstream effectors are 3, 6 and 7. Lysosomal cathepsins will also contribute within macrophages. Ceramide activates both upstream and downstream proteases. It activates proapoptotic Bad [21]. Bad is a bcl-2 or bcl-XL antagonist by virtue of heterodimer formation. The survival factor Akt (i.e., protein kinase B) sequesters Bad with protein 14-3-3 in the cytoplasm of cells, and so it protects against apoptosis [22]. In lymphocytes, bcl-2-interacting molecule Bim (Bcl-2 interacting mediator of cell death) is required for apoptosis, which is induced by cytokine withdrawal or calcium flux.

The death-promoting proteins bax and bad interact with the outer membrane proteins of the mitochondria to form pores so that holocytochrome C is released [23]. The megapore, spanning the inner and outer mitochondrial membranes, incorporates a porin, a voltage-dependent anion channel (VDAC) and the adenine nucleotide translocator. Mitochondrial permeability transition is determined by pore formation in the inner mitochondrial membrane [24]. Conversely, there are apoptosis-inhibiting proteins such as bcl-2 and bcl-XL, which stop outer membrane permeabilization of mitochondria [25] (Fig. 8.2). Bcl-2 stabilizes mitochondrial membranes and those of the ER and nuclear envelope [26].

Bcl-2 and Bcl-XL bind and inactivate Apaf1, which initiates the intrinsic pathway. Bcl-2 contributes to antiapoptosis in several ways [26, 27]: (a) It prevents

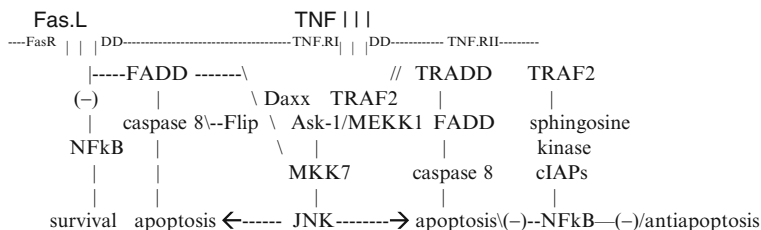


Fig. 8.3 Apoptosis signalling via Fas or the tumor necrosis factor- α (*TNF α*) RI. *DD* death domain; *IAP* inhibitor of apoptosis protein c-Flip thwarts caspase 8; *Ask-1* apoptosis signal-regulating kinase 1, thwarted by protein 14–3–3 [22]; *Ask-1* causes activation of Jun N-terminal kinase (*JNK*) and of p38 mitogen-activated protein kinase (*MAPK*). *Daxx*, a death or survival protein, also regulates the stress kinases *JNK* and p38 *MAPK* [82]. Receptor-interacting protein (*RIP*) is a serine-threonine protein kinase that competes with tumour necrosis factor receptor-associated death domain (*TRADD*) for recruitment to the *TNFR*I signal complex [83]. *RIP1* can lead to cell death. *A20* protein suppresses tumour necrosis factor (*TNF*) signalling by inhibiting recruitment of *RIP1*-*TRADD*-*TRAF2*. *TNFR*-*TRAF2*/5 E3 ligases activate *TAK1* (-*Tab2/3*) and thereby *IKK β* and nuclear factor kappa B (*NF- κ B*) (see also Fig. 12.6).

attachment triggers caspase activation and apoptosis, called in this context *anoikis*. An enzyme Bit1 transfer RNA (tRNA) hydrolase is released from the outer mitochondrial membrane [32]. Endothelial cells are protected from apoptosis by the stress response protein A20. It protects them from Fas- or *TNFR*I- (Fig. 8.3) induced apoptosis and from damage by NK cells. A20 stops release of cytochrome C from mitochondria [33]. A20 expression inhibits upstream caspases 8 and 9, and it inhibits downstream 3 and 6 caspases and stops Bid cleavage.

Neutrophils, albeit not macrophages, are protected from apoptosis by the antiapoptosis protein Mcl-1 [34].

8.1.2 Heat Shock Proteins and Apoptosis

Heat shock proteins like hsp27, hsp70 and hsp90 play several roles in controlling mitochondrial outer membrane permeabilization (MOMP) and apoptosome formation [35]. They inhibit translocation of bid and inhibit release of cytochrome C. They also support Akt survival kinase and *NF- κ B*.

8.2 Cellular Metabolism and Apoptosis

Since mitochondria regulate metabolism and control apoptosis, many of the mitochondrial core apoptotic proteins are actually regulators of cellular metabolism [36]. So, growth factor withdrawal causes decreased availability of substrates for

oxidative phosphorylation and hence ATP production and accordingly mitochondrial membrane depolarization and release of apoptotic factors. The VDAC is controlled by interactions with the Bcl-2 family proteins. Antiapoptotic Bcl-Xl can regulate VDAC, which in turn regulates proapoptotic protein Bak. Inactivation of Bcl-2 and Bcl-Xl or activation of Bax and Bak perturb VDAC function. Liver glucokinase is regulated by Bad phosphorylation. Following glucose or growth factor withdrawal, Bad is dephosphorylated, and it then promotes apoptosis. Factors that stimulate ATP production like Akt and Pim2 promote the association of hexokinase with the outer mitochondrial membrane. Akt and Pim2 promote growth factor-independent cell survival. Akt regulates metabolism and cell survival by phosphorylation of the Foxos (Forkhead transcription factors) (Chap. 4). Foxos could be important metabolic sensors. JNK is known to modulate apoptosis. Generally JNK/AP-1 (activator protein 1) mediate cell survival. One will recall that JNK helps survival of macrophages. The nature of the death signal and the cell type determine whether JNK will cause apoptosis [37] since it can cause release of mitochondrial cytochrome C. NF- κ B prevents prolonged JNK activation [38]. Yet, when ROS and JNK are involved in a mutual amplification system (as after TNFRI stimulation), then JNK will cause apoptosis [39–41]. Essentially, early transient JNK activation enables cell survival, but prolonged JNK activation leads to apoptosis. JNK causes apoptosis by mobilization of proapoptotic Bax and Bak, which act on the mitochondria [42, 43].

8.2.1 Endoplasmic Reticulum Stress

A high calcium concentration is required in the ER for its functioning. ER stress leads to transduction events that alter genes for host defence (NF- κ B) or transcription factors (TFs) leading to adaptation, like PERK, CHOP, and ATF4 (activating transcription factor 2) or Ask-1 (apoptosis signal-regulating kinase 1). Still, the result may be apoptosis [44, 45]. Thus, when C5b-9 complement damages cells, there is activation of cytosolic phospholipase A2 (cPLA2), and an “unfolded protein response” shows in the ER and causes stress. Trans-fatty acids in the diet can cause ER stress in adipocytes and other cells and thereby cardiovascular disease.

8.3 Inflammatory Caspases

There is a group of inflammatory caspases implicated in the cytokine responses to microbial pathogens [46]. Caspases 1 and 5 activate the proinflammatory cytokines IL-1 and IL-18 (Fig. 12.3). This is an intracellular nonapoptotic function of the caspases. Just as there is Apaf1 apoptosome-mediated activation of caspase 9, activation of the inflammatory caspases occurs on a molecular platform in macrophages and neutrophils termed the NALP inflammasome, which leads to release of

IL-1 (Fig. 12.3). A NALP is thus akin to Apaf1 [46]. The NALP inflammasome is a sensor that detects danger signals threatening cells [47, 476].

There are now techniques for monitoring caspase activity within cells [48]. Consider the apoptosis of polymorphonuclear leucocytes (PMNs), which is largely determined by ROS [49]. There is an early fall of reduced glutathione and, consequently, clustering of Fas (CD95) in surface lipid rafts and activation there of acid sphingomyelinase (ASMase), creating ceramide, which activates caspases.

8.4 Effect of Apoptotic Cells on Macrophages

When apoptotic cells are ingested by macrophages or when macrophages encounter released PS, Gc-binding protein is induced, which inhibits gene transcription of IL-12 p35 [50]. In general, ingestion of apoptotic cells stops the release of proinflammatory cytokines, and NO is released [49]. However, when pathogens cause apoptosis of neutrophils, heat shock proteins may be induced, and they then stimulate macrophages [51]. Furthermore, oxidised lipid epitopes on apoptotic cells will promote the inflammatory response [52, 53]. Note then that it is macrophage colony-stimulating factor (M-CSF)-driven IL-10 producing macrophages that preferentially clear apoptotic cells [54]. When macrophages ingest apoptotic cells, there is immediate activation of their PPAR γ nuclear receptors, and ROS formation is stopped [55].

8.5 Apoptosis of Neutrophils

The half-life of neutrophils is about 7 h before they are phagocytosed in the spleen. Their survival is helped by ERK/MAPK signalling and by NF- κ B activation, directed from the surface integrin Mac-1 (CD11b/CD18) [56]. Clustering of Mac-1 activates Akt and so the ERK pathway. Generally, the high level of reduced glutathione (GSH) aids their survival. Furthermore, induction of p38 MAPK leads to phosphorylation of caspases 8 and 3 and so their inactivation. The cytokines granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor GM-CSF [57] and leptin [58] support antiapoptosis, witness the expression of bcl-2 [59]. However, IL-17 attenuates the important action of GM-CSF [60]. One might expect Fas (CD95)-mediated neutrophil apoptosis. Yet, neutrophils in inflammatory exudates are protected by binding of Foxo3a to suppress the Fas promoter [61]. Both IL-2 and IL-15 confer some resistance to apoptosis. Neutrophils have antiapoptotic Mcl-1 [34]. In addition, the hypoxia-inducing factor 1 (HIF1) pathway protects against neutrophil apoptosis [62]. When neutrophils engage in phagocytosis, large amounts of ROS are created by their NADPH oxidases, so there is phagocytosis-induced cell death (PCID). NADPH oxidase stimulation of Lyn of neutrophils leads to binding of SHIP and SHP1 phosphatases to

Fas-associated death domains (FADDs) and so to apoptosis [63, 64]. Nitrosative stress inhibits NF- κ B [65]; hence, it sensitizes cells to TNF α apoptosis [66].

Neutrophils are more liable to apoptosis than monocytes. In monocytes, the I κ B α inhibitor of NF- κ B is degraded only in the cytosol (Chap. 11). In activated neutrophils, I κ B α is translocated into the nuclei, causing restraint over NF- κ B activity but liability to apoptosis [67].

8.6 Apoptosis of T Lymphocytes

One cannot expect T cell apoptosis soon after antigen-induced lymphocyte proliferation because that would thwart the immune response. Apoptosis occurs when there is exposure of activated T cells to large amounts of antigen, especially self-antigen, or when there is long-term antigenic stimulation [68, 69]. Following an acute viral infection, large numbers of CD8 cytotoxic T lymphocytes (CTLs) must be removed. Clearly, there is a need to silence CD4 or CD8 T cells that have become autoreactive.

Activation-induced cell death (AICD) occurs via Fas-ligand interaction with the Fas receptors of doomed lymphocytes. This depends on T cell receptor (TCR) stimulation by antigen, but there will be no antigen by the time that infection has resolved. Release of chemokine CCL19 (macrophage inflammatory protein 3 β , MIP-3 β) helps. AICD can occur when there is infection by parasites that cannot be killed [70].

Activated T cell autonomous death (ACAD) arises when TCR stimulation creates ROS, and thereby H₂O₂ that suppresses nuclear cAMP response element-binding protein (CREB), so that bcl-2 is lowered. Death of T cells at the termination of an immune response is initiated by Bim, a BH3 moiety-only Bcl-2 family member, that works along with proapoptotic Bax and Bak [70] (Fig. 8.2). Apoptosis is averted in T lymphocytes when they are adherent to type I collagen by integrin α 2 β 1. There is then PP2A phosphatase activation of the MAPK path [71].

8.7 Apoptosis of Dendritic Cells or Macrophages

After precursor immature DCs have encountered foreign antigen in the tissues, they migrate into lymph nodes as mature DCs, which are antigen-presenting cells. If *immature* DCs meet self-antigens, they stay alive since they have adequate bcl-2 and bcl-XL, and like this they can induce tolerant T cells. However, when *mature* DCs present processed antigen for activation of T cells, they show a decline of bcl-2 and a rise of Bim, which is proapoptotic, so they die [72]. Microbes often kill or cause apoptosis of activated macrophages. Yet, when Toll-like receptor 4 (TLR4) receptors activate both NF- κ B and CREB (via p38 MAPK), then genes PAI-2 and the Bfl-1/A1 are activated, with the result that cells can survive, as can DCs [73].

8.8 Cell Death via Fas or TNFRI Receptors

The response of cells to TNF α involves induction of genes for inflammation and often cell apoptosis or cell necrosis. Keeping a cell alive depends on the involvement of NF- κ B (Chap. 11). Actually, cell fate is determined by the cell type and local environmental factors. As part of immune regulation, the TNF receptors play vital roles in inducing lymphocyte apoptosis [74]. Also, Fas-L originating from T cells acts on Fas receptors of macrophages or B cells to create apoptosis. One has to be clear: TNF α induces apoptosis in normal cells only when NF- κ B is blocked.

Figure 8.3 shows the connections of these death receptors. The FasR and TNFRI have associated death receptors (DD), but TNFR2 do not. When TNF α binds, it trimerizes TNFRI, inducing association of the receptor death domains (DDs). Then, TRADD (TNFR-associated death domain) binds to the clustered receptor death domains. TRADD is a platform adaptor that recruits several signalling molecules to the activated receptor. FADD directs apoptosis by activating caspase 8, and TRADD via TRAF2 will activate Ask-1 and thereby JNK, which is an inducer of apoptosis. Interestingly, the matricellular protein CCN1 unmask the cytotoxic potential of TNF α . CCN1 binds to integrins and to syndecan 4 and thereby triggers formation of ROS via Rac1 activation of lipoxygenase. Hence, there is activation of JNK that leads to apoptosis of cells.

Whenever IAPs are induced, they suppress the apoptotic drive [68] by inhibiting caspases 3, 7 and 9 (Fig. 8.3). IAPs are formed from 1–3 baculovirus IAP repeats (BIRs), with homology to the baculovirus p35 protein, which is a caspase inhibitor. cIAPs consist of BIRs 1–3, whilst survivin has a single BIR [71]. Survivin is a nuclear protein that may function in cell cycle regulation rather than by controlling caspases.

Ask-1 is mentioned as a cause of stress-induced apoptosis, which it performs by activating p38 MAPK and JNK. ROS also activate JNK. The MAPK phosphatases (MKPs) that regulate JNK are inhibited by ROS. The antioxidant thioredoxin (TRX) inhibits Ask-1 kinase, but should there be S-nitrosylation of TRX, Ask-1 is then activated. One can best consider Ask-1 activation of JNK and p38-induced mitochondrial leak in the context of *ER stress-induced apoptosis* [75] when it acts alongside TF CHOP. As for CHOP, it plays its role in lipopolysaccharide (LPS)-induced inflammation of the lung, for CHOP knockout reveals that CHOP normally induces caspase 11 and thereby inflammation [76].

The Fas receptor operates in similar fashion to the TNFRs [77–79]. The three DDs, which are part of the receptor, bind FADDs and form the DISC (death-inducing signalling complex) (Fig. 8.1). So, caspase 8 is activated to promote apoptosis, but it is negated whenever there is available c-Flip [80]. Flip has to be down graded [80b]. Interestingly, Ask-1 is inhibited by Nef of human immunodeficiency virus (HIV), allowing that virus time to persist so it can mature and spread. There is additional control of Fas by Fas-associated protein phosphatase (FAP1). Fas causes caspase-dependent inactivation of IKK1 and therefore of NF- κ B. Fas also inactivates Nemo, so that IAPs are not formed (cf. Fig. 12.6) [81].

When NF- κ B is activated by TNF α , there is formation of XIAP (X chromosome inhibitor of apoptosis) and GADD45, which reduce JNK induction of apoptosis.

XIAP regulates the extrinsic and intrinsic pathways by inhibition of caspases 3 and 9. In addition, TRAF2 can stimulate sphingosine kinase and so NF- κ B to help cell survival (antiapoptosis) [84]. If statins are exhibited, antiapoptosis is abolished because statins thwart the action of NF- κ B [85].

It has been emphasized that the Fas ligand/Fas system terminates the action of T or B lymphocytes at the end of the immune response (Table 8.1), it eliminates cells carrying virus or intracellular bacteria, and it eradicates tumour cells. Another ligand called TRAIL (TNF-related apoptosis-inducing ligand) helps cytotoxic T cells and NK cells kill tumour cells. TRAIL interacts with apoptosis-inducing receptors DR4 or DR5, which are linked via DAP3 GTP-binding protein to FADD and hence to caspase activation. Interestingly, TRAIL removes effete neutrophils at the end of their lives when apoptotic cells induce tolerance that arises because CD8 T cells are then exhibiting TRAIL [86].

8.8.1 Death Receptor Functioning: Complexes I/II

In activated CD4 T cells, FasR (Fas receptor) is excluded from surface lipid rafts [87]. Accordingly, after engagement by FasL, FADD and caspase 8 (Flice) are recruited to FasR but do not signal efficiently. On TCR restimulation, Fas translocates into lipid rafts, and now there is efficient death signalling. Stimulation through FasRs results in rapid loss of lymphocyte cell surface glucose transporters.

The fact that TNFRI signalling results in the assembly of *two sequential and distinct signalling complexes* has enabled explanation of how and when cells remain alive or whether apoptosis ensues [88]. Within minutes of TNFRI activation, complex I is formed by the receptor along with TRADD, TRAF2 and RIP1 (receptor-interacting protein 1). Complex I leads to NF- κ B activation. Once it is activated, NF- κ B inhibits the proapoptotic JNK. Within 2 h, possibly after receptor internalization, TRADD-TRAF2-RIP1 dissociate from the receptor, and instead there is recruitment of FADD and caspase 8 into a secondary complex II [88]. In this way, caspase 8 is activated. Also, there is cleavage of bid to t-bid, and apoptosis is promoted.

8.9 Detecting Apoptosis

The morphological differences between necrotic and apoptotic cells have been indicated (Sect. 8.1). One can perform immunofluorescence on specific proteins like the caspases or death receptors. Binding of annexin V to cells indicates PS externalization. *Tunel* is a cytochemical technique for analysis of DNA fragmentation. Using terminal deoxynucleotidyltransferase (TdT), there is enzymatic labelling of the 3'-OH ends of fragmented DNA. If fluorescence labelling is used, fluorescein-12-dUTP labelled DNA is observed by microscopy. Reverse transcriptase polymerase chain reaction (RT-PCR) for apoptosis-related messenger RNAs

(mRNAs) is a sensitive indicator of apoptosis in a cell population but not individual cells.

Identification of the macrophage receptors that interact with PS on apoptotic cells has come in for scrutiny. MFGE8 is a bridging molecule expressed by macrophages. T cell immunoglobulin domain and mucin domain 4 (TIM4) receptors [89] and BAI-1 receptors will both mediate engulfment [90].

8.10 DNA Damage Leading to Cell Cycle Arrest or Apoptosis

DNA damage by radiation is used to study DNA repair mechanisms, the checkpoints in the cell cycle, the processes that lead to apoptosis or alternatively the genesis of cancer. To deal with the last briefly, when cells have mutations in DNA of damaged signalling pathways, this causes genomic instability. If, concomitantly, there are alterations to mitotic checkpoints or mutations that hinder apoptosis, then cancer can arise. Normally, minor *DNA damage activates the p53 protein*, so that there is growth arrest of cells, whilst DNA repair occurs [29]. Major damage to the DNA ensures that p53 instigates apoptosis of affected cells [91, 92]. p53 is a short-lived protein that is stabilized and transcriptionally activated by ATM-mediated phosphorylation [93] (Fig. 8.4). An enzyme pim1 attaches to phosphorylated p53 to change its configuration so that it becomes activated [94]. p53 rapidly initiates G1 and intra-S checkpoint arrest. The G1 checkpoint will curtail hyperactive Ras, Myc and E2F signalling. Checkpoint arrest occurs because (a) p53 phosphorylation induces p21^{cip1/waf1} inhibitor of cyclin-dependent kinases, which silences the G1/S-promoting cyclin E/cdk2 kinase; (b) ATM via chk2 (checkpoint kinase 2) causes the degradation of phosphatase cdc25A by the ubiquitin system, causing inhibitory phosphorylation of cdk2 and hence inhibition of cyclin E:cdk2 activity (Fig. 17.1, Table 16.2). It was revealing when ATM^{null} mice were examined because it was evident that their formation of ROS reflects an inability to control oxidative stress.

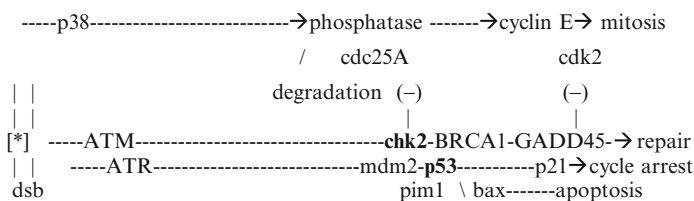


Fig. 8.4 Responses to DNA double-strand breaks. (a) Checkpoint kinases (chk1/2) block phosphatases needed for G1/S and G2/M. (b) p53 activates multiple proteins to block the G1/S and S/G2 checks. (c) Peptidyl-prolyl-isomerase pim1 stabilizes and activates p53 [94]. (d) p38 mitogen-activated protein kinase (MAPK), sensing DNA damage, phosphorylates/immobilizes cdc25 [96]. (e) The kinases ATM, ATR, DBA-PK phosphorylate histone H2AX protein, which enhances accurate repair or prevents inaccurate repair [97]. ATM is a regulator of oxidative stress. (f) After DNA damage, ATM phosphorylates Bid (of the apoptosis path) with resulting cell cycle arrest in the S phase [98].

Hence, abnormal DNA recombination in those mice led to immunodeficiency, and they were liable to develop tumours [95].

Cdc25 phosphatase isoforms are essential for G1-S and G2-M transitions [99]. The cdc25 isoforms connect to cell cycle regulation and are controlled by the appropriate checkpoints [100], such as ATM/ATR-(-) cdc25A, p38-MAPKAPK-2-(-) cdc25B, and pim-1-(-) Tak-1-(-) cdc25C. *Cdc25 overexpression is reported in many cancers.* The three phosphatases reverse inhibitory cyclin-dependent kinase (CDK) phosphorylation [100]. Cdc25A is responsible for cdk2 activation. Yet, it is degraded after any DNA damage. Normally, unbound E2F and c-Myc jointly activate the key target gene cyclin E, whose product activates the cdk2 kinase required for initiation of DNA replication [101].

Note (Fig. 8.4) how a PI3 kinase that is the product of the ataxia-telangiectasia ATM gene activates a kinase Chk2, which phosphorylates p53 protein [102], which is the essential component of the DNA damage checkpoint response [102, 103]. p53 activation will lead to cell cycle arrest or cell apoptosis. Chk2 inhibits cdc25 phosphatases. ATM recognises double-strand breaks (in Fig. 8.4) caused by ionizing radiation, whilst ATR is activated by ultraviolet irradiation. ATR has been implicated in cell responses to hypoxia [103].

DNA damage causes Chk2 to phosphorylate breast cancer gene protein BRCA1, which is located in small foci within nuclei with the protein Rad51, which can repair chromosome breaks [104]. Expression of BRCA1 leads to GADD45 and p21^{cip.1}, which are implicated in apoptosis or cycle arrest [105, 106]. BRCA1 (relevant in breast cancer) is actually an ATM-related mediator [107]. It is a large, multidomain protein with two tandem BRCT domains at the C-terminus, and they serve as protein-phosphoprotein binding modules. BCRA1 facilitates ATM signalling. The related BRCA2 is inactivated in Fanconi's anemia. Suppressor gene p53 encodes TF p53 for multiple genes (Sect. 17.5). When there is cell stress, phosphorylation inhibits p53 interaction with murine double minute 2 (mdm-2), which is an E3 ubiquitin ligase that (a) blocks p53 transactivation of DNA and (b) promotes p53 nuclear export and ubiquitination. Hence, p53 is free to bind to specific DNA sequences to induce protective genes. In fact, p19 ARF (ADP ribosylation factor) will block mdm-2, removing its block of p53. For example, this happens when NO induces ARF and thereby determines p53-induced apoptosis [108]. p53 is regulated via mdm-2-directed proteasomal degradation and via an atypical ubiquitin ligase E4F1 [109].

One can now understand how p53 induces apoptosis (Fig. 8.5). p53 prevents the generation of genetically altered cells. (a) p53 induces the caspases and cathepsin D,

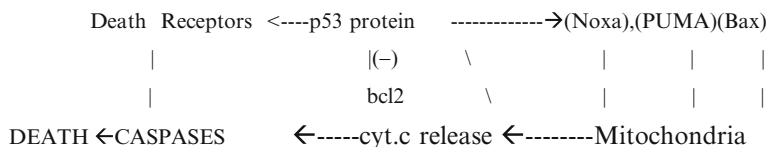


Fig. 8.5 How p53 causes apoptosis of cells

so that cell apoptosis occurs. (b) p53 lowers Bcl-2 and induces proapoptotic Bax [110]. p53 also incites Puma, another BH3-only protein [111]. (c) p53 induces cell cycle arrest at G1 by inhibiting cyclins D and E (Chap. 17) and by elevating p21, cyclin-dependent kinase inhibitor. (d) p53 induces G2 arrest by inhibiting cdc2 and cyclin B. By promoting p21, p53 inhibits cdc25c, and it promotes the action of GADD45 and of 14-3-3 σ scaffold protein. (e) p53 induces PTEN (protein tyrosine phosphatase) for PI3 kinase, impairing Akt activation. Moreover, it promotes caspase-mediated Akt degradation. (f) p53 induces MKP1, dual-specificity phosphatase, so that MAPKs are abated [112]. Obviously, p53 is subject to controls. Mdm-2 [113] causes its degradation. Silent information regulator 2 (Sir-2) deacetylates p53 and silences it [114]. In T cells, p53 favours ATP production by oxidative phosphorylation, which emphasizes a control over events in the mitochondria [115]. The structure of p53 protein [115b] appears in Fig. 17.7 (Chap. 17).

Note how p53 protein inhibits formation of Bcl-2 (Fig. 8.5), and it induces Bax/Puma/Noxa and so on, which cause apoptosis. Furthermore, p53 causes formation of miRNA-34, which causes cell cycle arrest and apoptosis of cells [116].

8.11 Apoptosis versus Carcinogenesis

DNA damage by radiation, chemicals or oxygen radicals creates genomic instability of cells so that cancer can arise. Cancer is common in the skin, in the lungs and in the gut, where epithelial cells are exposed continuously to environmental insults. In these situations, it is thought that stem cells that become involved in repeated repair are at risk [117]. However, the fact that genotoxic insults lead to a halt in ongoing DNA synthesis via the ATM/ATR machinery (Fig. 8.4), leading to activation of p53, means also that apoptosis of such cells will usually result. Depending on a network of interactions, there is an apoptotic threshold that varies with the tissue and the sequence of mutations [118]. When cancer does arise, p53 is often found to be mutated. Akt protects cells from apoptosis. Yet, if Akt is overexpressed and localized by membrane myristoylation, its three isoforms have the capacity to transform cells.

The wild-type breast cancer gene BRCA1 (Chap. 18) responds to DNA damage in breast epithelium, so that there is expression of GADD45 and p21^{cip.1}, which mediate cell cycle arrest and apoptosis. BRCA1 also recruits activated ATM to sites of DNA damage with the result that there is then phosphorylation of the cohesin protein SMC1 and that induces cell cycle arrest.

There must be sensors for aberrant proliferation of cells. C-Myc along with Ras-MAPK incite cell proliferation (Fig. 4.18). C-Myc regulates cell cycle genes like ornithine decarboxylase for polyamine biosynthesis, cad for pyrimidine biosynthesis, eIF-4E translation factor and cdc25A cell cycle phosphatase. The tumour suppressor ARF is induced in response to cell proliferation caused by c-Myc. c-Myc, via the ARF-mdm-2 pathway [119], activates the p53 damage monitor (Fig. 17.5), which can provoke either senescence or apoptosis of cells. Hence, for cancer

to arise there must be inactivation of the pathways: (a) p16(Ink4)-cyclinD/cdk-pRb-E2F and (b) p19 ARF-mdm-2-p53. This information will become clear after reading Chap. 17 on the cell cycle. What can be stressed here is that it is not actually DNA damage that puts p53 into action as a tumour suppressor, but rather “oncogenic stress” affecting the upstream regulator ARF (cf. Fig. 17.5) [120].

References

1. Huynh M-L, Fadok VA, Henson PM. Phosphatidylserine ingestion of apoptotic cells promote TGF β 1 secretion and regulation of inflammation. *J Clin Invest* 2002;109:41–50
2. Liu G, Wu C, Wu Y, Zhao EY. Phagocytosis of apoptotic cells and immune regulation. *Scand J Immunol* 2006;64:1–9
- 3a. Li M, Carpio DF, Zheng Y, Bruzzo L et al. Essential role of NF- κ B? Toll pathway in induction of inflammatory and tissue repair gene expression by necrotic cells. *J Immunol* 2001;166:7128–7135
- 3b. Zong WX, Thompson CB. Necrotic cell death. *Genes Dev* 2006;20:1–15
- 4a. Sen P, Wallet MA, Yi Z et al. Apoptotic cells induce Mer tyrosine kinase dependent blockade of NF κ B activation in dendritic cells. *Blood* 2007;109:653–660
- 4b. Ren G, Su J, Zhac X et al. Apoptotic cells induce immunosuppression through dendritic cells; critical roles of IFN α and nitric oxide *J Immunol* 2008;181:3277–3284
- 4c. Jennewein C, Kuhn A.M, Schmidt MV et al. Sumoylation of Peroxisome Proliferator-Activated Receptor γ by apoptotic cells. *J Immunol* 2008;181:5646–5652
5. Li J, Lee B, Lee AS. ER stress induced apoptosis. *J Biol Chem* 2006;281:7260–7270
6. Granville DJ, Carthy CM, Hunt D, McManus BM. Apoptosis: molecular aspects of cell death and disease. *Lab Invest* 1998;78:893–903
7. Reed JC. Mechanisms of apoptosis. *Am J Pathol* 2000;157:1415–1430
8. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. *Science* 1998;281:1305–1307
9. Green DR. Mitochondria and apoptosis. *Science* 1998;281:1309–1312
10. Jiang X, Wang X. Cytochrome C mediated apoptosis. *Annu Rev Biochem* 2004;73:87–106
11. Ravagnon L, Roumier T, Kroemer G. Mitochondria: the killer organelles and their weapons. *J Cell Physiol* 2002;192:131–137
12. Rodriguez J, Lazebnik Y. Caspase 9 and Apaf.1 form an active holoenzyme. *Genes Dev* 1999;13:3179–3184
13. Verhagen AM, Ekert PG, Pakusch M, Silke J et al. Identification of Diablo a protein that promotes apoptosis by binding to IAP proteins. *Cell* 2000;102:43–53
14. Jabs T. Reactive oxygen intermediates as mediators of programmed cell death in plants and animals. *Biochem Pharmacol* 1999;57:231–245
15. Chakraborti I, Das S, Mondol M et al. Oxidation, mitochondria and calcium. *Cell Signaling* 1999;11:77–85
16. Yu S-W, Wang H, Poitras MF et al. Mediation of PARP dependent cell death by apoptosis inducing factor. *Science* 2002;297:259–263
17. Kolthur-Seetharam U et al.. Control of AIF-mediated cell death by the functional interplay of SIRT1 and PARP-1 in response to DNA damage. *Cell Cycle* 2006;5:873–877
18. Nakagawa T, Yuan J. Activation of caspase by calpain in apoptosis. *J Cell Biol* 2000;150:887–894
19. Kim Y-M, Bombeck CA, Billiar TR. Nitric oxide as a bifunctional regulator of apoptosis. *Circ Res* 1999;84:253–256
20. Martin SJ, Green DR. Protease activation during apoptosis. *Cell* 1995;82:349–352
21. Tepper AD, de Vries E, van Blitterswijk WJ, Borst J. Ordering of ceramide formation, caspase activation and mitochondrial changes. *J Clin Invest* 1999;103:971–978

22. van Henert MJ, Steensma HY, van Heusden GPH. 14-3-3 proteins: regulators of cell division, signalling, apoptosis. *Bioessays* 2001;23:936–946
23. Marzo I, Brenner C, Zamzani N et al. Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis. *Science* 1998;281:2027–2031
24. Armstrong JS. The role of the mitochondrial permeability transition in cell death. *Mitochondrion* 2006;6:225–234
25. Hengartner NO. The biochemistry of apoptosis. *Nature* 2000;407:770–776
26. Yang J, Liu X, Bhalla K et al. Prevention of apoptosis by Bcl2: release of cytochrome C from mitochondria is blocked. *Science* 1997;275:1129–1132
27. Lucken-Ardjomande S, Martinou J-C. Regulation of Bcl-2 proteins and of the permeability of the outer mitochondrial membrane. *C R Biol* 2005;328:616–631
28. Zhuang S, Schnellmann RG. A death-promoting role for extracellular signal-regulated kinase. *J Pharmacol Exp Ther* 2006;319:991–997
29. Woods DB, Vousden KH. Regulation p53 function. *Exp Cell Res* 2001;264:56–66
30. Hiromura K, Pippin JW, Fero ML et al. Modulation of apoptosis by the cyclin dependent kinase inhibitor p27. *J Clin Invest* 1999;103:597–604
31. Hingorani R, Bi BY, Das T et al. CD95/Fas signaling in T lymphocytes induces cell cycle control protein p21 which promotes apoptosis. *J Immunol* 2000;164:4032–4036
32. Stupoch DG, Cheresh DA. A Bit role for integrins in apoptosis. *Nat Cell Biol* 2004;6:388
33. Daniel S, Aruelo MB, Patel VI et al. A20 protects endothelial cells from TNF α , Fas and natural killer mediated cell death by inhibiting caspase 8 activation. *Blood* 2004;104:2376–2384
34. Dzhagalov I, St John A, He Y-W. The anti-apoptotic protein Mcl-1 is essential for survival of neutrophils but not macrophages. *Blood* 2007;109:1620–1626
35. Beere HM. Death versus survival: functional interaction between the apoptotic and stress-inducible heat shock protein pathways. *J Clin Invest* 2005;115:2633–2639
36. Hammerman PS, Fox CJ, Thompson CB. Beginnings of a signal transduction pathway for bioenergetic control of cell survival. *Trends Biochem Sci* 2004;29:586–592
37. Konda H, Miura M. Regulatory roles of JNK in programmed cell death. *J Biochem* 2004;136:1–6
38. Luo J-L, Kamata H, Karin M. The anti-death machinery in IKK/NF κ B signaling. *J Clin Immunol* 2005;25:541–549
39. Papa S, Bubici C, Phan CG et al. NF-kappaB meets ROS. *Cell Death Differ* 2005;12:1259–1262
40. Kanayama A, Miyamoto Y. Apoptosis triggered by phagocyte related oxidative stress through FLIP downregulation and JNK activation. *J Leukoc Biol* 2007;82:1344–1352
41. Eisenberg-Lerner A, Kimeli A. DAP kinase regulates JNK signaling by binding and activating protein kinase D under oxidative stress. *Cell Death Differ* 2007;14:1908–1915
42. Davis RJ. Signal transduction by the JNK MAPkinases. *Cell* 2000;103:239–252
43. Weston CR, Davis RJ. The JNK signal transduction pathway. *Curr Opin Cell Biol* 2007;19:142–149
44. Xu C, Bailly-Maitre B, Reed JC. Endoplasmic reticulum stress: cell life and death decisions. *J Clin Invest* 2005;115:2656–2664
45. Mariniak SJ, Ren S. Endoplasmic reticulum stress in disease. *Physiol Rev* 2006;86:1133–1149
46. Matinon F, Tschopp J. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell* 2004;117:561–574
47. Drenth JPH, van der Meer JWM. The inflammasome—a linebacker of innate defense. *N Engl J Med* 2006;355:730–732
48. He L, Wu X, Meylon F et al. Monitoring caspase activity in living cells using fluorescent proteins and flow cytometry. *Am J Pathol* 2004;164:1901–1913
49. Scheel-Toellner D, Wang K, Craddock R et al. Reactive oxygen species limit neutrophil life span by activating death receptor signaling. *Blood* 2004;104:2557–2564
50. Kim S, Elkon KB, Ma X. Transcriptional suppression of IL-12 gene expression following phagocytosis of apoptotic cells. *Immunity* 2004;21:643–653
51. Shibata T, Nagata K, Kubayashi Y. A critical role of NO in preventing inflammation upon apoptotic cell clearance. *J Immunol* 2007;179:3407–3411
52. Zheng L, He M, Long M et al. Pathogen induced apoptotic neutrophils express heat shock proteins and elicit activation of human macrophages. *J Immunol* 2004;173:6319–6326.

53. Chang M-K, Binder CJ, Miller YI, et al. Apoptotic cells with oxidised specific epitopes are immunogenic and proinflammatory. *J Exp Med* 2004;200:1359–1370
54. Xu W, Roos A, Schlagwein N et al. IL-10 producing macrophages preferentially clear apoptotic cells. *Blood* 2006;107:4930–4937
55. Johann AM, van Knethen A, Lindemann D, Brune B. Recognition of apoptotic cells by macrophages attenuates the oxidation burst. *Cell Death Differ* 2006;13:1533–1540
56. Mayadas TN, Cullere X. Neutrophil b2 integrins: moderators of life or death decisions. *Trends Immunol* 2005;26:388–395
57. Kotone-Miyahara Y, Yamashita K, Lee K-K et al. Short term delay of Fas-stimulated apoptosis by GM-CSF as a result of temporary suppression of FADD recruitment in neutrophils. *J Leukoc Biol* 2004;76:1047–1056
58. Bruno A, Conus S, Schmid I, Simon H-U. Apoptotic pathways are inhibited by leptin receptor activation in neutrophils. *J Immunol* 2005;174:8090–8096
59. Villunger A, O'Reilly LA, Holler N et al. Fas-ligand, bcl-2, G-CSF and p38 MAPK Regulators of distinct cell death and survival pathways in granulocytes. *J Exp Med* 2000;192:747–658
60. Dragon S, Saffer AS, Shan L. IL-17 attenuates the antiapoptotic effects of GM-CSF in neutrophils. *Mol Immun* 2008;45:160–168
61. Jonsson H, Allen P, Peng SL. Inflammatory arthritis requires Foxo3a to prevent Fas-ligand induced neutrophil apoptosis. *Nat Med* 2005;11:666–671
62. Walmesly SR, Cadwallader KA, Chilvers ER. The role of HIF-1a in myeloid cell inflammation. *Trends Immunol* 2005;26:434–438
63. Gardai S, Whitlock BB, Helgasen C. Activation of SHIP by NADPH oxidase stimulated Lyn leads to enhanced apoptosis in neutrophils. *J Biol Chem* 2002;277:5236–5246
64. Daigle D, Yousefi S, Colonna M et al. Death receptors bind SHP-1 and block cytokine induced antiapoptotic signaling in neutrophils. *Nat Med* 2002;8:61–67
65. Marshall JE, Stamler JS. Nitrosative stress induced apoptosis through inhibition of NFkB. *J Biol Chem* 2002;277:34223–34228
66. Salamane G, Trevoni S, Martinez D et al. Analysis of mechanisms involved in stimulation of neutrophil apoptosis by TNFa. *Immunology* 2004;113:355–362
67. Castro-Alcaraz S, Miskoloci V, Kalaspudi B et al. NFkB regulation in human neutrophils by nuclear IkB: correlation to apoptosis. *J Immunol* 2002;169:3947–3953
68. Deveraux QL, Reed JC. IAP family proteins: suppressors of apoptosis. *Genes Dev* 1999;13:239–252
69. Stennicke H, Ryan CA, Salveson GS. The molecular basis of caspase inhibition. *Trends Biochem Sci* 2002;37:94–98
70. Strasser A, Pellegrini M. T lymphocyte death. *Trend Immun* 2004;25:610–615
71. Gendron S, Couture J, Aoudjit F. Integrin a2b1 inhibits Fas mediated apoptosis in T lymphocytes. *J Biol Chem* 2003;278:48633–48643
72. Hou W-S, van Parijs L. A Bcl-2 dependent molecular timer regulates the lifespan and immunogenicity of dendritic cells. *Nat Immunol* 2004;5:583–589
73. Park JM, Greten FR, Wang A et al. Signaling pathways and genes that inhibit pathogen induced macrophage apoptosis. *Immunity* 2005;23:319–329
74. MacKay F, Kalled SL. TNFa ligands and receptors. *Curr Opin Immunol* 2002;14:783–790
75. Gotoh T, Mori M. Nitric oxide and endoplasmic reticulum stress. *Arterioscler Thromb Vasc Biol* 2006;26:1439–1446
76. Endo M, Mori M, Akira S, Gotoh T. CHOP is crucial for induction of caspase 11 and the pathogenesis of LPS induced inflammation. *J Immunol* 2006;176:6245–6253
77. Stennicke RH, Ryan CA, Salveson GS. The molecular basis of caspase inhibition. *Trends Biochem Sci* 2002;27:94–98
78. Scaffidi S, Fulda S, Srinivasan A. Two CD95(Fas) signalling pathways. *EMBO J* 1998;17:1675–1678
79. Chan FK-M, Siegel RM, Lenardo MJ. Signaling by TNF receptor family. *Cell* 2000;13:419–422

80. a. Chang DW. cFLIP is a dual function regulator for caspase 8 activation and CD95 mediated apoptosis. *EMBO J* 2002;21:3704–3714
80. b. Wang L, Azad N, Kongkaneramt L et al. The Fas Death Signaling pathway connecting ROS generation and FLICE: inhibitory protein down regulation. *J Immunol* 2008;180:3072–3080
81. Frelin C, Imbert V, Bottero V et al. Inhibition of NFkB survival via caspase cleavage of IKK scaffold protein and NFkB modulator Nemo. *Cell Death Differ* 2008;15:152–160
82. Salomi P, Khelifi AF. Daxx: death or survival protein. *Trends Cell Biol* 2006;16:97–104
83. Zheng L, Bidere N, Staudt D et al. Competitive control of independent programs of TNF receptor-induced cell death by TRADD and RIP1. *Mol Cell Biol* 2006;26:3505–3510
84. Xia P, Wang L, Moretta PAE et al. Sphingosine kinase interacts with Traf and dissects TNFa signaling. *J Biol Chem* 2002;277:7996–8003
85. Ahn KS, Sethi G, Aggarwal BB. Simvastatin potentiates TNF induced apoptosis through down-regulation of NF-kB. *J Immunol* 2007;178:2507–2516
86. Griffith TS, Kazana H, van Oosten RL et al. Apoptotic cells induce tolerance by generating CD8 T cells that produce Trail. *J Immunol* 2007;178:2679–2687
87. Sianov RV, Haupt Y. The cellular response to p53: decision between life and death. *Oncogene* 1999;18:6145–6157
88. Muppidi JR, Tschopp J, Siegel RM. Life and death decisions. *Immunity* 2004;21:461–465
89. Miyanishi M. Identification of Tim4 as a phosphatidylserine receptor. *Nature* 2007;450:435–439
90. Park D, Tosello-Trampant A-C, Elliott MR et al. BAI-1 is an engulfment receptor for apoptotic cells upstream of the Elmo/Dock 180/Rac module. *Nature* 2007;450:30–34
91. Bartek J, Lukas J. Mammalian G1 and S-phase checkpoints. *Curr Opin Cell Biol* 2001;13:738–747
92. Levitt NC, Hickson ID. Caretaker tumour suppressor genes that defend genome integrity. *Trends Mol Med* 2002;8:179–186
93. Vogelstein B, Lane D, Levine AJ. Surfing p53 network. *Nature* 2000;408:307–311
94. Zheng H, You H, Zhou XZ et al. The prolyl isomerase Pim.1 is a regulator of p53 in the genotoxic response. *Nature* 2002;419:849–885
95. Ito K, Takubo K, Arai F et al. Regulation of ROS by ATM is essential for proper response to DNA double-stranded breaks in lymphocytes. *J Immunol* 2007;178:103–110
96. Pearce AK, Humphrey TC. Integrating stress response and cell cycle checkpoint pathways. *Trends Cell Biol* 2001;11:426–432
97. Downs AJ, Jackson SP. Protective packaging for DNA. *Nature* 2003;424:732–734
98. Kastan MB. A bid for the pathway. *Nature* 2005;437:1103
99. Boutros R, Dozier C, Ducommun B. The when and wheres of cdc25 phosphatases. *Curr Opin Cell Biol* 2006;18:185–191
100. Karlsson-Rosenthal C, Millar JBA. cdc25: mechanisms of checkpoint inhibition and recovery. *Trends Cell Biol* 2006;16:285–292
101. Rixh T, Allen RL, Wyllie AH. Defying death after DNA damage. *Nature* 2000;408:433–439
102. Zhou B-B, Elledge SJ. The DNA damage response. *Nature* 2000;408:433–439
103. Massague J. G1 cell-cycle control and cancer. *Nature* 2004;432:298–306
104. Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. *Nature* 2004;432:316–323
105. Scully RS, Chen J, Plug A et al. Association BRCA1 with Rad51. *Cell* 1997;88:265–275
106. Li S, Ting NSY, Zhang L et al. Functional link of BRCA1 and ataxia telangiectasia gene product in DNA damage response. *Nature* 2000;406:210–215
107. Harkin DP, Bean JM, Miklos D et al. Induction of GADD45 and JNK/SAPK dependent apoptosis following inducible expression of BRCA1. *Cell* 1999;97:575–586
108. Zein M, Traves PG, Lopez-Fontal R et al. Specific contribution of p19ARF to nitric oxide dependent apoptosis. *J Immunol* 2006;177:3327–3336
109. Le Cam L, Linares LK, Paul C et al. E4F1 is an atypical ubiquitin ligase that modulates p53 effector functions. *Cell* 2006;127:775–788

110. Brenner C, Le Bras M, Kroemer G. Insights into mitochondrial signaling pathway and apoptosis. *J Clin Immunol* 2003;23:73–80
111. Chipuk JE, Bouchier-Hayes L. Puma and proapoptotic p53. *Science* 2005;309:1732–1735
112. Yin Y, Liu Y-X, Jin YJ et al. PAC.1 phosphatase is a transcription target of p53 in signalling apoptosis and growth suppression. *Nature* 2003;422:527–531
113. Guimaraes DP, Hainaut P. TP53: a key gene in human cancer. *Biochimie* 2002;84:83–93
114. Smith JS. Human Sir2 and silencing of p53 activity. *Trends Cell Biol.* 2001;12:404–406
115. a. Jones RG, Thompson CB. Signal transduction fuels T cell activation. *Immunity* 2007; 27:173–178
- 115b. Joerger AC, Fersht AR. Structural biology of the tumor suppressor p53. *Ann Rev Biochem* 2008;77:557–582
116. Ravera-Shapira N. miRNA-34a and p53 apoptosis. *Mol Cell* 2007;26:731–743
117. Beachy PA, Karhadkar SS, Berman DM. Tissue repair and stem cell renewal in carcinogenesis. *Nature* 2004;432:324–331
118. Lowe SW, Cepero E, Evan G. Intrinsic tumour suppression. *Nature* 2004;432:307–315
119. van Dyke T. p53 and tumor suppression. *N Engl J Med* 2007;356:79–81
120. Zindy F, Williams RT, Baudino T. ARF tumor suppressor promoter monitors latent oncogenic signals in vivo. *Proc Natl Acad Sci U S A* 2003;100:15930–15935.

Chapter 9

Control of the Cytoskeleton

Abstract How the GTPases control the cytoskeleton and thus cell migration or phagocytosis is a fascinating topic that engages some cell biologists fully. Immunologists need to know how immune defence cells discharge mediator granules and how cells adhere to endothelium or to extracellular matrix. The beneficial statin drugs curb the activities of monocyte macrophages by affecting GTPases.

9.1 The LowMolecular Weight Monomeric GTPases

In Fig. 5.1, there is a depiction of the working of a low molecular weight guanosine triphosphatase (GTPase). The GTPases are covalently attached to membrane phospholipids [1], and these monomeric G proteins include Rho GTPases like Rac, Ras cdc42, and the adenosine triphosphate (ADP) ribosylation factors (ARFs). The diversity of their actions is apparent in Fig. 9.1. Like Ras (Fig. 5.1), they are active when guanosine triphosphate (GTP) is bound. Then, their intrinsic GTPase activity hydrolyses GTP to GDP, so they return to the inactive state. GTPase-activating proteins (GAPs) enhance the GTPase activity [2]. The p85 of PI3 kinase (PI3K) counts as one of these.

Recruitment of the stimulating guanine-nucleotide exchange factors (GEFs) is controlled by receptor phosphorylations or binding to membrane phospholipids [3–5]. Study of mice with GEF mutants has laid groundwork for understanding the differential activation of surface receptors. The GEFs allow diverse upstream signals to culminate in downstream cellular events that are equally diverse and specific. For example, interaction of GEFs with scaffold proteins selects downstream targets for the various Rho GTPases. There are also the individual GAPs, which contain unique domains that mediate downstream effects. The GEF Tiam that affects Rac GTPase can fire MLK3 and MKK6 attached to scaffolds JIP2 or JIP1, and thereby p38 mitogen-activated protein kinase (MAPK) or Jun N-terminal kinase (JNK), respectively (cf. Fig. 4.4) [6].

The small GTPases of white cells control movement and phagocytosis (Fig. 9.1). In neutrophils and macrophages, the small GTPases help the formation of superoxide

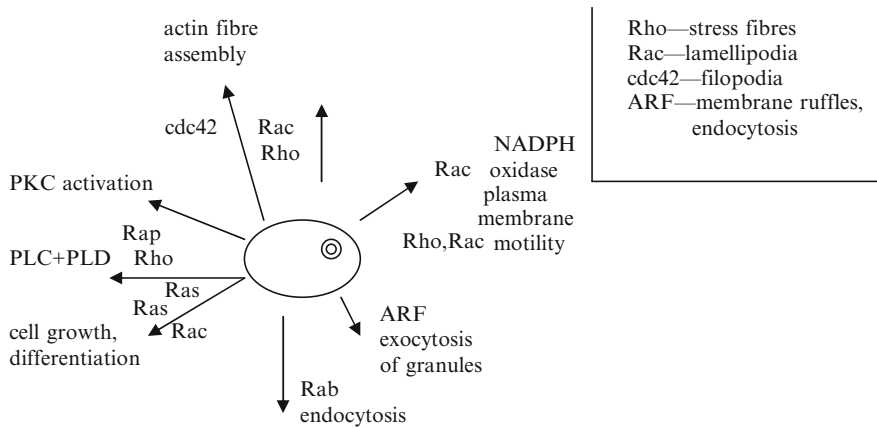


Fig. 9.1 Low molecular weight guanosine triphosphatases (GTPases) and phagocyte activation

anions that aid bacterial killing, that is, Rac1 and Rac2 translocate to the cell membrane simultaneously with, but independently of, translocation of p67/p47^{phox} (Fig. 10.2). ARF (ADP ribosylation factor) or Rho stimulate the phospholipase D pathway [7–9], and the phosphatidic acid (PtdOH) that is formed stimulates NADPH oxidase, so that reactive oxygen species (ROS) are produced. Rap mediates signals that regulate surface integrins from inside the cell. Rap is also implicated in cadherin-mediated cell junction formation.

The ARFs were identified as GAPs. ARF proteins regulate both phosphatidylinositol phosphate kinases (PIPKs) and phospholipase D. ARFs and other G proteins are implicated in vesicle-mediated protein trafficking in cells [7–9]. ARFs mediate some of the functions of Rac. ARFs affect the sorting of membrane proteins and actin filament formation when membrane protrusions are forming (Fig. 9.1). ARFs induce PIP5 kinase (Fig. 2.4), so that PIP2 is formed, and phospholipase D is operative [9].

9.2 Rho GTPases

The Rho GTPase regulators determine cell migration, formation of focal adhesion contacts and stress fibres, membrane blebbing and endocytosis/exocytosis and even glucose transport and functioning of ion channels [10]. Rho activates genes via extracellular signal-related kinase (ERK), but not JNK or p38, and it can activate nuclear factor kappa B (NF- κ B). In macrophages, Rho and Rac are essential for macrophage colony-stimulating factor (M-CSF)-induced migration, whereas *cdc42* is utilised for sensing of a chemical gradient. In leucocytes, RhoA and *cdc42* regulate the location of the control enzyme PTEN (Fig. 2.6) [11].

Some signalling cascades are extremely complex; thus, they have been difficult to elucidate. Indeed, networks are often involved. Figure 9.2 indicates that PI3K

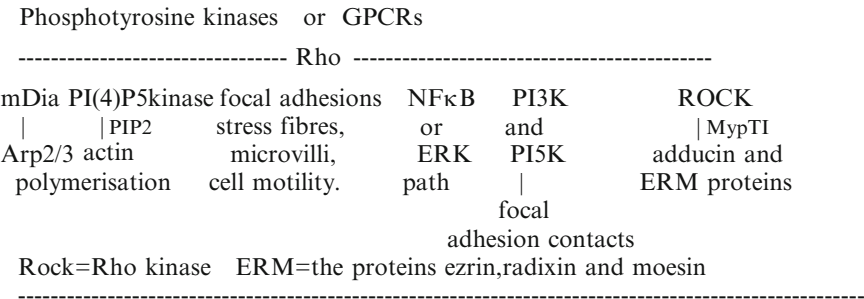


Fig. 9.2 Targets of Rho guanosine triphosphatase (GTPase)

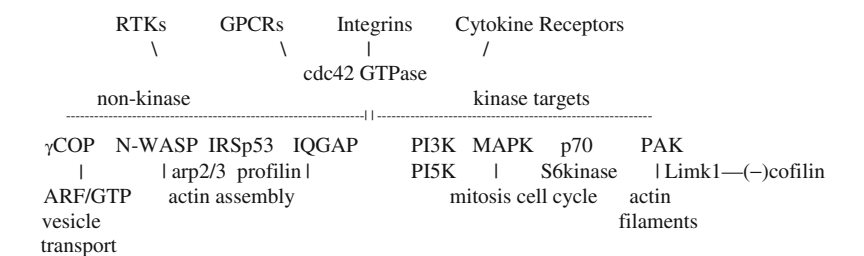


Fig. 9.3 Targets for Cdc42 (Rho family guanosine triphosphatase [GTPase]). IQGAP has been reviewed [19] and cell stage change induced by cdc42 [20]

aids the formation of focal adhesion contacts. Integrin-mediated adhesion activates Src tyrosine kinases, which in turn activate PI3K. PI3K can then activate the Vav GEF; thus, small GTPase Rho [12] induces membrane ruffles. Rho also activates PI(4)P5K. Its product PtdIns(4,5)P2 regulates interaction of the actin-binding proteins like α-actinin or gelsolin.

Rho GTPases regulate aspects of cytoskeletal organization, cell adhesion to the substratum, cell polarity and transcriptional activation. Ras, Rac and Rho determine actin microfilament-dependent processes [13–15]. Indeed, as indicated, the small GTPase RhoA activates ERM (ezrin, radixin, moesin) proteins that are concerned with cortical cytoskeleton remodelling, including cell–cell boundaries and membrane ruffles. Cdc42 regulates proteins that determine cell motility (Fig. 9.3). In in vitro studies of fibroblasts, cdc42 activation leads to formation of filopodia, Rac causes formation of lamellipodia and membrane ruffling and Rho induces stress fibres [13], for Rac-GTP activates PAK (p21-activated kinase), and then guanylate cyclase will form cGMP. Furthermore, Rho via ROK ROCK kinase stimulates myosin light-chain kinase (MLCK) and myosin phosphatase. Interestingly, in macrophages of mouse models of autoimmunity there is abnormal regulation of Rho GTPase; thus, those macrophages show increased adhesiveness and greater spreading [16].

When integrins connect to the extracellular matrix (Fig. 10.8), Rac1 is activated and thereby MEKK1 → MKK4/7→JNK1/2/3→c-Jun, supporting cell growth and differentiation.

Obviously, the organization of actin skeletal dynamics is very important in macrophages, and that is actually coordinated by sphingosine kinase 1 [17]. Cdc42 activates MRCK α/β kinases and so formation of filopodia. Cdc42 activates WASp protein (Wiskott-Aldrich syndrome protein) in haemopoietic cells, and WASp also is implicated in actin polymerization and formation of filopodia. WASp was characterized in neutrophils [18] as a cdc42-selective target concerned with induction of Arp2/3 and thus initiation of actin polymerization (Fig. 9.5).

In Fig. 9.3, note PAK [21], which helps to direct cytoskeletal reorganization and cell motility. PAK links the small GTPases like cdc42 and Rac to MLCK and to the MAPKs (Fig. 4.3). Adherence of leucocytes to the extracellular matrix [22] has crucial effects for their cytoskeleton (Fig. 9.3). For example, the serine-threonine kinase PAK is stimulated when fibroblasts are adherent but not when they are in suspension. In macrophages, cdc42 directs and stabilizes Ras/Rac activity at the forefront. Cdc42 regulates the orientation of the microtubule organizing centre. In T lymphocytes, cdc42 is active in the immunological synapse (IS), where there is contact with the antigen-presenting cell.

Figure 9.3 shows in addition that cdc42 activates the γ -subunit of coatamer protein complex (COP), which will bud and form vesicles that will be used for the transport of proteins [23]. Note how ARF controls their assembly.

9.3 Actin Bundles in Neutrophils

The nucleation factor proteins (NFPs) include WASP and WAVE (WASP family verprolin homologous protein (class I) and cortactin (class II). WASP (Fig. 9.5) is activated by GTP using WASP interacting protein (WIP) when stimulated by PtdIns(4,5)P₂ together with cdc42 or Rac small GTPases. Either WASP or Wave/Scar activate the Arp2/3 complex, so that there will be actin polymerisation in neutrophils [18, 24, 25]. Arp2 and Arp3 use ATP to effect actin filament y-branching. In T lymphocytes, WASP and Scar/Wave, by their effects on actin, regulate the form of the immunological synapse (Fig. 9.2) [26].

Turnover of F-actin occurs by depolymerisation of the pointed ends of fibrils and barbed-end polymerisation. Barbed-end growth is effected by ATP-G-actin and profilin-actin in various arrays. There are new names involved in actin reorganization (Table 9.1).

Table 9.1 PIP2-binding proteins associated with actin reorganization

For actin polymerization:	
α -Actinin	Links F-actin to integrins or bundles of actin
Profilin	Addition of actin monomers to filaments
For actin depolymerization	
Cofilin	Severing or depolymerisation of actin filaments
Gelsolin	Caps the barbed ends of actin filaments
WASp, N-WASp, or Scar/Wave recruit Arp2 plus Arp3, which activate actin filaments	
<i>N-WASP</i> neural WASP, <i>WASP</i> Wiskott-Aldrich syndrome protein, <i>Wave</i> WASP family verprolin homologous protein	

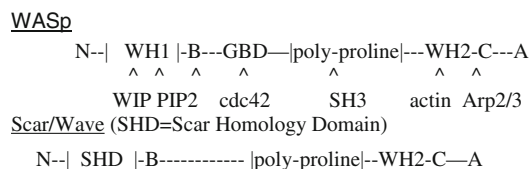


Fig. 9.4 The structure of Wiskott-Aldrich syndrome protein (WASP) and Scar/Wave. The c-terminal VCA binds actin and Arp2/3[28]

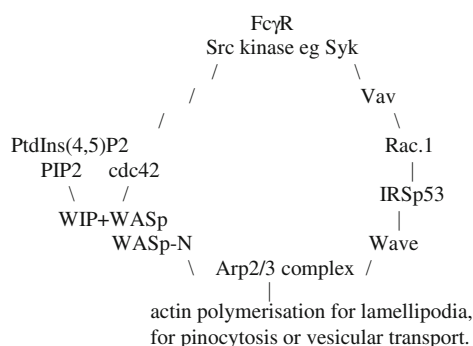


Fig. 9.5 WASp and WAVE proteins from scaffolds of multiprotein complexes.

N-Wasp[18] has regions that bind cdc42, or Rac GTPase activated by Vav and PIP2, and it attaches Arp2/3 ATPase protein which forms actin filaments from actin monomer [28] and so lamellipodia. N-Wasp (with Abi1) regulates actin-based vesicular transport [29]. So does Scar/Wave. Wave=WASp family verprotein homologous protein. Wave (with Abi1) mediates Rac-dependent membrane protrusion and macropinocytosis [29]. Similarly cortactin is activated by RacGTPase to induce formation of lamellipodia [30]. As in other situations, phosphatase SHP-1 regulates tethering of receptors to the actin cytoskeleton by its ability to modulate the phosphorylation of β -actinin [31]. WIP = WASp interacting protein, as in the adhesion and spreading of fibroblasts [32] Activation of Wiskott-Aldrich syndrome protein (WASP) Arp2/3 and lamellipodia formation. WASp and WASp family verprolin homologous protein (WAVE) proteins form scaffolds of multiprotein complexes

Neural Wasp (N-Wasp) has an extra copy of the G-actin-binding sequence WH2 at its C-terminus VCA domain (Fig. 9.4). N-Wasp recruits tyrosine phosphorylated proteins into phagocytic cups [24]. N-Wasp effects endocytosis of surface receptors and actin-mediated transport of vesicles. In essence, N-Wasp gathers Rac GTPase and directs the Arp2/3 complex to assemble actin filaments in association with profiling [25, 26]. Arp2/3 interacts with barbed (+) ends to initiate barbed (−) end actin fibre elongation or branching. The combination of filament anchoring by Arp2/3 branching activity and monomer addition to newly nucleated filaments provides mechanical force to push the membrane into protrusions.

Cofilin is activated by dephosphorylation. Cofilin phosphorylation is regulated by Slingshot (Ssh). The interest in cofilin is that it regulates the production of actin monomers; thus, it helps to determine formation of lamellipodia [27]. Removal of cofilin allows actin growth (Fig. 9.5).

N-Wasp [18] has regions that bind cdc42, or Rac GTPase activated by Vav and PIP2, and it attaches Arp2/3 adenosine triphosphatase (ATPase) protein, which forms actin filaments from actin monomer [28] and so lamellipodia. N-Wasp (with Abi1) regulates actin-based vesicular transport [29], as does Scar/Wave. -Wave (with Abi1) mediates Rac-dependent membrane protrusion and macropinocytosis [29]. Similarly, cortactin is activated by Rac GTPase to induce formation of lamellipodia [30]. As in other situations, phosphatase SHP-1 regulates tethering of receptors to the actin cytoskeleton by its ability to modulate the phosphorylation of α -actinin [31]. WIP mediates the adhesion and spreading of fibroblasts [32].

In the resting state, actin filaments are stable, and they have their growing ends (+) covered by capping protein CapG. Vav activation of cdc42 or Rac GTPase activates Wasp/N-Wasp, which then severs actin filaments. Hence, the uncapped ends are exposed. Polymerisation of globular G-actin leads to formation of filamentous F-actin, two molecules of which coil around one another. Actin polymerisation occurs at the barbed (+) ends of filaments, whilst depolymerisation happens at the pointed (−) end [33, 34]. Wasp is in an autoinhibited state, but when cdc42 binds to it, the active c-terminal VCA is exposed.

Alternatively, receptor stimulation activates Rac, and Wave1 is liberated from an inactive complex of proteins [35]. As N-Wasp assembles the required GTPases, substrate PIP2 and protein Arp2/3, it promotes polymerisation of actin in its

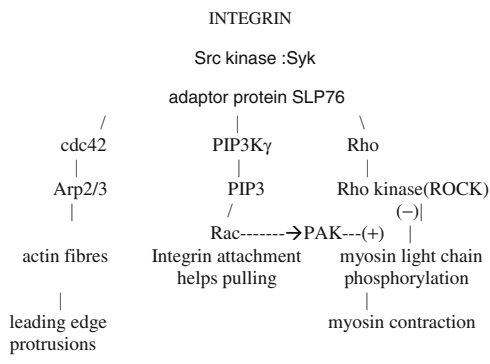


Fig. 9.6 Neutrophil migration. At the leading edge of moving neutrophils are C5a receptors. The forward membrane contains PI(4,5)P2 and Rac and cdc42 (GTPases) [43] and underneath in the anterior cortex is p21-activated kinase (PAK) and F-actin filaments. Posteriorly in the cortex, there is myosin and PAK acting as a motor. Rac, Ras and PI3 kinase form feedback loops leading to robust production of PIP3 [49]. Its production is also helped by an ARF-GAP called GIT2, which promotes neutrophil chemotaxis but stops formation of superoxide anions [50]. There is RhoA in the cell body and some in the protrusions. There is actually a reciprocal balance between Rac and RhoA (Fig. 9.7) [51] since Rac protrusive activity is suppressed by Rho [52]. PtdOH (phosphatidic acid) or the Rho GTPases also induce PI-phosphate kinase, which generates PtdIns(4,5)P2, which plays a major role in cell migration[53].

submembranous position, forming a lamellipodium [35] or a dendrite [36]. Alternatively, Rac activation of Arp2/3 proteins increases the amount of G-actin available for filament polymerization at the leading edge of cells (Fig. 9.5 and 9.6) [35, 36]. The molecule profilin accelerates exchange of ATP for ADP on G-actin monomers and sustains high-affinity ADP-Pi G-actin units at the barbed (+) ends.

In the meantime, Ras activation of PAK leads to LIM (actin binding) kinase activation and so inhibition of the G-actin-binding protein cofilin, allowing actin filament growth. To achieve membrane *ruffling*, activated Rac stimulates IRSp53 (of the insulin receptor family), and that activates Wave [37], which like Wasp binds Arp2/3, activating it (Fig. 9.5). Wave activates nucleation of actin filaments for membrane protrusions [38]. VASP (vasodilator-stimulated phosphoprotein) is another Arp2/3-binding protein that recruits G-actin. Naturally, Arp2/3 mediates actin polymerisation during platelet shape change [39]. The rate-limiting step is assembly of three actin monomers into a trimer. One site on Arp2/3 binds actin filaments and another binds the ends of actin monomers.

Recent attention has been focussed on WIP, which aids Wasp stability and function. In T cells, WIP helps convey Wasp to areas of actin assembly after antigen receptor stimulation. In macrophages, WIP/Wasp help regulate the formation of phagocytic cups.

9.4 Cell Migration

Cell motility derives from localized actin polymerization at the leading edge of cells. Since the pool of actin is limited, the polymerization/depolymerization must be under dynamic control. Essentially, there are five distinct steps:

1. Extension of the leading edge of the cell.
2. Integrin adhesion to contact points on the matrix. This means that paxillin and vinculin (Fig. 9.11) enable “focal contacts” with the extracellular matrix to connect internally with actin stress fibres.
3. Contraction of the cytoplasm by organised actin filaments.

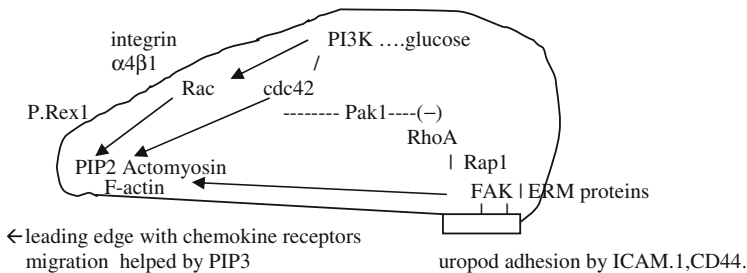


Fig. 9.7 Rap1 mediates surface adhesions [40] via integrins $\alpha4\beta1$. When Rac is operating, RhoA is being inhibited [51]. Rac activates PAK. Akt-2 is a negative regulator of PAK, and so restrains migration. FAK is for formation/diassembly of actin cytoskeletal networks.

P.Rec 1:3 or GEF to Rac

For details on migration machinery consult ref [57]

4. Release of the rear contact sites (Fig. 9.7).
5. Finally, recycling of membrane integrins from the rear to the front using Rab GTPase-mediated endosomal and membrane trafficking.

Activated integrins at the front edge of a cell enable adhesion, so that it can be pulled forwards. Rap1 GTPase within the cell mediates integrin-mediated adhesion [40]. Calpain-mediated proteolysis of talin regulates the adhesion process [41]. Rho GTPases generate the forces required for forward movement of migrating cells [42]. Rho regulates the submembranous ERM proteins, whilst Rac and cdc42 have a supporting role [43]. Thus, PtdIns(3,4,5)P₃ acting on Rac GTPases will induce polymers of actin at the leading edge. PIP₃ binding to the membrane and proteins helps the formation of lamellipodia at the leading edge of the cell, whilst cdc42-stimulated filopodia sense chemotactic gradients and enable directed movement [44, 45]. The Rac GTPases influence PAK, which directs the state of MLCK [46] and thereby affects stress fibres and the formation of focal adhesions [47].

β -Arrestins (Fig. 1.2) have turned out to be important general regulators of chemotaxis [48] (Fig. 9.6).

The way in which Rho, acting via Rho kinase (Rock), leads to the suppression of Rac protrusive activity is a complicated arrangement (Fig. 9.7) [52]. In cells like lymphocytes, Rock phosphorylates the ERM proteins and helps cell polarization and formation of a uropod. The ERM proteins connect surface adhesion molecules with cortical F-actin. Yet, Rock suppresses protrusion in various other cell types. Rock phosphorylates FilGAP (filamin) to control actin remodelling. Rho $\rightarrow$ Rock $\rightarrow$ FilGAP inhibits Rac activity. Hence, FilGAP stops formation of lamellae [52]. Even so, Rock phosphorylates and activates myosin II, motivating cell contractility. In fact, Rac1 at the leading edge is able to promote Rho GTPase and thus myosin contraction at the trailing edge (Fig. 9.7) [54].

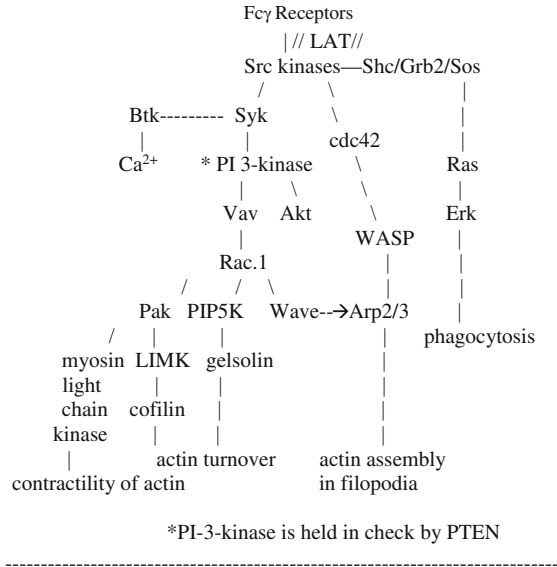
When one looks at the means of propulsion of T lymphocytes, the leading edge chemokine receptors connect with CasL, which in turn activates Rap1 so that there is integrin-induced adhesion. The phosphorylated CasL is monitored by Chat-H, signal transducer, which facilitates the process [55]. T cell chemotaxis is driven by phospholipase C β , a molecule that is not used in neutrophils. Chemokine receptors also orchestrate F-actin rearrangements through a dedicator of cytokinesis 2 (DOCK2)-Rac-dependent path linked to PI3K drive [56]. Recently it was noted that the PIIOY isoform of PI3k requests movement of T cells into inflammatory sites [56b].

As Fig. 9.7 shows, adhesion receptors at the rear of the cell trigger local activation of ERM proteins, which operate myosin light chain (MLC) phosphorylation through a RhoA-Rock pathway.

9.5 The Mechanics of Phagocytosis

Clustering of adhesion receptors at the phagocytic cup generates a signal that leads to the local polymerization of actin microfilaments, and thus an encircled bacterium will be endocytosed into a phagosome, which has to mature into a

Fig. 9.8 The mechanics of phagocytosis



degradative and microbicidal vacuole. This all arises from the negativity of the inner aspect of the cell membrane being an anchor for key signalling and effector molecules, such as various Rho-GTPases, and control by phosphoinositide molecules and sphingosine-1-phosphate [58]. Rho activates protein kinase C (PKC), and RhoA is implicated in early F-actin recruitment at the site of phagocytosis. Cdc42 regulates the extension of membrane over the edges of engulfed particles. Rac1 with PI3K helps membrane fusion and closure of the phagocytic cup [59a] in response to β 1-integrin engagement [59b]. Fc receptors are ubiquitinated and then can interact with the ESCRT (endosomal trafficking) complex to facilitate inward membrane budding, and events occur as shown in Fig. 9.8. WASP and WIP (wasp interacting protein) form a complex at the phagoextic cup [60].

9.6 Endocytosis

Akin to the situation in Fig. 5.5, the GTPase dynamin aids the endocytosis of clathrin-coated pits and formation of endosomes that contain effete receptors or particles [61, 62]. Monoubiquitination or multiubiquitination is used for sorting of membrane proteins into the interior of endosomes. The dynamin uses GTP to polymerise and form rings or spirals around the neck of the pits [63]. Recruitment of clathrin-coated proteins is helped by PtdIns(4,5)P₂, produced from PtdOH when ARF6 GTPase stimulates phospholipase D. ARF is regulated by SMAP, which is a GAP. As clathrin is invaginated, it binds with clathrin adaptor protein AP-2, and accessory factors like epsin, which aids membrane curvature when PI(4,5)P₂ gives

the impetus [64, 65]. Ral-GTPases are relevant to endocytosis by driving filamin induced actin formation and by promoting Ant phosphorylation [65b].

Arp2/3 actin remodelling participates at various stages of endocytosis, which is helped by PDZ adaptor proteins. The small GTPase Rab5 regulates interaction of endosomes with the network of microtubules to regulate their movements in the cell [66]. Rab5 is itself controlled by a GAP called Rab5 GAP5, which limits the amount of activated Rab5. More detail is available [67, 68]. So, phosphoinositides help to form early and late endosomes, or lysosomes [61], using membrane fusions directed by various GTPases. Dissociation of Rab5 and association of Rab7 can mature early to late endosomes. Those endosomes are associated with actin microfilaments and with microtubules to ensure appropriate movements within the cells. The lumina of such vacuoles are influenced by their pH and by the effects of intraluminal oxygen and nitrogen radicals.

There has been a concentration of effort at understanding how enteric bacteria enter the gut mucosal cells and then move through. Salmonellae, shigellae and other bacteria have means of enlisting the Rho GTPases so that there is reorganization of the actin cytoskeleton [69, 70]. Listeria bacteria hijack the clathrin-dependent endocytic machinery [71]. When a streptococcus enters pharyngeal epithelial cells, its M1 protein adheres to $\alpha 5\beta 1$ -integrins; thus, there is activation of PI3K, so that phosphoinositides are produced, and there are actin fibre rearrangements [72]. Various bacteria have opacity protein molecules (OPAs), and those proteins interact with carcinoembryonic antigen adhesion molecules like CEACAM3 on neutrophils [73]. Accordingly, there is activation of Rac GTPase and formation of surface membrane extensions, leading to phagosome formation. Class I PI3K is essential for particle engulfment. One can detect PtdIns(3,4,5)P₃ in the phagocytic cups and PtdIns(3)P in sealed phagosomes. Within phagolysosomes, pathogens are destroyed by a combination of low pH, hydrolysis by enzymes and free-radical attack. The bacterial pathogen-associated molecular patterns (PAMP), which interact with Toll-like receptors (TLRs) on the surfaces of phagocytes, promote the TLRs via p38 MAPK to ensure phagosome maturation [74]. Other receptors that bind pathogens include triggering receptors expressed on myeloid cells (TREM), Siglecs and the C-type lectins like Dectin-1, BDCA and DCAL-2 [75]. Dectin-1 activates Syk tyrosine kinase so that there is (a) activation of CARD9 (adaptor transducer) and so NF- κ B, (b) the MAPKs and (c) activation of GTPases Rac1 and cdc42 and thus ingestion of pattern recognition receptors. Syk activity produces ROS. Some C-type lectins are ITAM coupled, such as the BDCA2 of plasmacytoid dendritic cells, and others are ITIM coupled, like DCAL-2 on dendritic cells, which via ERK and p38 engenders interleukin (IL) 10.

9.7 Exocytosis: Discharge of Vesicles

Neutrophils and macrophages defend against microbial invaders by discharging the content of their granules. There are general principles that determine vesicle transport and exocytosis. (a) A vesicle is formed at a donor membrane, (b) the vesicle is

targeted to an acceptor membrane, (c) the vesicle must dock at the acceptor membrane and (d) the vesicle fuses with that acceptor external membrane. The stages are controlled by Rab GDP/GTP cycle proteins, whose action integrates with the membrane association–dissociation process [76]. The triggering of exocytosis is by Ca^{2+} ions arriving via voltage-operated channels (VOCs) via ryanodine receptors or by the action of InsP_3 receptors. SNAP synaptogamin ATPases are involved in the fusion and their receptors are called SNAREs. A vesicle v-SNARE (of synaptobrevin, actually vesicle-associated membrane protein 2 [VAMP2]) creates a ternary trans-SNARE complex [77] with a t- (target membrane) SNARE (such as syntaxin and SNAP-25) (Fig. 9.9).

During *exocytosis* of neutrophils, VAMPs mediate the process; that is, the v-SNARE is VAMP1/2 protein attached to the granules, and the m-SNARE is syntaxin 4-SNAP23 attached beneath the cell membrane [78–80]. The propensity for exocytosis is related to the density of fusogenic protein VAMP2 and is high in secretory vesicles but lower in specific granules [81]. The phosphatase PTP-MEG2 (PTPN9) is localised to secretory vesicles. It dephosphorylates *N*-ethylmaleimide sensitive factor (NSF). Whereas specific and tertiary granules are discharged externally, azurophilic granules are moved into the phagosome, and that process occurs later than discharge of specific and gelatinase granules.

Lymphocytes use a similar process [82]. The Q- and R-SNARE components for cell surface fusion of secretory lysosomes of cytotoxic T cells have not been identified (Chap. 13), but there are regulatory proteins Munc13-4 and Lyst (lysosomal trafficking regulator) and Rab27a for CD8 and CD4 T cells. The T cell receptors (TCRs) are continually endocytosed and recycled to the plasma membrane. VAMP2/3 mediate

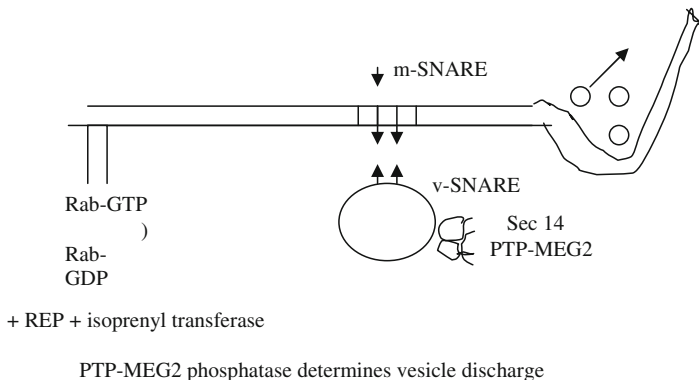


Fig. 9.9 Rab proteins as regulators of vesicle exocytosis. Protein kinase A stimulates synthesis of vesicle carriers, whose intracellular movement is controlled by Rab guanosine triphosphatases (GTPases). The membrane association of Rab proteins depends on hydrophobic modifications by REP (Rab escort protein) and an isoprenyl transferase. PTP-MEG2 phosphatase determines vesicle discharge. It dephosphorylates *N*-ethylmaleimide sensitive factor (NSF), which mediates vesicle fusion. Membrane anchor proteins [86, 87] enable vesicle discharge through the membrane. Nitric oxide (NO) donors inhibit exocytosis, like discharge of von Willebrand factor from endothelial cells

delivery of TCRs from recycling endosomes to the immunological synapse and the partners are syntaxin STX4 and SNAP23. *In mast cells*, the cholesterol-rich lipid raft domains regulate the interaction of Q-Snare proteins STX3/4 by separating them from regulatory Sec/Munc-like proteins in non-lipid-raft domains [77]. SNAP23 is phosphorylated on cell activation to become functional for degranulation [83]. Doc2 α colocalised with Munc 13-4 regulates granule exocytosis. *Platelets* have α -granules that contain coagulation factors, dense granules carrying serotonin and lysosomal granules. SNAP23 and syntaxin 2 are exposed on platelet surfaces. MARCKs, a platelet PKC substrate, is required for the secretion of dense granules. Regarding effector granule discharge, a v-SNARE (VAMP3) must interact with syntaxin 4 for α -granules and for dense granules. Various Rab GTPase molecules are implicated [84, 85].

Eosinophils are known for the toxicity of their granules. Regulated exocytosis occurs by means of a docking complex of SNAP receptors on the vesicles (i.e. VAMP2) and a target membrane t-SNARE (SNAP23 and syntaxin 4) on their plasma membrane [86].

Monocyte-macrophages do not contain granules, but a continuous stream of carrier vesicles (bearing VAMP3) transports cytokines for innate immune responses [87]. They can release large quantities of cytokine IL-1 β when the hormone is cleaved by IL-1 β -converting enzyme (ICE), which is the same as caspase 1. Caspases have to be controlled. Thus, the IL-1 β meets caspase 1 within secretory lysosomes, which undergo exocytosis (cf. Fig. 12.3). iPLA2 activates processing, whilst cPLA2 (cytosolic phospholipase A2) determines endosomal release [88]. When macrophages discharge tumour necrosis factor- α (TNF α), surface delivery from lipid rafts occurs by means of syntaxin 4 and SNAP23 [89].

9.7.1 Interleukin 8 Chemokine and Discharge of Neutrophil Granules

β -Arrestins have been described as agents that uncouple activated G protein-coupled receptors (GPCRs) and drive receptor internalization (Chap. 1). They have even more potential because, when IL-8 stimulates neutrophils using chemokine receptor CXCR1, the internalised β -arrestin associates with Src family tyrosine kinases Hck and c-Fgr, and that association is critical for release of granules [90].

9.8 Cell Adhesion Receptors and the Cytoskeleton

9.8.1 Signalling from Integrins

Adhesion of cells to extracellular matrix proteins is provided by the Integrin molecules that form sites for focal adhesions and contacts. Their α/β heterodimers initiate several signal processes [91, 92]. It has been explained how surface integrins

and molecules like CD44 lead to ROCK-mediated phosphorylation of the ERM proteins that determine actin polymerization and so cell adhesion or migration. Platelet spreading depends on $\beta 3/1$ -integrin ligands, as does neutrophil release of ROS or the activation of macrophages. Furthermore, various immunoreceptors that have ITAMs that activate Syk determine the “inside-out” activation of integrins [93]. These could be Dap12 and FcR γ partners.

9.8.1.1 Focal Adhesion Kinase

Focal adhesion kinase (FAK) in Fig. 9.10 (or Fig. 4.4) is a nonreceptor tyrosine kinase that is linked to integrins via talin or to platelet-derived growth factor/epidermal growth factor (PDGF/EGF) receptors. Essentially, the activity of FAK is determined by integrin-mediated cell adhesion. FAK engenders PIP2, which regulates interaction of vinculin with α -actinin so that actin fibrils are organized.

Understandably, surface receptors connect to FAK and thence to Rac1 and other GTPases during phagocytosis [94–96]. Integrin-stimulated FAK phosphorylation means that it forms an FAK-Src signal complex, which acts to control cell shape and focal contact turnover. FAK connects then with the scaffold protein p130Cas [95] in combination with Crk to promote Rac GTPase activity to create lamellipodia. In Fig. 9.10, we see again how FAK and the p130Cas help to determine release of metalloproteinases [96], for example, from T lymphocytes.

FAK will activate metalloproteinases at the leading edge of moving cells, ensuring proteolysis that releases integrin-matrix contacts and thereby focal contact turnover is facilitated. FAK can also promote signalling from Ras to ERK and from Rac to JNK (cf. Fig. 4.3). FAK enhances PI3K activation of Akt and so cell survival. In Chap. 18, we consider how FAK supports carcinogenesis and protease expression that helps cell invasion and metastasis.

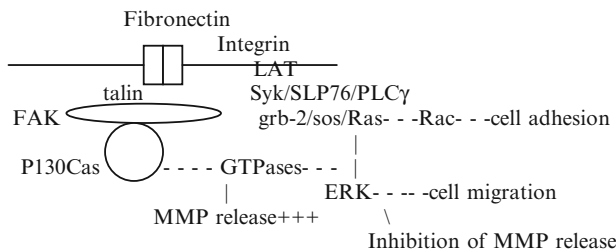


Fig. 9.10 FAK is recruited to integrins through integrin associated proteins talin and paxillin. Formation of an FAK-Src complex and cooperation with p130 Cas-Crk promotes various activities. Abl phosphorylation of Crk is a negative regulator. So FAK is a signal platform that can promote phosphorylation of N-Wasp, alter the cytoskeleton for cell migration, or effect Rac GTPase activation for cell proliferation or cell survival. Focal adhesion kinase and p130 Cas scaffold

9.8.1.2 Integrins Signal via Rho GTPases

Integrins signal *outside-in* in response to ligands that regulate (a) cell shape, (b) cell migration and (c) cell growth and survival. Conversely, there is *inside-out* signalling for display of integrins, which determines cell adhesiveness, cell polarization or even migration of leucocytes. One can envisage how display of surface integrins will enable leucocytes that are rolling along endothelium to be arrested by firm adhesion. Activation of integrins (Fig. 9.11) for ligand to bind occurs in response to Rap1 GTPase inciting actin-binding protein talin to bind to between the cytoplasmic tails of the integrins [97]. Agonist $\rightarrow$ Ca^{2+} or diacylglycerol (DAG)/PKC $\rightarrow$ Rap-1—RIAM adaptor talin $\rightarrow$ integrin activation. Thus, Rap-1 translates chemokine signals into activation of integrins, which enables cell polarization and cell motility as cells cross vascular endothelium under flow conditions [98, 98b].

FAK (Fig. 9.10) participates in *focal contacts* between cells and their matrix and connects with the Rho GTPases Rho, Rac or cdc42, which are then linked to the actin cytoskeleton. Hence, there can be formation of actin stress fibres by means of Rho kinase and myosin phosphatases, formation of filopodia via cdc42-WASp-Arp (Fig. 9.5) and formation of lamellipodia via Rac and cdc42 (Fig. 9.5). Rho GTPases make as many as 40 connections. Integrin-mediated cell anchorage and consequent actin-cytoskeletal reorganization determine upstream-to-downstream coupling of the Ras/Raf/MEK—ERK signalling cascade [98].

Talin is found at the leading edge of cytokine-stimulated lymphocytes. Talin has a FERM domain, which activates the β -integrins and thus mediates inside-out signalling.

Vinculin stabilizes focal adhesions and suppresses migration of cells [99]. It has many binding partners, like talin and paxillin. Engagement of extracellular matrix by integrins can induce p27 cell cycle inhibitor degradation and so cell cycle activ-

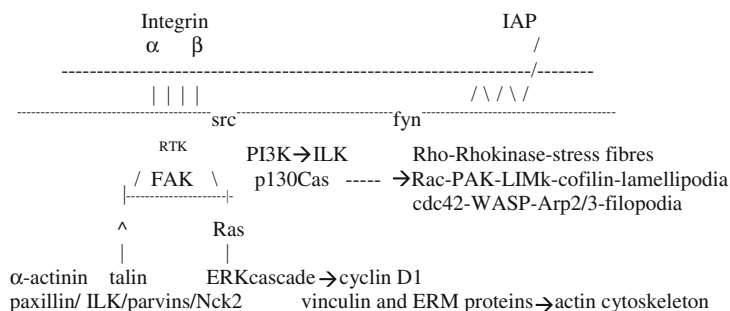


Fig. 9.11 Talin etc in focal adhesion complexes connect Integrins to the actin cytoskeleton

RTK=receptor tyrosine kinase IAP=Integrin Associated Protein

FAK=focal adhesion kinase ILK=Integrin linked kinase

Talin is found at the leading edge of cytokine stimulated lymphocytes. Talin has a FERM domain which activates the β -integrins and thus mediates inside—out signalling. Talin is activated by Rap1 GRPase via RIAM (Rap1-GTP interacting adaptor molecule)

Integrin-initiated signalling

ity. Conversely, integrins are downregulated by Ephrin receptor tyrosine kinases or by semaphorin [100]. H-Ras and PLCε suppress integrin activation [101]. The integrin-associated protein (IAP, CD47) aids transmigration of neutrophils or monocytes when it interacts with SIRP of leucocytes [102]. When TNFα activates IAP and causes thrombospondin 1 (TSP-1) to bind to it, ICAM1/VCAM1 adhesion molecules appear on monocytes so that they bind to endothelial cells. Like integrins, IAP is represented on many cells and tissues. On smooth muscle cells, the action of integrin-α2β1 in increasing chemotaxis toward collagen is augmented by the IAP association [102].

9.8.1.3 Integrins Promote Cytoskeletal Contraction

Obviously, integrins lead not only to cytoskeletal remodelling but also to contraction of the actin filaments. This is effected by means of the sequence integrin → Rho → Rock-MLCP → myosin II contractility, which was discussed in relation to Fig. 5.10.

9.8.2 Detail about Focal Adhesion Kinase

FAK activity stems from integrin-mediated cell adhesion [103] and its association as a signal complex with Src family kinases (SFKs) (Fig. 9.12). FAK-mediated regulation of the Rho family GTPases means that it affects the formation and disassembly of actin cytoskeletal frameworks. Accordingly, FAK is important for cell motility and cancer cell invasion. Furthermore, when transforming growth factor-β (TGFβ) upregulates myofibroblasts to become fibrous tissue forming fibroblasts in lungs, liver or kidneys, that is because surface integrins are linked via FAK to cell differentiation [104]. The connections of FAK to adaptor protein p130 Cas/Crk help the coordinated activation of Rac at cell membrane extensions.

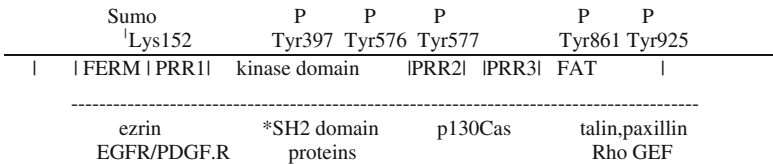


Fig. 9.12 Focal adhesion kinase (FAK) domain structure. Observe the focal adhesion targeting (FAT) domain that recruits FAK to focal contacts by integrin-associated proteins like talin and paxillin. FAT also links FAK to the activation of Rho guanosine triphosphatases (GTPases). Within the kinase domain, phosphorylation of Tyr576 and Tyr577 is needed for maximum catalytic activity. The FERM domain refers to protein 4.1, ezrin, radixin, moiesin affinity sites. Proline-rich regions (PRRs) 1–3 bind Src-homology domain 3 (SH3) domain proteins like the p130 Cas adaptor

The module structure of proteins was indicated in Fig. 5.6 (Sect. 5.4). Consideration of the structure of FAK can be used to illustrate further the contribution of protein modules. There are three proline-rich regions PRR1–3. FAK becomes autophosphorylated at Try397 in its central kinase domain, so that a motif is created for various Src-homology domain 2 (SH2) domain proteins. They are the SFKs, phospholipase C γ , PI3K, the Shc adaptor protein and RasGAP.

9.8.3 *Signalling from Immunoglobulin CAMs*

Members of the immunoglobulin-like cell adhesion molecules (Ig CAMs) like ICAM, VCAM, NCAMs, CEA and PECAM-1 play important roles in transmitting information into cells from the cell surface of haemopoietic cells. The interaction of ICAM-1 of endothelial cells with LFA-1 of leucocytes is depicted in Fig. 10.9. ICAM-1, which is upregulated on endothelial cells in inflammation, interacts with the leucocyte integrins LFA-1 (α L β 2) and Mac-1 (α M β 2). The Ig CAMs connect with the cytoskeleton to enable migration. PECAM-1 (CD31) is found at the junctions between endothelial cells, where it aids the extravasation of leucocytes. The cytoplasmic domain of PECAM-1 contains two ITIMs, which serve as docking sites for the dual SH2 domains of phosphatases SHP-1, SHP-2, and SHIP. Hence, this protein can provide a negative influence over other cell surface receptors [105].

VCAM-1 has a positive role in the extravasation of cells like monocytes because, when it is activated by leucocyte contact, it stimulates endothelial cell NADPH oxidase to produce ROS [106], which can then activate local metalloproteinases.

9.8.4 *Signalling from Selectins*

Platelets have special adhesion molecules called P-selectins, endothelial cells have E-selectins and leucocytes have L-selectins. Each has a terminal carbohydrate recognition domain (CRD), which is a mucin with O-linked sugars on its side chains. Selectins regulate the trafficking of leucocytes between the blood and the tissues by interaction with glycoprotein counterreceptors like P-selectin glycoprotein ligand 1 (PSGL1) that are akin to sialyl Lewis X. PSGL1 occurs on all leucocytes but is only functional when correctly glycosylated. Selectins can activate β 2-integrins. Selectins signal via MAPKs and connect with the actin cytoskeleton of their cells. Consider that cross-linking of PSGL (Fig. 9.13) leads to c-Abl-mediated (Fig. 5.8) alteration of the PMN cytoskeleton [107]. Perhaps, not surprisingly, lipid raft disruption in leucocytes by cholesterol-chelating agents stops vital interactions involving PSGL and L-selectin, and strongly reduces L-, P- and E-selectin-dependent rolling of leucocytes on the endothelial cells [98b, 108]. Syk is implicated in leucocyte rolling, as revealed by its thwarting by RNA interference [109].

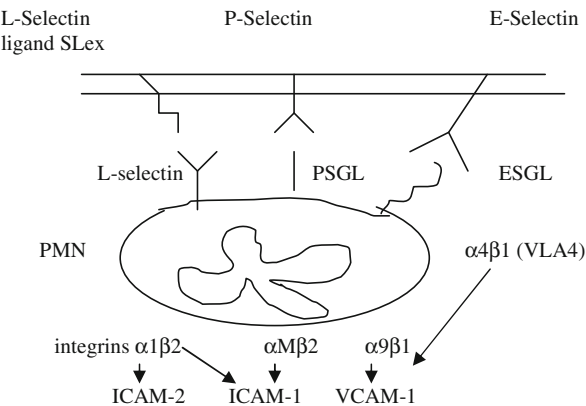


Fig. 9.13 Selectins in leucocyte interactions. (a) The selectins have specific ligands that interact with them. (b) Integrins connect with ICAM/VCAM molecules. (c) PIR-B are inhibitory molecules that negatively regulate neutrophil and macrophage signalling by integrins [110]

Table 9.2 Selectins in leucocyte interactions

Name	Tissue	Ligand
L-selectin (CD62L)	All white cells	Sulphated sialyl Lewis
P-selectin (CD62P)	GlyCAM1, CD34, MadCAM1	
Sialyl Lewis X	Endothelium	P-selectin glycoprotein ligand 1 (PSGL1)
E-selectin (CD62E)	Endothelium	L-selectin, sialyl Lewis X

Thus, this is an alternative integrin activation pathway to signalling through GPCRs (cf. Chap. 1). One recalls that leucocyte transmigration through venules involves (a) selectin-mediated rolling, (b) chemokine-triggered leucocyte activation and (c) integrin-dependent arrest (Table 9.2). There has been a detailed recent review by Alon and K Ley [98b].

9.8.5 Cadherins and Adhesion of Epithelial Cells

The cadherins (**Fig. 18.3**) are a family of receptors that use Ca²⁺ to form stable cell–cell adhesion when cells form a layer of epithelium. In fact, cadherins help determine the cell phenotype [111, 112] Furthermore p120-Catenin mediates cross-talk between the Rac and Rho GTPase signal pathways [113]. Polarization of epithelial cells, so that there is an apical side to each cell and an opposing basolateral surface, stems from cadherin-induced cytoskeletal organization utilising the small GTPases

Rho, Rac and cdc42. The Rho GTPases contribute by affecting actin fibre arrangements [87, 103], organelle distribution and gene expression. E-cadherin expression (Chap. 18) is lost in cancers.

9.9 The Use of Statins

Statin drugs as high-mobility group coenzyme A (HMG-CoA) reductase inhibitors lower the blood cholesterol. They also inhibit the small GTPases Rho, Rac and Ras because Ras translocation is dependent on farnesylation, and Rho translocation is dependent on geranylgeranylation [114]. To be precise, the Ras proteins are farnesylated at a terminal CAAX motif, in which C is cysteine. K-Ras and N-Ras undergo geranylgeranylation. So, statins will curb the functions of monocyte-macrophages, T and B lymphocytes and NK cells. Thus, the statins exert a variety of anti-inflammatory effects [115]. Lovastatin even induces apoptosis of macrophages [116]. Statins curb the ability of dendritic cells to induce T cell proliferation, and they stop their secretion of proinflammatory cytokines [117]. Inhibition of T cells is derived directly from inactivation of Rac and Ras activities [118]. Atorvastatin lowers activation of NF- κ B induced by angiotensin II, and accordingly it reduces expression of IL-8 and MCP-1 in damaged kidneys. When it was used for autoimmune encephalomyelitis in mice, lovastatin inhibited the formation of Th1 lymphocytes and their cytokines [119]. Statins stop LFA-1-mediated adhesion of lymphocytes (Fig. 13.3), preventing costimulation of lymphocytes with suppression of the inflammatory response [120]. Statins are beneficial for atherosclerosis since they counteract the endothelial dysfunction by increasing the expression of endothelial nitric oxide synthase (eNOS) and so basal and induced NO production [121]. Recent articles emphasized the ability of the statins to ameliorate the relentless fibrosis of the kidneys, arising from macrophage activation, that accounts for the development of end-stage kidney disease [122]. Statins ameliorate arthritis since formation of IL-1, IL-6 and TNF α is reduced, and chemokines CCL5/CCL2 are lowered [123]. CCL5 is Rantes. Statins inhibit cyclooxygenase 2 (Cox-2) expression in human monocytic cells [124].

References

1. Casey PJ. Protein lipidation in cell signaling. *Science* 1999;268:221–224
2. Takai Y, Sasaki T, Matozaki T. Small GTP binding proteins. *Physiol Rev* 2001;81:153–208
3. Symons M, Settleman J. Rho family GTPases: more than simple switches. *Trends Cell Biol* 2000;10:415–420
4. Patel VA, Longacre A, Hsiao K et al. Apoptotic cells trigger characteristic signaling events. *J Biol Chem* 2006;281:4663–4670
5. Behnia R, Munro S. Organelle identity and the signposts for membrane traffic. *Nature* 2005;438:597–604
6. Marinissen MJ, Gutkind JS. Scaffold proteins dictate Rho GTPase signalling specificity. *Trends Biochem Sci* 2005;30:423–426

7. Quinn MT, Low MW. GTP binding proteins and leukocyte signal transduction. *J Leukoc Biol* 1995;58:263–276
8. Benard V, Bokoch GM, Diebold BA. Small GTPases that regulate neutrophil function. *Trends Pharm Sci* 1999;20:365–369
9. Donaldson JG. Multiple roles for ARF6. *J Biol Chem* 2003;278:41573–41576
10. San VP, Seasholtz TM, Sagi SA, Brown JH. The role of Rho in G protein coupled receptor signal transduction. *Annu Rev Pharmacol Toxicol* 2000;40:459–489
11. Li Z, Dang X, Wang Z et al. Regulation of PTEN by Rho small GTPases. *Nat Cell Biol* 2005;7:399–411
12. Ridley AJ, Hall A. The small GTP binding protein Rho regulates assembly of focal adhesions and actin stress fibres in response to growth factors. *Cell* 1992;70:389–393
13. Etienne-Manneville S, Hall A. Rho GTPases in biology. *Nature* 2002;420:629–635
14. Schmitz AA, Govek E-E, Bottner V, van Aelst L. Rho GTPases: signaling, migration and invasion. *Exp Cell Res* 2000;261:1–12
15. Ridley AJ. Rho family proteins coordinate cell responses. *Trends Cell Biol* 2001;11:471–477
16. Fan H, Patel VA, Longove A, Levine JS. Abnormal regulation of the cytoskeletal regulator Rho typifies macrophages of the major murine models of spontaneous autoimmunity. *J Leukoc Biol* 2006;79:155–165
17. Kusner DJ, Thompson CR, Melrose NA et al. The localization and activity of sphingosine kinase 1 and coordinated regulation with actin cytoskeletal dynamics in macrophages. *J Biol Chem* 2007;282:23147–23162
18. Zigmund SH. How Wasp regulates actin polymerization. *J Cell Biol* 2000;150:F117–F120
19. Brown MD, Sacks DB. IQGAP in cellular signaling: bridging the GAP. *Trends Cell Biol* 2006;16:242–249
20. Disanza A, Mantocni S, Hertzog M et al. Regulation of cell shape by cdc42 is by the synergic actin-bundling activity of the Eps8-IRSp53 complex. *Nat Cell Biol* 2006;8(12):1337–1347
21. Kumar R, Vadlamudi RK. Emerging functions of p21 activated kinases in human cancer cells. *J Cell Physiol* 2002;193:133–144
22. Lowell CA, Berton G. Integrin mediated signal transduction in myeloid leukocytes. *J Leukoc Biol* 1999;65:313–320
23. Scoles SJ, Gomex M, Kreis TE. Coat proteins regulating membrane traffic. *Int Rev Cytol* 2000;195:67–144
24. Loenzi R, Brickell PM, Katz DR et al. Wiskott-Aldrich syndrome protein is necessary for IgG mediated phagocytosis. *Blood* 2000;95:2943–2946
25. Goley ED, Welch MD. The Arp2/3 complex: an actin nucleator comes of age. *Nat Rev Mol Cell Biol* 2006;7:713–725
26. Dupre L, Aiuti A, Trifani S et al. Wasp regulates lipid raft dynamics during immune synapse formation. *Immunity* 2002;17:157–166
27. Kiuchi T, Ohashi K, Kurita S, Mizuno K. Cofilin promotes stimulus induced lamellipodium formation by generating actin monomers. *J Cell Biol* 2007;177:465–476
28. Higgs HN, Pollard TD. Regulation of actin filament network formation through Arp2/3 complex. *Annu Rev Biochem* 2001;70:649–676
29. Innocenti M, Gerboth S, Rottner K et al. Abi-1 regulates the activity of N-Wasp and Wave in distinct actin based processes. *Nat Cell Biol* 2005;7:969–976
30. Daly RJ. Cortactin signalling and actin networks. *Biochem J* 2004;382:13–25
31. Lin S-Y, Raval S, Zhang Z et al. The protein tyrosine phosphatase SHP-1 regulates the phosphorylation of α -actinin. *J Biol Chem* 2004;279:25755–25764
32. Lanzardo S, Curcio C, Forni G, Anton IM. A role for WIP in fibroblast adhesion, spreading and migration. *Int J Biochem Cell Biol* 2007;39:262–265
33. Carlier M-F, Pantaloni D. Control of actin assembly dynamics in cell motility. *J Biol Chem* 2007;282:23005–23009
34. Cory GOC, Ridley AJ. Braking Waves. *Nature* 2002;418:732–738
35. Carlier MF. Control of actin dynamics. *Curr Opin Cell Biol* 1998;10:45–51

36. Jones GE. Cell signaling in macrophage migration and chemotaxis. *J Leukoc Biol* 2000;8:93–102
37. Miki H, Yamaqudi H, Suetsuqi S, Takenawa T. IRSp53 is an essential intermediate between Rac and Wave in the regulation of macrophage membrane ruffling. *Nature* 2000;408:732–736
38. Yamazaki D, Suetsuqi S, Miki H et al. Wave 2 is required for directed cell migration and cardiovascular development. *Nature* 2003;424:452–456
39. Li Z, Kim ES, Bearer EL. Arp2/3 complex is required for actin polymerization during platelet shape change. *Blood* 2002;99:4466–4474
40. Bos JL. Linking Rap to cell adhesion. *Curr Opin Cell Biol* 2005;17:123–128
41. Franco SJ, Rodgers MA, Pevin BJ et al. Calpain mediated proteolysis of talin regulates adhesion dynamics. *Nat Cell Biol* 2004;6:977–983
42. Rafelski SM, Theriot JA. Crawling toward a unified model of cell motility. *Annu Rev Biochem* 2004;73:209–239
43. Ivetic A, Ridley AJ. ERM proteins and RhoGTPase signaling in leucocytes. *Immunology* 2004;112:165–176
44. Ridley AJ. Cell migration: interacting signals from back to front. *Science* 2003;302:1204–1207
45. Bokach GM. Regulation of innate immunity by Rho GTPases. *Trends Cell Biol* 2005;15:163–171
46. Bokach GM. Biology of p21 activated kinases. *Annu Rev Biochem* 2003;72:743–781
47. Niggli U. Signaling to migration in neutrophils. *Int J Biochem Cell Biol* 2003;35:169–171
48. DeFea KA. Beta-arrestins as regulators of chemotaxis. *Annu Rev Physiol* 2007;69:535–560
49. Charest PG, Firtel RA. Feedback signaling controls leading-edge formation during chemotaxis. *Curr Opin Genet Dev* 2006;16:339–347
50. Mazaki Y, Hashimoto S, Tsujimura T et al. Neutrophil direction sensing and superoxide production linked by the GTPase activating protein G172. *Nature Immunol* 2006;7:724–731
51. Pertz O, Hodgson L, Klemke RL, Hahn KM. Spatiotemporal dynamics of RhoA activity in migrating cells. *Nature* 2006;440:1069–1072
52. Burridge K, Doughman R. Front /back by Rho and Rac. *Nat Cell Biol* 2006;8:781–782
53. Ling K, Schill NJ, Wagoner MP, Sun Y, Anderson RA. The role of PtdIns(4,5)P2 in cell migration. *Trends Cell Biol* 2006;16:276–284
54. Pestonjamas KN, Forster C, Sun C et al. Rac1 links leading edge and uropod events through Rho /myosin activation during chemotaxis. *Blood* 2006;108:2814–2820
55. Regelman AG, Denzl NM, Wanjalla C et al. The hematopoietic isoform of Cas-Hef1 associated signal transducer (Chat1) regulates chemokine induced inside-out signaling. *Immunity* 2006;25:907–918
- 56a. Viola A, Gupta N. Regulation of membrane raft dynamics by actin binding proteins. *Nat Rev Immunol* 2007;7:889–896
- 56b. Thomas MS, Mitchell JS, De Nucci CC et al. The p110 δ isoform of PI3 kinase regulates migration of effector CD4 T lymphocytes into peripheral inflammatory sites. *J Leukoc Biol* 2008; 89:814–823
57. Liao J, Planchon SM, Wolfman JC, Wolfman A. Growth factor dependent Akt activation and cell migration requires c-k(B)Ras. *J Biol Chem* 2006;281:29730–29738
58. Yeung T, Grinstein S. Lipid signaling and the modulation of surface charge during phagocytosis. *Immunol Rev* 2007;219:17–36
59. (a) Crespo P, Schuebel KE, Ostram AA et al. Phosphotyrosine dependent activation of Rac-1 GDP/GTP exchange by Vav protooncogene product. *Nature* 1997;385:169–172; (b) Wang Q-Q, Li H, Oliver T et al. Integrin β 1 regulates phagosome maturation in macrophages through Rac expression. *J Immunol* 2008;180:2419–2428
60. T Suboi S, Meerloo J. Wiskott-Aldrich protein is a key regulator of the phagocytic cup formation in macrophages. *J Biol Chem* 2007;282:34194–34203
61. Perrat E, Lakkaraju A, Deborde S et al. Evolving endosomes. *Curr Opin Cell Biol* 2005;17:423–434

62. Aniento F, Emons N, Griffiths G, Greenberg J. Cytoplasmic dynein-dependent vesicular transport from early to late endosomes. *J Cell Biol* 1993;123:1373–1387
63. Takei K, Yoshida Y, Yamada H. Regulatory mechanisms of dynamin-dependent endocytosis. *J Biochem* 2005;137:243–247
64. Takei K, Haucke V. Clathrin mediated endocytosis. *Trends Cell Biol* 2001;11:385–391
- 65a. Ford M, Mills IG, Peter BJ et al. Curvature of clathrin coated pits driven by epsin. *Nature* 2002;491:361–364
- 65b. Hao Y, Wong R, Feig LA. RalGDS couples growth factor signaling to Akt activation. *Mol Cell Biol* 2008;28:2851–2859
66. Nielsen E, Severin F, Backer JM et al. Rab5 regulates motility of endosomes on microtubules. *Nat Cell Biol* 1991;1:376–382
67. Jones MC, Caswell PT, Norman JC. Endocytic recycling pathways. *Curr Opin Cell Biol* 2006;18:549–557
68. von Zaslow M, Sorkin A. Signaling on the endocytic pathway. *Curr Opin Cell Biol* 2007;19:436–445
69. Donnenberg MS Pathogenic strategies of enteric bacteria. *Nature* 2000;408:768–775
70. Boquet P, Lemichez E. Bacterial virulence factors targeting Rho GTPases. *Trend Cell Biol* 2003;13:238–246
71. Veiga E, Cossart P. *Listeria* hijacks the clathrin dependent endocytic machinery to invade mammalian cells. *Nat Cell Biol* 2005;7:894–900
72. Purushothaman SS, Wang B, Clearly PP. M1 protein triggers a phosphoinositide cascade for group A streptococcus invasion of epithelial cells. *Infect Immun* 2003;71:5823–5830
73. Schmitter T, Agerer F, Peterson L et al. Granulocyte CEACAM3 is a phagocytic receptor of the innate immune system that eliminates human specific pathogens. *J Exp Med* 2004;199:36–46
74. Blands JM, Medzhitov R. Regulation of phagosome maturation by signals from Toll like receptors. *Science* 2004;304:1014–1018
75. Robinson MJ, Sancho D, Slack EC et al. Myeloid C-type lectins in innate immunity. *Nat Immunol* 2006;7(12):1258–1264
76. Pu QQ, Streuli CH. Integrin control of cell cycle. *Bioessays* 2002;24:17–21
77. Stow JL, Manderson AP, Murray RZ. SNAREing immunity: the role of SNAREs in the immune system. *Nat Rev Immunol* 2006;6:919–929
78. Mertin Marlin B, Nabokisc SM, Lasi JB, Lazo PA, Mollinedo F. Involvement of Snap 23 and syntaxin 6 in human neutrophil exocytosis. *Blood* 2000;96:3334–3340
79. Pelham HRB. Snares and the specificity of membrane fusion. *Trends Cell Biol* 2001;11:99–101
80. Mollinedo F, Calafat J, Janssen H et al. Combinatorial SNARE complexes modulate secretion of cytoplasmic granules in neutrophils. *J Immunol* 2006;177:2831–2841
81. Brumell JH. Subcellular distribution of docking /fusion proteins in neutrophils, secretory cells with multiple exocytic compartments. *J Immunol* 1995;155:5750–5759
82. Braga VM. Cell-cell adhesion and signalling. *Curr Opin Cell Biol* 2002;14:546–556
83. Hepp R, Puri N, Hohenstein AC et al. Phosphorylation of Snap23 regulates exocytosis from mast cells. *J Biol Chem* 2005;280:6610–6620
84. Chen D, Lemons PP, Schraw T, Whiteheart SW. Molecular mechanisms of platelet exocytosis. *Blood* 2000;96:1782–1787
85. Reed GL, Fitzgerald ML, Polgar J. Molecular mechanisms of platelet exocytosis. *Blood* 2000;96:3334–3340
86. Holt OJ, Gallo F, Griffiths G. Regulating secretory lysosomes. *J Biochem* 2006;140:7–12
87. Logan MR, Odemuyiwa SO, Moqbel R. Understanding exocytosis in immune and inflammatory cells. *J Allergy Clin Immunol* 2003;111:923–932
88. Wewers MD. Il-1 β : an endosomal exit. *Proc Natl Acad Sci USA* 2004;101:10241–10242
89. Kay JG, Murray RZ, Pagan JK, Stow JL. Cytokine secretion via cholesterol-rich lipid raft-associated SNAREs at the phagocytic cup. *J Biol Chem* 2006;281:11949–11954
90. Barlic J, Andrews J, Kelvin AA et al. Regulation of tyrosine kinase activation and granule release through β -arrestin by CXCR1. *Nat Immunol* 2000;1:227–233

91. Juliano RL. Signal transduction by cell adhesion receptors and the cytoskeleton. *Annu Rev Pharmacol Toxicol* 2002;42:283–323
92. Larsen M, Artym VV, Green JA, Yamada KM. Extracellular matrix remodeling and integrin signaling. *Curr Opin Cell Biol* 2006;18:463–471
93. Kahn ML, Koretzky GA. Integrins and ITAMs. *Nat Immunol* 2006;7:1286–1288
94. Wu Y, Tibrewal N, Birge RB. Phosphatidylserine recognition by phagocytes. *Trends Cell Biol* 2006;16:189–197
95. Defilippi P, DiStefano P, Cabodi S. p130Cas: a versatile scaffold in signaling networks. *Trends Cell Biol* 2006;16:257–263
96. Segarra M, Vilardell C, Matsumoto K et al. Dual function of focal adhesion kinase in integrin induced metalloproteinase release by human T cells. *FASEB J* 2005;19:1875–1877
97. Burbach BJ, Medeiros R. TCR signaling to integrins. *Immunol Rev* 2007;218:65–81
- 98a. Li W, Metcalf DG et al. Rap1 translates chemokine signals to integrin activation. *J Cell Biol* 2003;161:417–427
- 98b. Alon R, Ley K. Shear-regulated integrin activation in leukocyte rolling and arrest an endothelial cells. *Curr Opin Cell Biol* 2008;20:525–532
99. Ziegler WH, Liddington RC, Critchley DR. The structure and regulation of vinculin. *Trends Cell Biol* 2006;16:453–440
100. Halloran MC, Wolman MA. Repulsion or adhesion: receptors make the call. *Curr Opin Cell Biol* 2006;18:533–540
101. Lad Y, McHugh B, Hodgkinson PS et al. Phospholipase C ϵ suppresses integrin activation. *J Biol Chem* 2006;281:29501–29512
102. Wang XQ, Frazier WA. The thrombospondin receptor CD47(IAP) modulates and associates $\alpha 2\beta 1$ integrin in vascular smooth muscle cells. *Mol Biol Cell* 1998;8:865–874
103. Mitra SK, Hanson DA, Schlaepfer DD. Focal adhesion kinase: in command and control of cell motility. *Nat Mol Cell Biol* 2005;6:56–66
104. Thannickal VJ, Lee DY, White ES et al. Myfibroblast differentiation by TGF β 1 is dependent on cell adhesion and integrin signaling. *J Biol Chem* 2003;278:12384–12389
105. Mamdouh Z, Chen X, Pierini LM, Maxfield FR, Muller WA. Targeted recycling of PECAM from endothelial surface-connected compartments during diapedesis. *Nature* 2003;421:748–753
106. Cook-Mills JM, Deem TL. Active participation of endothelial cells in inflammation. *J Leukoc Biol* 2005;77:487–495
107. Ba X, Chen C, Gao Y, Zeng X. Signaling function of PSGL-1 in neutrophils: c-Abl involved alteration in actin cytoskeleton. *J Cell Biochem* 2005;94:367–373
108. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. The leukocyte adhesion cascade updated. *Nat Rev Immunol* 2007;7:678–689
109. Abbal C, Lambelet M, Bertaggia D et al. Lipid raft adhesion receptors and Syk regulates selectin-dependent rolling under flow conditions. *Blood* 2006;108:3352–3359
110. Periera S, Zhang S, Takai T, Lowell CA. The inhibitory receptor PIR-B negatively regulates neutrophil and macrophage integrin signaling. *J Immunol* 2004;173:5757–5765
111. Wheelock MJ, Johnson KR. Cadherins as modulators of cellular phenotype. *Annu Rev Cell Dev Biol* 2003;19:207–235
112. Braga VMM, Yap AS. Epithelial junctions and small GTPase signalling. *Curr Opin Cell Biol* 2005;17:466–474
113. Niessen CM, Yap AS. Another Job for the talented p120-Catenin cell 2006;127:875–876
114. Mason JC. Statins and their role in vascular protection. *Clin Sci* 2003;105:251–266
115. Blanco-Colio LM, Tunon J, Martin-Ventura JL, Egido J. Antiinflammatory and immunomodulatory effects of statins. *Kidney Int* 2003;63:12–23
116. Liang S-L, Liu H, Zhou A. Lovastatin induced apoptosis in macrophages through the Rac1/cdc42/JNK pathway. *J Immunol* 2006;177:651–656
117. Yilmaz A, Reiss C, Weng A et al. Differential effects of statins on relevant functions of human macrophage-derived dendritic cells. *J Leukoc Biol* 2006;79:529–538
118. Ghittani R, Patrussi L, Pirozzi K et al. Simvastatin inhibits T cell activation by impairing the function of Ras superfamily GTPases. *FASEB J* 2005;19:605–607

119. Nath N, Giri S, Prasad R et al. Potential targets of 3hydroxy-3-methyl-glutaryl coenzyme A reductase inhibitor for multiple sclerosis therapy. *J Immunol* 2004;172:1273–1286
120. Weitz-Schmidt G, Welzenbach J, Brinkmann V et al. Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. *Nat Med* 2001;7:687–692
121. Laufs U, La Fata V, Plutzky J, Liao JK. Upregulation of eNOS by HMG CoA reductase inhibitors. *Circulation* 1998;97:1129–1135
122. Epstein M, Campese VM. Pleiotropic effects of 3hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors on renal function. *Am J Kid Dis* 2005;45:2–14
123. Barsante MM, Roffe E, Yokoro CM et al. Anti-inflammatory and analgesic effects of atorvastatin in rat model of adjuvant-induced arthritis. *Eur J Pharm* 2005;516:282–289
124. Habib A, Shamseddeen I, Nasrahah MS et al. Modulation of Cox2 expression by statins in human monocytic cells. *FASEB J* 2007;21:1665–1674

Chapter 10

Leucocyte Activation and Behaviour

Abstract How neutrophils or eosinophils are activated and how they also adhere using integrins has to be appreciated.

10.1 Activation of Neutrophils

Neutrophils are attracted to inflammatory sites by chemokines like interleukin 8 (IL-8). They produce a range of cytokines and chemokines, which implies that they may never work alone. To combat microbial invasion, they are activated (a) to produce reactive oxygen species (ROS) by means of their membrane NADPH oxidase [1] and (b) for discharge of their lysosomal enzyme granules. Azurophilic granules contain myeloperoxidase, defensins and hydrolases, and the specific granules contain lactoferrin (an antiendotoxin), lysozyme and metalloproteinases like gelatinase.

Neutrophils are activated by the bacterial peptide *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) [2], IL-8, tumour necrosis factor- α (TNF α) [3], complement component C5a, platelet-activating factor (PAF) or leukotriene B4 (LTB4), 5-oxoHETE and defensins. It has been difficult to sort out the many processes involved in their activation [4]. It turns out that sphingosine kinase (*) (Fig. 10.1) is a point of convergence for priming agents like PAF, TNF α , granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-8 and substance P [5]. The priming cytokines induce only a weak oxidative response in neutrophils, but they strongly enhance a response to a secondary stimulus like bacterial *N*-formyl peptides.

Neutrophils express receptors that function in pathogen recognition and uptake, such as mannose receptors, scavenger receptors, Fc and complement receptors and Toll-like receptors. In Fig. 10.1, we note the familiar sequence of phospholipase C (PLC) hydrolysis of membrane PtdIns(4,5)P₂, leading to InsP₃, which causes influx of Ca²⁺ ions into the cytosol, and formation of diacylglycerol (DAG), which activates protein kinase C (PKC). Note also how phospholipase D produces phosphatidic acid (PtdOH) and how that activates membrane NADPH oxidase so that superoxide anions [6] are formed for killing of bacteria. Yet, the real killing agents are proteases like cathepsin G and elastase, which are activated by K⁺ ions that

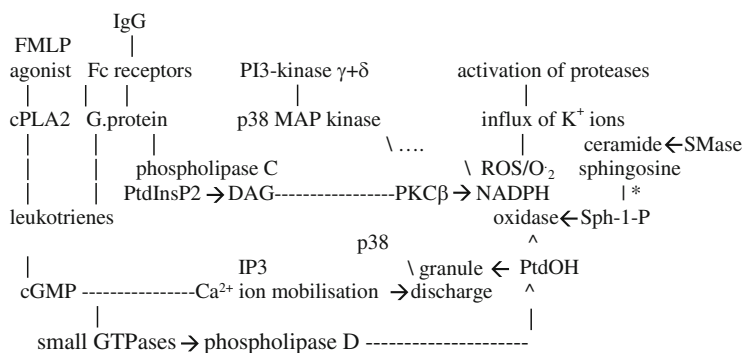


Fig. 10.1 Activation of neutrophils. p38 mitogen-activated protein kinase (*MAPK*) is very important in neutrophils. *FMLP* *N*-formyl-methionyl-leucyl-phenylalanine [2]. *Sphingosine kinase converts sphingosine to sphingosine-1-phosphate

move into the endocytic vacuole in response to the anionic charge created by superoxide anions [7, 8]. Granule proteases and antimicrobial peptides and even nitric oxide ($\text{NO}\cdot$) production are supported by hypoxia-inducing factor 1 α (HIF1 α) in response to bacterial infection [9]. Perhaps, not surprisingly, polymorphonuclear leucocyte (PMN) neutrophils deliver antigenic molecules to dendritic cells (DCs) [10].

The PI3 kinase (PI3K) of neutrophils supports Akt and thereby mTOR Target of Rapamycin (Fig 11.6). mTOR limits production of pro-inflammatory cytokines as a safety mechanism! [11]. The FMLP receptor connects to PI3K γ , and its action is supported secondarily by PI3K δ , which is induced by integrins. So this explains PMN responses to bacteria.

PtdIns(3,4,5)P3 signalling in neutrophils is curbed competitively by PtdIns(1,3,4,5)P4 [12], so preventing over-charging of responses. Of course, PI3K generated PtdIns(3,4,5)P3 induces F-actin reorganization needed for cell migration, as directed by p38 MAPkinase and its effector kinase MK2 (Table 12.12). Thus one appreciates that PTEN phosphates in the uropod plays an important role in directed PMN migration towards stimuli [12b].

PtdOH causes the discharge of neutrophil granules. Sphingosine-1-P formed in response to TNF α also helps. In many cell types, agents that stimulate cAMP formation (like adenosine, the PDE inhibitors or E prostaglandins) have a dampening effect on the activation process. PGE2 via Epac-1 activation enhances SHP-1 phosphates activity, so resulting in increased PTEN activity [13]. Lipoxin A4 inhibits neutrophil-mediated proinflammatory responses. Polyisoprenyl phosphates are endogenous regulators [14a, 14b].

When FMLP causes neutrophil chemotaxis and activation, the cascade Ras-Raf-MEK1-MAPK is activated [15, 16]. If there is activation by G-CSF, the extracellular signal-related kinase (ERK) path alone is activated, and when activation is by GM-CSF, there is ERK activation and weak p38 promotion. When TNF α acts, ERK activation is weak, and p38 MAPK activation is strong. TNF α activates

NF- κ B (Chap 11), but G-CSF or GM-CSF does not. When PAF acts, the p38 cascade incites chemotaxis [17]. Neutrophil transepithelial migration makes use of the Mac1 Integrins (CD11b/CD18). This glycoprotein bears sialyl Lewis X epitopes.

Actually, *p38 dominates in neutrophil activities*. One could make additions to Fig. 10.1. The receptors for FMLP connect with PI3K (actually PI3K γ) and with PLC β , and an associated G protein causes CD38 to activate cyclic adenosine diphosphate (ADP)-ribose (Sect. 2.5) to release internal Ca²⁺ ions and to boost entry of Ca²⁺ by plasma membrane channels [18]. ROS and thus hydrogen peroxide reinforce the entry of calcium ions. When FMLP acts, Vav1 in its role as exchange factor (GEF) induces the Rho and Rac (GTPases) [19]. Formation of actin filaments is required for PMN chemotaxis and for phagocytosis of bacteria (Fig. 9.8). PAK (p21-activated kinase) [20] plays a part in actin polymerization, as does Rac with its associated lipid kinase [21]. Rac2 helps to generate free barbed ends of actin filaments, and Rac1 uncaps existing barbed ends.

Rab5 is an important regulator of early events in endocytosis and phagocytosis [22]. LTB₄-induced neutrophil degranulation, via the BLT1 receptors, involves activation of PKC and the Yes kinase [23].

In view of its importance, one must focus on the *membrane NADPH oxidase* that catalyses one electron reduction of molecular oxygen to superoxide anions [3]. Its preferred electron donor is NADPH and not NADH. The cytochrome b558 is unusual for it is a heterodimer of a 22-kDa α -subunit and a highly glycosylated β -subunit (76–92 kDa). Membrane cytochrome b oxidase requires the support of special cytosolic proteins. They are p47^{phox} and p67^{phox}, phox meaning phagocytic cell oxidase. There is also a p40^{phox}, p22 Rac.2 [24] and an associated GDP dissociation inhibitor (GDI) i.e. Rho GDI. The onset of the respiratory burst during phagocytosis is provoked by phosphorylation of the phox components [25] and their translocation into phagosomes. GM-CSF and TNF α cause phosphorylation of Ser345 on the p47^{phox} [25]. There is also a RhoG, which supports regulation of the NADPHox [26].

One would expect the receptors for phagocytosis, the Fc γ Rs, to connect with the NADPH oxidase. In fact, Vav proteins incite Rac2 GTPase and thereby NADPHox activity [27]. The neutrophil Src kinases called Hck and Fgr connect FMLP receptors via their G proteins to Vav1 and hence to Rac2, which regulates the neutrophil respiratory burst [28]. In addition Dock2, which is activated by PtdIns-3P created by PI3K, activates the Rac-2.

One will know how the hypertensive agent angiotensin II (Ang II) acts on smooth muscle cells and in the kidneys. Ang II via p38 MAPK also stimulates the NADPHox of monocytes and neutrophils [29]. As shown in Fig. 10.3, (a) there is ROS formation (intracellularly and extracellularly) associated with translocation of NADPHox to the cell membrane and its activation; (b) the process is promoted by PI3K activation; (c) there is intracellular mobilisation of Ca²⁺ ions; (d) the Ca²⁺ stimulates CaN (calcineurin), and that stimulates the ERK1/2, Jun N-terminal kinase (JNK) and p38 MAPKs, and they stimulate NF- κ B (Sectn 11.1), which explains the proinflammatory effects of Ang II. Look at the scheme in Fig. 10.3. There is an NADPHox also in the membranes of vascular smooth muscle cells, which explains Ang II action in inducing oxidative stress.

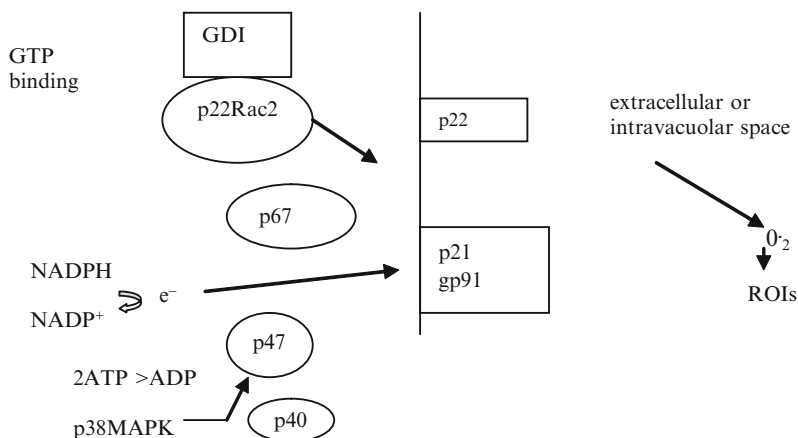


Fig 10.2 Components of the *NADPH* oxidase complex. *p* phox proteins. Superoxide release in suspended human neutrophils is stimulated by tumour necrosis factor- α (*TNF* α) and granulocyte-macrophage colony-stimulating factor (*GM-CSF*) but not by (*G-CSF*). Cdc42 stops *NADPHox* formation of reactive oxygen species (*ROS*). In contrast to Rac2 of neutrophils, monocytes utilise Rac1. Note that unoxidized phospholipids boost *ROS* and work synergistically with FMLP to potentiate the oxidative burst. Yet oxidized phospholipids act as inhibitors of the oxidative burst! [29b]

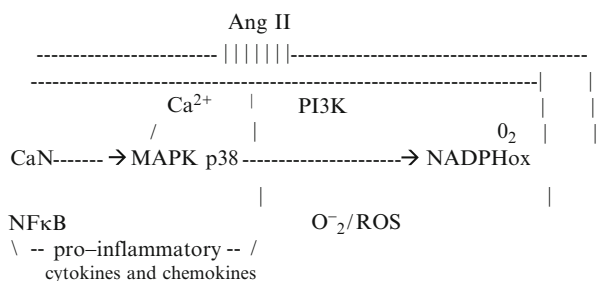


Fig 10.3 Activation of neutrophils by angiotensin II (*Ang II*) calcineurin (CaN) acts positively to stimulate p38 in neutrophils and positively in lymphocytes so that calcineurin NFAT stimulates IL-2 production (Fig. 13.6). So calcineurin inhibitors are used in transplantation.

Yet, one will find that CaN works also in an opposite sense since it negatively regulates Toll-like receptor-mediated activation pathways. In that case, CaN negatively regulates macrophage NF- κ B, the MAPKs and interferon response factor.

10.1.1 *Ca²⁺Balance and Neutrophils*

After initial activation, restoration of Ca²⁺ balance in neutrophils prevents hyperactivity. So, when Ca²⁺ is released from the endoplasmic reticulum (ER) stores, it is removed (a) by plasma membrane Ca-ATPase (PMCA) and (b) by endomembrane

Ca-ATPase back into internal stores. The former PMCA is helped by protein kinase A and will be supported by use of phosphodiesterase 4 (PDE4) inhibitors or adenosine A2a agonists.

10.2 Neutrophil Hyperactivation

Neutrophil activation occurs by Ca^{2+} -dependent and -independent means. Ca^{2+} dependence refers to how Ca^{2+} ions entering the cell through Ca^{2+} channels, or those released from the ER in response to IP_3 , activate PKC and hence the NADPH oxidase. Priming of resting neutrophils by the cytokines $\text{TNF}\alpha$, GM-CSF or G-CSF or activation of primed neutrophils by FMLP, C5a, LTB_4 , PAF or IL-8 acting on 7-TM receptors leads to activation of Src kinases and so to induction of PI3K, which will activate the membrane NADPH oxidase (Fig. 10.1). In addition, the surface Fc receptors for immunoglobulin G (IgG) (Fig. 9.10) or $\beta 2$ -integrins (Fig. 9.11) cause activation of phosphotyrosine kinases (PTKs), and so PLC, which releases DAG that stimulates PKC to cause NADPH oxidase activation. Movement of Rac2 to the NADPH oxidase triggers activity (Fig. 10.2). Primed activated neutrophils adhere to the endothelium of capillaries and release superoxide anions as they move out into the extravascular tissue at sites of inflammation. To achieve this, Ras, PI3K, the ERKs and p38 MAPK are all activated [30]. FMLP stimulates the p38 MAPK and hence the respiratory burst [31]. When p38 MAPK is activated, there is also induction of cytosolic phospholipase A2 (Fig. 6.2) so that there is liberation of arachidonic acid, which is used to form leukotrienes.

In the course of sepsis, complement component C5a stimulates its receptors on neutrophils, inducing chemotaxis, production of ROS [32] and cell survival (Fig. 10.4). The action of C5a on neutrophils is to activate PI3K so that antiapoptotic Bad is formed, and that stops release of cytochrome C from mitochondria. p38-MAPK signals survival by phosphorylating the caspases 8 and 3. There is NF- κ B activation but preservation of mitochondrial polarisation [33] and reduced activity of caspases.

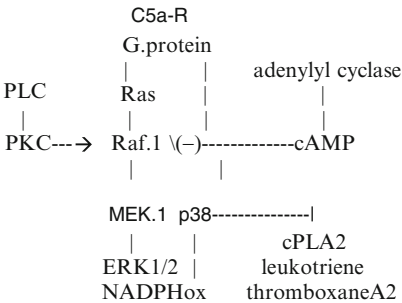


Fig 10.4 Stimulation of neutrophils by C5a. With C5a as agonist, there is no use of phospholipase D, as is the case for *N*-formyl-methionyl-leucyl-phenylalanine (FMLP)

Of course, means of offsetting neutrophil hyperactivation are contemplated. A combination of hypertonic saline infusion with pentoxifylline will increase intracellular cAMP and so restrain the oxidative burst. There is still some MAPK phosphorylation. C5a receptor blockage is imminent [33b].

There is now appreciation that IL-67 drives PMN activation [33c]. Neutrophils that have been activated as a consequence of trauma or burns demonstrate enhanced Ca^{2+} elevation and increased signalling via PKC, thereby explaining their exaggerated superoxide anion generation [34, 35]. These findings indicate how damage to vital organs in multiorgan failure (MOF) will be mediated by ROS. It is well known that adult respiratory distress syndrome (ARDS) arises from entry into the lungs of activated neutrophils [36]. Endotoxaemia is a common cause. Lipopolysaccharide (LPS) (endotoxin) primes the neutrophil respiratory burst in two separate ways [37]. When serum is present, LPS interacts with CD14 receptors on the neutrophils, and so the NADPH oxidase produces superoxide (which cannot be suppressed by IL-10). Otherwise, independently of serum, LPS interacts with CD14 receptors to release PAF and LTB₄, and there is induction of NADPH oxidase (inhibited by IL-10).

Nrf2 has been found to be a critical regulator of oxidative stress in sepsis [38]. By regulating glutathione S-transferase and intracellular glutathione (GSH), Nrf2 controls intracellular ROS and thereby NF- κ B activation. Nrf2^{null} mice show enhanced expression of TNF α and thus severe lung injury after administration of LPS. Nrf2 regulates various proinflammatory cytokines and associated chemokines.

When LPS interacts with neutrophils, surface lipid rafts are implicated, for the p38 MAPK and the Rho family GTPase cdc42 enter rafts, and there also are CD14 receptors and the Toll-like receptors 4. So, there is cell activation from the cholesterol-rich environment of the raft [39]. Interestingly, LPS inhibits any tendency to apoptosis of neutrophils by induction of cIAP-2, with resulting degradation of caspase 3 by the proteasomes [40]. Yet, in patients with septic shock there is elevation of ceramide in proportion to the plasma TNF α [41]. Elevation of ceramide within phagocytes will certainly lead to apoptosis, albeit that might be offset if ceramide stimulates PI3K and thereby Akt [42].

10.2.1 Stimulation of Phagocytes by Colony-Stimulation Factors

GM-CSF or G-CSF stimulate PI3K and thence Akt, so they protect neutrophils from apoptosis (Sect 8.5). Furthermore, GM-CSF stops the recruitment of Fas-associated death domain (FADD). The regulator Lyn is recruited to lipid rafts in PMNs, albeit the action of SHP-1 phosphatase keeps its activity low. GM-CSF action releases SHP-1 [43]. However, that action does not occur in PMNs of the elderly, which are weakened. Macrophage colony-stimulating factor (M-CSF) acts on monocyte macrophages to regulate immediate and delayed “early response

genes” for cell proliferation. There is a brake on its action when SHIP2 (phosphatase) associates with the receptor and prevents too much Akt or NF-κB activation [44].

10.3 Fc Receptor-Mediated Phagocytosis

The Fc receptors FcγRIIA have particular importance for protection against pathogens, even more so if there is complement deficiency (Fig. 10.5). Release of arachidonic acid by cPLA2 (cytosolic phospholipase A2) in myeloid cells leads to formation of prostaglandin E2 (PGE2), which can act on EP4 receptors so that there is activation of protein kinase A, which phosphorylates cAMP response element-binding protein (CREB) (Fig. 4.8). CREB-CRE (cAMP response element) binding on DNA activates the FcγRIIA gene and so protein expression [45].

FcγRs like FcγRI, RIIA and RIIA use immunoreceptor tyrosine activation motifs (ITAMs), and they activate cells. Yet, the most widely expressed receptors are the inhibitory FcγRIIB, which are linked to ITIMs. They recruit SHP-1 and other phosphatases to stop tyrosine phosphorylation of Syk (spleen tyrosine kinase) [46] and thereby dampen reactivity of their cells. Deficiency (or polymorphism) of FcγRIIB receptors could be a basis for autoimmune disease [47]. Thus, FcγRIIB knockout mice develop autoantibodies and strain-dependent glomerulonephritis.

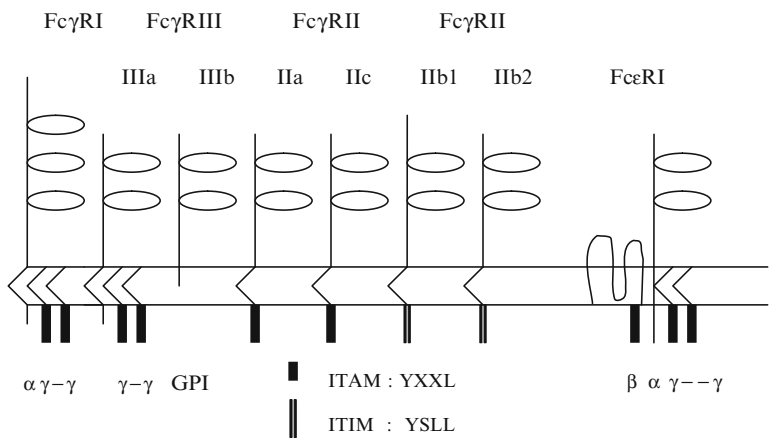


Fig 10.5 Fc receptors on neutrophils and macrophages. Fcγ receptors for immunoglobulin G (*IgG*) complexes act as triggers for inflammatory, cytolytic, allergic, endocytic and phagocytic activities of human effector cells. FcγRI are receptors for IgE-mediated reactions. An immunoreceptor tyrosine activation motif (*ITAM*) indicates an activating receptor. An ITAM has a Tyr in a motif YXXL that is target for phosphorylation by Src family tyrosine kinases (*SFKs*) and subsequent binding of proteins with -SH2 (Src-homology domain 2) domains. An ITIM (YSLL) means that the receptor will inhibit cellular activation

One can demonstrate both activating and inhibitory Fc γ receptors on monocytes [48]. Cross-linking of the Fc γ RIIA leads to cytokine production or phagocytosis, whilst cross-linking of Fc γ RIIB receptors leads to a reduction of monocyte effector functions. IL-10 upregulates all the receptors. TNF α decreases the inhibitory Fc γ RIIBs. IL-4 or IL-13 downregulate the activating receptors [49].

There is modulation of Fc γ R-mediated signalling by other membrane molecules called *signal regulators* [50]. Thus, the Siglecs (sialic acid-binding Ig-like lectins) have ITIM sequences. Siglecs protect cells from aggressors [51].

Receptors for the Fc tail of IgG belong to the immunoglobulin superfamily. They work with adhesion molecules CD11/CD18 and other integrins. On neutrophils, there are Fc γ RII (CD32) receptors (40 kDa, 20–40,000 per cell) and Fc γ RIII (CD16) (50–80 kDa, 100–300,000 per cell). There are also Fc γ RI that are induced by cytokines G-CSF and interferon- γ (IFN γ). When IgG opsonizes bacteria to facilitate their phagocytosis, this *type I phagocytosis* uses Fc γ Rs, so that Rac1 and cdc42 (Fig. 9.8) induce actin extensions that close around in a zipper mechanism. Not surprisingly, LTB₄ enhances Fc γ receptor phagocytosis [52]. In *type II phagocytosis*, complement receptors CR1, CR3 or CR4 help a particle sink through the plasma membrane, helped by just a little actin polymerisation induced via Vav1/3 $\rightarrow$ RhoA [53].

In the process of phagocytosis, bacteria will be coated by IgG antibody (Fig. 10.6). Hence, cross-linking of Fc γ RIIA and Fc γ RIIIB receptors [54] leads to ingestion of opsonized pathogens or agglutinated immune complexes. The process stimulates secretion of ROS and release of enzymes from the granules of the phagocytic cells.

Actually, Fc γ RIIIB receptors are short GPI-linked proteins that float in the lipid bilayer of the cell membrane. They are recruited into membrane lipid rafts with

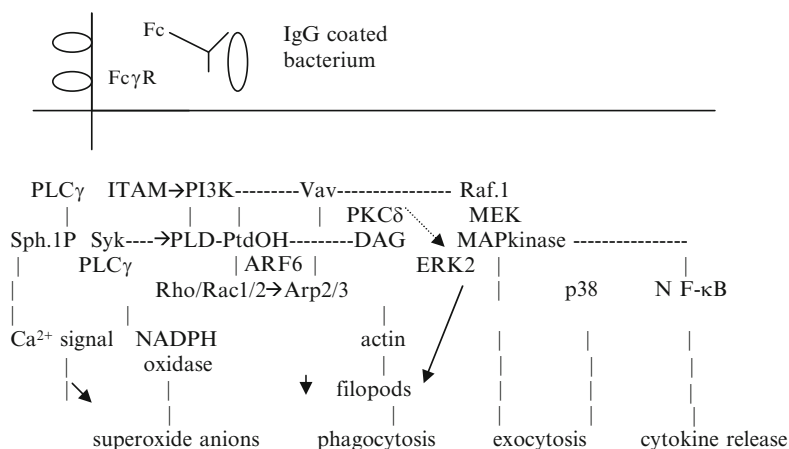


Fig. 10.6 Fc receptors stimulate phagocytosis. The guanine-nucleotide exchange factor (GEF) Vav activates Rho, Rac, cdc42 guanosine triphosphatases (GTPases) $\rightarrow$ actin formation. The adaptor Caprin (a Ras GTPase-activating protein [GAP]) brings cdc42 and Rac to the Fc γ receptor

the FcγRIIA receptors that have ITAMs on their cytoplasmic tails; thus, they are able to transmit a signal [55]. Moreover granule proteins of PMNs trigger macrophage phagocytosis [55b].

Figure 10.6 shows how surface Fcγ receptors connect with the MAPK cascade. There is activation of phospholipase D and hence PKCδ and ERK2, which phosphorylates and activates myosin light-chain kinase, leading to the formation of filopods and phagocytosis [56]. Study of murine Rac1/2^{null} bone marrow macrophages revealed deficiency of both FcγR- and CR3-mediated phagocytosis [57]. Actually, FcγR-mediated phagocytosis leads to Rac1/2 activation and so Arp2/3 recruitment and actin fibre assembly. Vav proteins serve as Rac GEFs in the process of CR3 phagocytosis [58, 59].

p38 MAPK is implicated in neutrophil degranulation [60]. Release of cytokines is not part of phagocytosis. Cytokine inhibition occurs because the adaptor protein Shc connects the ITAM of the FcγRIIA to SHIP phosphatase, which leads to inhibition of NF-κB (Chap. 11). However, murine macrophages performing FcγR phagocytosis do have oxidase activation [61] and some cytokine release [62].

One will note that cytokines IL-1β, IL-6 and TNFα are not released by neutrophils, but M-CSF, IL-8 and IL-1Ra are [63] and so are chemokines macrophage inflammatory protein 1α (MIP-1α), MIP-1β and GROα.

When neutrophils are activated by their FcγRIIA or FcγRIIIB receptors, ERK1/2 and p38 kinases are activated. PI3K is implicated in both forms of activation. FcγRIIA activates Src tyrosine kinase Fgr, whilst FcγRIIIB activates Hck but not Fgr. FcγRIIA cross-linking causes greater ERK activity, and FcγRIIIB cross-linking stimulates p38 more [61]. Both FcγRIIA and FcγRIIIB stimulate the respiratory burst, and FcγRI stimulation yields more hydrogen peroxide. Ingestion of bacteria by means of FcγRII induces the microbicidal response better than FcγRIII binding. FcγRIIIB receptors, which are highly expressed by human neutrophils, induce both β2- and β1-integrins, whilst cross-linking of FcγRIIA upgrades only β2-integrins [63]. Integrins signal by mechanisms comparable to immunoreceptors [64].

Therefore, note the role of Syk (Fig.10.6) as initiating tyrosine kinase during ITAM-dependent phagocytosis in both PMNs and macrophages [65, 66]. ROS aid Syk activation. When IgG-coated targets are involved, so is LTB₄, which is produced when Syk-induced Ca²⁺_i activates 5-lipoxygenase. LTB₄ acting on external receptors then amplifies Syk activation. The usual Syk-induced activation of PLCγ and PI3K leads to production of phosphatidyl-inositol (PtdIns) messengers and a sustained increase of cytosolic Ca²⁺. Syk leads to the assembly of actin cytoskeleton for surface movements, a process that is controlled by GTPases [67, 68] (Chap. 9). Syk also promotes the role of β-integrins in the phagocytic activation process [69]. Integrins lead to (a) actin rearrangements, (b) generation of ROS and (c) discharge of granules.

Involvement of inhibitory FcγRIIB for IgG in opposition to stimulatory receptors determines the state of cell activation during interaction with immune complexes. When an inhibitory receptor is coligated, SHIP is recruited, and it hydrolyses PIP₃ to PIP₂, and there is negation of Ca²⁺ influx. SHIP causes dissociation from

the membrane of the pleckstrin homology (PH) domain containing proteins PLC γ and Bruton's tyrosine kinase (Btk). SHIP, by its hydrolysis of PIP₃, also stops recruitment of survival factor Akt. Lyn also is a negative regulator of integrin signalling. Lyn phosphorylates p62 negative regulatory Dok proteins, which then recruit SHIP.

Sphingosine phosphate [70] mobilises Ca²⁺ ions, helping the formation of superoxide anions, phagocytosis or exocytosis of granules. Conversely, buildup of ceramide terminates phagocytosis. ERK1/2 are then inhibited [71].

There is another aspect. A molecule of the Ig superfamily called *Trem* has been identified on PMNs and monocytes. Trem works in association with a Dap12 molecule (Fig. 15.3). Stimulation of Trem [72] leads to neutrophil degranulation and release of TNF α and chemokines IL-8 and MCP-1. Generally, Trem amplifies cytokine-induced inflammation.

Activated neutrophils adhere to endothelial cells or to extracellular matrix proteins. When β 2-integrin (CD11/18) leads to neutrophil adhesion (i.e. spreading and shape change), phosphorylation of c-Cbl plays its role [73]. Cbl src-kinases with PI3K are then translocated to the plasma membrane. The PIP₃ that is formed helps spread the phagocyte. During sepsis, C5a-dependent induction of β 2-integrin enhances the ability of neutrophils to pass through the capillary walls [74].

10.4 Movement of Leucocytes in Response to Chemoattractants

Migration of leucocytes to sites of bacterial invasion is determined by the local release of chemokines [75], which sit strategically on endothelial cells. The chemoattractants include sphingosine-1-P and lysophosphatidic acid and chemokine IL-8 attracts neutrophils (PMNs). As discussed in Sect. 9.4, the IL-8 response is mediated by a G protein-coupled receptor (GPCR). The neutrophil response to bacterial peptide FMLP is similar (cf. Fig. 10.1). (a) When a chemokine binds to its leucocyte receptor, a GPCR, there is homo- or heterodimerisation of receptors. A tyrosine is thus exposed in the receptor DRYLAIV motif. Accordingly, Jak2 causes phosphorylation of that receptor, and the receptor and its G protein are activated [76, 77]. The action of most chemokines is inhibited by pertussis toxin since Gi protein is involved in signalling. (b) G α i activate Src kinases [78] and so PI3K, thus leading to rapid and transient formation of PtdIns(3,4,5)P₃. PI3K γ is used to recruit neutrophils during inflammation [79]. Other PI3Ks are activated by G protein $\beta\gamma$ -units [80]. (c) PI3Ks form 3-phosphorylated lipids that activate PKC, Akt, Ras and via Vav the small GTPases Rho, Rac and cdc42. Cdc42 causes actin formation for directional migration of leucocytes [81]. (d) G $\beta\gamma$ -units activate PLC so that DAG and IP₃ are formed. Intracellular calcium is mobilised, and adenylyl cyclase is inhibited. (e) G $\beta\gamma$ -units cause Ras activation and hence the MAPKs and cytoskeletal elements at the leading edge of the cell. Ras activates

Rac and so JNK. (f) Chemokine-triggered signals lead to the activation of integrins [82a, 82b] (Fig. 10.8). (g) $G\alpha_{16}$ couples chemoattractant receptors to NF- κ B activation [83]. In brief, small GTPase-regulated pathways like RhoA activity have emerged as regulators of the adhesiveness provided by integrins (Sects. 10.6 and 10.7). Deadhesion of uropods is attributed to RhoA. It would be useful in therapeutics to have inhibitors of the GPCRs CXCR1 and CXCR2. The pepducins are peptide inhibitors derived from the third intracellular loop of GPCRs. When neutrophils are migrating, their chemokine receptors are focussed within the lipid rafts at the leading edge [81], and at the front there is a sharp gradient of $\text{PtdIns}(3,4,5)\text{P}_3$ and hence accumulation of F-actin (Fig. 9.7). PI3K products will lead via adaptor protein SLP76 to the activation of p44/42 MAPKs and the p38 MAPK. Recall that neutrophils are really p38 dependent. A new Rac GEF called P-Rex-1 mediates Rac activation in response to PIP3 (Fig. 9.7). At the rear end of the cell is PAK, which is implicated in the assembly of myosin II, so that the cell is driven forwards [80, 81].

Lymphocyte adhesion and migration are determined by the G12/13 effector RhoA (Fig. 10.7). Also Rap GTP binds the effector molecule RapL, which associates with LFA-1 integrins to determine cell adhesion [84] and polarisation [85–87]. Thus, the chemokine SDF1 α (CXCL12) is known to activate LFA-1 adhesion to ICAM via PLC γ -mediated formation of DAG and PKC. The ICAM1-Rho signal path, by using the adaptor proteins LAT (Linker of Activated T cells) and SLP76, will also support lymphocyte migration [88]. Furthermore, there is in lymphocytes a scaffold protein Cybr that (a) regulates LFA-1 and so mediates attachments and (b) binds cytohesin-1 so that there is actin cytoskeleton remodelling during cell migration [89]. The mechanism of lymphocyte migration differs from that of neu-

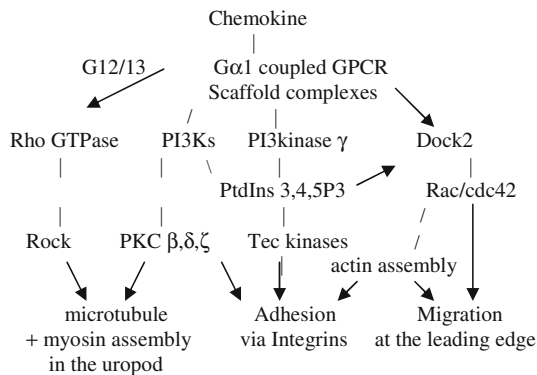


Fig 10.7 Lymphocyte migration in response to chemokine. This is a hypothetical scheme; the details are being worked out [94, 95]. Rock (*Rho kinase*) is a downstream effector of Rho guanosine triphosphate (*GTP*) that regulates the synthesis of $\text{PtdIns}(4,5)\text{P}_2$ by phosphatidylinositol phosphate kinase 1 (*PIPK1*) [96]. Dock2 (dedicator of cytokinesis 2) stirs lateral mobility of lymphocytes [97] or adherence to VCAM1

trophils [82b] for in lymphocytes there is a Dock2 (dedicator of cytokinesis 2), which is activated by chemokine receptors to induce Rac/cdc42 and thereby actin formation so that there can be attachment to VCAM1 on endothelium [90].

As for *production of chemokines* by monocyte macrophages, activated endothelial cells or other cells responding to microbial invasion, those cells utilise receptor activation of Ras and hence $\text{ERK} \rightarrow \text{Elk1}$, along with activation of CaN and so the NFATc and thus chemokine genes [91]. The chemokine promoters are also stimulated by PI3K-mediated activation of $\text{Raf} \rightarrow \text{ERK}$, by p38 MAPK and by NF- κ B. When neutrophils are activated by $\text{TNF}\alpha$ (and other factors), they release chemokine MCP-1, which ensures that monocytes and lymphocytes are attracted, as in the delayed hypersensitivity reaction [92]. For actual neutrophil recruitment (e.g. into the peritoneum), obtained by studies on mice, chemokine MIP-2 determines release of MIP-1 α , thence $\text{TNF}\alpha$ and so LTB4 formation [93].

P-Rex is a Rac GEF that is abundant in neutrophils and monocytes but not in lymphocytes. So wiring of Rac GEF signal networks must differ. Yet as in lymphocytes (Fig. 10.7) Dock 2 contributes to F-actin formation [82b].

10.5 Activation of Eosinophils

The eosinophils are proinflammatory granulocytes dedicated to allergic reactions and parasitic infections. Often, they are attracted by macrophage inhibitory factor (MIF). Like the neutrophils, they undergo enhanced oxidative metabolism [98a]. Adhesion and activation of eosinophils utilises β 2-integrin CD11b in company with CD66b of the carcinoembryonic antigen family CEACAM8 [98b]. Discharge of their granules releases toxic eosinophil cationic protein and major basic protein [99]. Cellular adhesion by means of α M β 2 (CD11b/CD18) and integrin α 4 β 1 (VLA4) is crucial for their degranulation [100]. That process involves a phosphotyrosine kinase activating PLC γ 2, so that there is formation of DAG to activate PKC, and IP3, which leads to release of Ca^{2+} ions. Involvement of PI3K (Fig. 10.1) leads to activation via Ras of the ERK pathway and the p38 MAPK path. By the use of specific pathway inhibitors, it has been shown that the p38 MAPK plays the greater role in granule discharge [101]. PI3K activates cPLA2, so that released arachidonate can form leukotriene C4 (LTC4) [102]. Rho and ROCK (i.e. Rho-associated coiled-coil forming protein kinase), play their part in actin rearrangements for the extrusion [96]. The regulated exocytosis uses the v-SNARE/t-SNARE docking system, meaning that VAMP (vesicle-associated membrane protein) on the vesicles interacts with Snap23 and syntaxin 4 at the cell membrane [103] [Sect. 9.7].

Eosinophil trafficking is determined by adhesion molecules. Whereas neutrophils bind C-X-C chemokines, eosinophils bind C-C chemokines. PAF induces five times the generation of superoxide in eosinophils compared with adherent neutrophils. IL-5 is a principal attractant and activator of eosinophils, as is eotaxin. Inhibitors of the eotaxin/CCR3 pathway are being developed. IL-5 induces PI3K, as

do other chemoattractants PAF, C5a and Rantes [104]. In turn, PI3K activates 5-lipoxygenase and thereby the eosinophils [105].

Eosinophils possess IDO (indole 2,3 di-oxygenase), which enables them to maintain Th2 cells around them and even to determine Treg cells [106]. Actually, integrins can determine both Th1 and Th2 cells.

10.6 Integrin-Mediated Adhesion of Phagocytes

When PMNs are activated, they shed their surface sialoprotein CD43 called leuko-sialin. Hence, they lose their negative charge, and they can adhere to surfaces. So, there is adherence to endothelial cells of the postcapillary venules prior to emigration into any inflammatory focus, and there will later be adherence to extracellular matrix proteins. When PMNs are adherent via their integrins, they perform much better. Platelet-derived microparticles actually transfer GPIIb/IIIa receptors to neutrophils, and they work along with β 2-integrins [107]. Integrins connect to intracellular actin (Fig. 10.8) via the proteins talin-filamin-14-3-3, and the process is regulated by Rap1 small GTPase [108, 109]. Also, surface L-selectin on microvilli (Fig. 9.13) connects with the ERM (ezrin, radizin, moiesin) protein moiesin and so with α -actinin. Similarly, PSGL-1, ligand for endothelial P-selectin, is anchored to the actin cytoskeleton via the ERM protein ezrin.

Neutrophil-priming agents like LTB4, C5a, FMLP, PAF or IL-8 and the cytokines TNF α and GM-CSF all upregulate PMN adhesion molecules like the integrin α M β 2 (Mac-1, CD11b/CD18) within minutes. Performance is enhanced. The integrins lack intrinsic enzyme activity, but they utilise adaptor molecules like p130 Cas for their signalling [110–112] (Fig. 10.8). Cas provides connections to actin polymerisation. p130 Cas silencing by RNA interference inhibits Src-dependent lamellipodial extensions and migration. Stimulation of β 2-integrins (CD11/18) leads to phosphorylation of c-Cbl and so to adhesion, spreading and cell shape change [76]. In some cells, c-Abl tyrosine kinase leads to transient activation of MAPK as cells form attachments to fibronectin (Fig. 5.8).

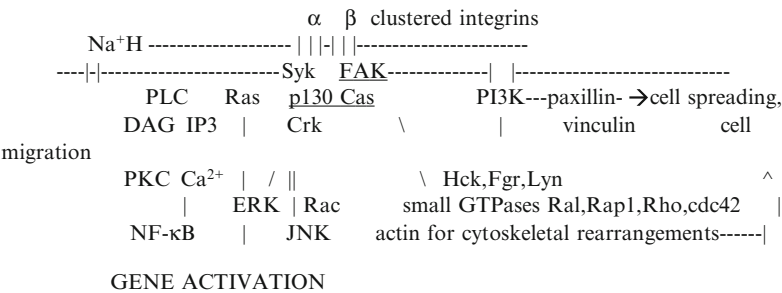


Fig 10.8 Integrin outside-in signalling

Wasp (Wiskott-Aldrich syndrome protein) (Fig. 9.5) helps to determine clustering of integrins on phagocytes and DCs. Such clustering stems from actin rearrangements, which also mediate migration, degranulation and respiratory burst activity of leucocytes. Src tyrosine kinases like Hck, Fgr and Lyn control some events [28, 113]. Fgr or Hck can negatively regulate responses to chemokines. Csk is a negative regulator of cell adhesion [114].

Phosphorylation of focal adhesion kinase (FAK), which is attached to the intracellular domain of $\beta 1$ -integrins, leads to (a) spreading and migration of cells; (b) expression of cyclins, c-fos and c-myc and thus cell proliferation; and (c) release of IL-1 and TNF α proinflammatory cytokines. There is an FAK-related protein Pyk2, protein-rich tyrosine kinase-2, whose phosphorylation helps integrin-dependent activation of the neutrophils. Rho family GTPases are intermediaries in adhesion-dependent cell cycle progression. Thus, integrins connect with Rac/cdc42 so that cyclin D1 is activated (Fig. 17.1), and integrin acting via RhoA stops the inhibitory p27^{kip.1} so that cyclin E is activated.

Stimulation of small GTPases by cell adhesion (Fig. 9.2) enhances integrin functioning. Rac and cdc42 are activated rapidly in cells like fibroblasts [115, 116] and likewise in leucocytes. Both contribute to formation of filopodia and lamellipodia and thus to spreading on a matrix. cdc42 will activate PI3K and so Akt, and PAK, MAPK and JNK are induced. Paxillin [117] is an adaptor protein, with both SH2 and SH3 binding domains, that has a facilitating action on integrin-mediated adhesion via its connection to focal adhesion kinase.

When cells are treated with botulinum C3 exoenzyme, which ADP ribosylates and inactivates Rho GTPase, integrin-mediated adhesion is blocked in various cell types [118]. Clearly, Rho GTPase helps to activate adhesion by integrins. Integrins and syndecan cooperate to regulate Rho [118]. When cells are plated on fibronectin, Rho activity increases as focal adhesion contacts and stress fibres are formed (cf. Fig. 9.2). The proteoglycan syndecan 4 acts as a coreceptor for cell adhesion to extracellular matrix [119]. One will ask how integrin LFA-1 (CD11/CD18) can be expressed on white cells in response to a chemokine like MIP [82b]. Chemokine receptors activate Ras together with PI3K, so there is

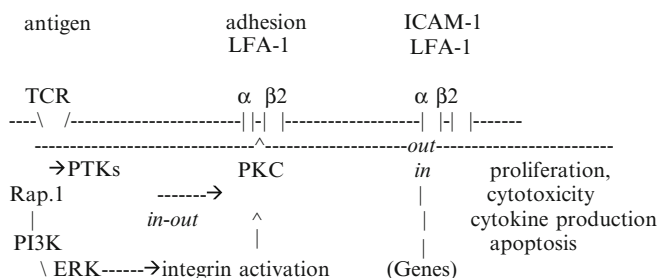


Fig. 10.9 The bidirectional function of leucocyte function-associated antigen 1 (LFA-1) molecules. LFA-1 (CD11a,CD18) is a 180-kDa dimer, α -chain plus $\beta 2$ -chain, that is, $\alpha L\beta 2$

F-actin polymerisation and expression of LFA-1 [76, 120]. This is inside-out signalling.

10.7 Integrin LFA-1 as Regulator of Leucocyte Adhesion

Leucocyte function-associated antigen 1 (Fig. 10.9) has two-way signalling [120]. Accordingly, LFA-1 mediates T lymphocyte adhesion; concurrently, T cells are activated (Fig. 10.9). Signalling is in-to-out when activation of the T cell receptor (TCR) leads to activation of PTKs, so that LFA-1 participates in adhesion. Conversely, there is out-to-in signalling when LFA-1 binds to endothelial or tissue ICAM molecules, generating intracellular signal transmission that affects lymphocyte gene transcription and thereby cell proliferation or cytotoxicity. ICAM1/LFA-1 interactions could form a “T cell sensor”, especially as LFA-1 acts as costimulator for antigen-induced activation of T lymphocytes [121].

In the nonactive state, LFA-1 integrin is tethered by its β -chain, bearing a TTF motif, to the cytoskeletal actin. There is a regulatory protein MacMARCKS (macrophage-enriched myristoylated alanine-rich C-kinase substrate) in a submembranous position. TCR activation generates PKC and activates MacMARCKS. The rise of Ca^{2+} induces local calpain so that LFA-1 is freed from constraint by the cytoskeleton. This allows LFA-1 to form clusters; thus, ligand ICAM1 binds. The *adhesisome* mediates signalling from outside-to-inside the cell [122]. Thereby, MacMARCKS and associated scaffold protein Rack-1 (receptor for activated PKC 1) and cytoadhesin-1 induce cortical actin fibre formation. The activation of LFA-1 is promoted by (a) PKC, (b) PI3K, (c) Rho GTPase and (d) Rap1 small GTPase (Fig. 10.9). Interestingly, ICAM1 is necessary for the activation of CD28-deficient T lymphocytes [123] which otherwise have no costimulation.

Whereas neutrophils adhere by means of their β 2-integrins, LFA-1 to ICAM1, macrophages use the β 1- and β 3-integrins, such as VLA4, which is α 4 β 1 to VCAM1. Importantly, when C1q acts on PMNs, inducing ROS, β 2-integrins synergise with ICAM1 to enhance ROS formation [124]. However, when there is much ICAM1 on stromal cells, C1q will bind there, so formation of superoxide by PMNs will be restricted [125].

10.8 Th17 cells via IL-17 drive neutrophils and inflammation

Despite its late discovery, this cytokine is one of the oldest, and a serious cause of concern. Th17 cells secrete a range of cytokines including IL-17, which causes chemokine induced attraction of PMNs and macrophages, so causing inflammation as in auto-immune diseases [126–128] and allergy [129]. Even NK cells and $\gamma\delta$ T cells can produce IL-17 [130]. IL-17A homodimer and IL-17 F/A heterodimers account for the accumulation of neutrophils in airways inflammation [128].

10.9 Communication between Neutrophils and Dendritic Cells

There are two-way communications between neutrophils and DCs [131]. DCs can prolong the life span of neutrophils when DCs play a role in recruitment of neutrophils to inflamed tissues. Via the surface receptor C-type lectin DC-Sign, DCs interact with CD11b/CD18 (Mac1) and with CEACAM on the neutrophils so that apoptosis is offset. Conversely when neutrophils are in lymph nodes, they can stimulate the DCs to release IL-12 so that development of Th1 lymphocytes is promoted.

References

1. Dewald B, Baggiolini M. Activation of NADPH oxidase in polymorphonuclear leukocytes: synergism between FMLP and neutrophil products PAF and leukotriene B₄. *Biochem Biophys Res Commun*. 1985;128:297–304
- 2a. Fu H, Karlsson J, Bylund J et al. Ligand recognition and activation of formylpeptide receptors in neutrophils. *J Leukoc Biol* 2006;79:247–256
- 2b. Paruch S, El-Benna J, Djerdjuri B. Role of MAP kinases in FMLP mediated phospholipase D activity and antioxidant production. *FASEB J* 2006;20:142–144
3. Niwa M, Kozawa O, Matsuno H et al. Involvement of sphingomyelin metabolites in the priming of TNF α on FMLP stimulated superoxide production. *Life Sci* 2000;66:245–266
4. Condliffe AM, Kitchen E, Chilvers ER et al. Neutrophil priming: pathophysiologic consequences and underlying mechanisms. *Clin Sci* 1998;94:461–471
5. Mackinnon AC, Buckley A, Chilvers ER et al. Sphingosine kinase: a point of convergence in the action of diverse neutrophil priming agents. *J Immunol* 2002;169:6394–6400
6. Clark RA. Human neutrophil respiratory burst. *J Infect Dis* 1990;161:1140–1147
7. Reeves EP, Lu H, Jacobs HL et al. Killing activity of neutrophils is mediated through activation of proteases by K⁺ flux. *Nature* 2002;416:291–297
8. Roda BK, Geiszt M, Koldi K et al. Dual role of phagocytic NADPH oxidase in bacterial killing. *Blood* 2004;104:2947–2953
9. Peyssonnaud C, Datta V, Cramer T et al. HIF-1 α expression regulates the bactericidal capacity of phagocytes. *J Clin Invest* 2005;115:1806–1815
10. Megiovanni AM, Sanchez F, Robledo-Sarmienter M et al. PMN neutrophils deliver activation signals and antigenic molecules to dendritic cells. *J Leukoc Biol* 2006;79:977–988
11. Weichhart T, Costantino G, Poglitsch M et al. The TSC-mTOR signaling pathway regulates the innate inflammatory response *Immunity* 2008;29: 565–577
- 12a. Jia Y, Subramanian KK, Erneux C et al. Inositol 1,3,4,5 tetra-kisphosphate negatively regulates PI3,4,5 trisphosphate signaling in neutrophils. *Immunity* 2007;27:453–467
- 12b. Billadeau DD. PTEN gives neutrophils direction *Nat. Immunol* 2008;9:716–718
13. Canetti C, Serezani CH, Atrasz RG et al. Activation of PTEN on chromosome 10 mediates inhibition of Fc γ R phagocytosis by prostaglandin E₂. *J Immunol* 2007;179:8350–8356
- 14a. Levy BD, Hickey L, Morris AJ et al. Novel PIPs block phospholipase D and human neutrophil activation. *Br J Pharmacol* 2005;146:344–351
- 14b. Bonnans C, Fukunaga K, Keledjian R et al. Regulation of PI3kinase by polyiso-prenylphosphates in neutrophil mediated tissue injury. *J Exp Med* 06,203:857–863
15. Thompson HL, Shiroo M, Saklatvala O. The chemotactic factor N-FMLP activates microtubule associated protein-2 and MAP kinase in polymorphonuclear leucocytes. *Biochem J* 1993;200:483–488

16. Dewas C, Fay M, Gougert-Pocidallo M-A et al. MAPK ERK 1–2 pathway is involved in FMLP induced p47phox phosphorylation in neutrophils. *J Immunol* 2000;165:5238–5244
17. Nick JA, Audi NJ, Young SK et al. Common and distinct pathways in human neutrophils used by PAF and FMLP. *J Clin Invest* 1997;99:975–986
18. Le Y, Murphy PM, Wang JM. Formyl peptide receptors revisited. *Trends Immunol* 2002;23:541–548
19. Kim C, Marchal CC, Penninger J, Dinauer MC. The hemopoietic Rho/Rac guanine nucleotide exchange factor Vav1 regulates N-FMLP activated neutrophil function. *J Immunol* 2003;171:4425–4430
20. Daniels RH, Bokach GM. p21 activated protein kinase, a crucial component of morphological signaling. *Trends Biochem Sci* 1999;24:350–355
21. Carpenter CL, Tolias KF, van Vogt A, Hartwig J. Lipid kinases are novel effectors of the GTPase Rac.1. *Adv Enzyme Regul* 1999;39:299–312
22. Roberts RL, Barbieri MA, Ullrich J, Stahl PD. Dynamics of Rab activation in endocytosis and phagocytosis. *J Leukoc Biol* 2000;68:627–632
23. Gaudreault E, Thompson C, Stankowa J, Rola-Pleczynski M. Leukotriene B4 induced neutrophil degranulation. *J Immunol* 2005;174:3617–3625
24. Carstanjen D, Yamauchi A, Koorneef A et al. Rac2 regulates neutrophil chemotaxis, superoxide production and myeloid colony formation through distinct effector pathways. *J Immunol* 2005;174:4613–4620
25. Dang PMC, Stensballe A, Boussetta T et al. A specific p47 phox-serine phosphorylated by convergent MAPKs mediates neutrophil NADPH oxidase priming at inflammatory sites. *J Clin Invest* 2006;116:2033–2043
26. Condliffe AM, Webb LM, Ferguson GJ et al. Rho G regulates neutrophil NADPH oxidase. *J Immunol* 2006;176:5314–5320
27. Utumo A, Cullero X, Glogauer M et al. Vav proteins in neutrophils are required for FcγR mediated signaling to Rac GTPases and NADPHox p40. *J Immunol* 2006; 177:6388–6397
28. Fumagalli L, Zhang H, Baruzzi A et al. The Src family kinases Hck and Fgr regulate neutrophil responses to N-FMLP. *J Immunol* 2007;178:3874–3885
29. El Bekay R, Alvarez M, Monteseirin J et al. Oxidative stress is a critical mediator of the angiotensin II signal in human neutrophils. *Blood* 2003;102:662–671
- 29b. Blüml S, Rosc B, Lorincz A et al. The oxidation state of phospholipids controls the oxidative burst in neutrophil granulocytes. *J Immunol* 2008;181:4347–4353
30. Fearn C, Kline L, Gram H et al. Coordinate expression of endogenous p38α,β,γ and by inflammatory stimuli. *J Leukoc Biol* 2000;67:705–711
31. Rane MJ, Carrithers SL, Arthur JM, Klein JH, McLeish KR. Formyl peptide receptors are coupled to multiple MAP kinase cascades by distinct signal transduction pathways: role in activation of reduced NADPH oxidase. *J Immunol* 1997;159:5070–5078
32. Guo R-F, Riedemann NC, Bernacki KD et al. Neutrophil C5a receptors and outcome in a rat model of sepsis. *FASEB J* 2003;17:1889–1891
- 33a. Taneja R, Parodo J, Jia SH et al. Delayed neutrophil apoptosis in sepsis is associated with maintenance of mitochondrial transmembrane potential and reduced caspase activity. *Crit Care Med* 2004;32:1460–1469
- 33b. Rittirsch D, Flierl MA, Ward PA. Harmful molecular mechanisms in sepsis. *Nat Rev Immunol* 2008;8:776–787
- 33c. Flierl MA, Rittirsch D, Gao H et al. Adverse functions of IL-17A in experimental sepsis. *Faseb J* 2008;22:2198–2205
34. Sayeed MM. Exuberant calcium signaling in neutrophils: cause for concern. *News Physiol Sci* 2000;15:130–136
35. Anderson BO, Brown JM, Harken AH. Mechanisms of neutrophil mediated tissue injury. *J Surg Res* 1991;51:130–136
36. Neuhofer H. Actions and interactions of mediator systems and mediators in the pathogenesis of ARDS and multiorgan failure. *Acta Anaesthesiol* 1991;35(s95):71–74

37. Sibelius U, Hattar K, Hoffmann S et al. Distinct pathways of LPS priming of human neutrophil respiratory burst: role of lipid mediator synthesis and sensitivity to IL-10. *Crit Care Med* 2002;30:2306–2312
38. Kolls JK. Oxidative stress in sepsis: a redox redux. *J Clin Invest* 2006;116:860–863
39. Fessler MB, Arndt PG, Frasch SC et al. Lipid rafts regulate LPS-induced activation of cdc42 and inflammatory functions of the human neutrophil. *J Biol Chem* 2004;279:39989–39998
40. Mica L, Harter L, Trentz O, Keel M. Endotoxin reduces CD95 induced neutrophil apoptosis by cIAP-2 mediated caspase 3 degradation. *J Am Coll Surg* 2004;199:595–602
41. Delogu G, Famulano G, Amati F et al. Ceramide concentrations in septic patients: a possible marker of multiorgan dysfunction syndrome. *Crit Care Med* 1999;27:2413–2417
42. Monich MM, Mallampalli RK, Carter AB et al. Ceramide regulates LPS induced PI3-kinase and Akt activity in human alveolar macrophages. *J Immunol* 2001;167:5977–5985
43. Fortin CF, Larbi A, Lesur O et al. Impairment of SHP1 downregulation in lipid rafts of human neutrophils under GM-CSF stimulation contributes to age-related altered functions. *J Leukoc Biol* 2006;79:1061–1072
44. Wang Y, Keogh RJ, Hunter MG et al. SHIP2 is recruited to cell membranes upon M-CSF stimulation and regulates M-CSF induced signaling. *J Immunol* 2004;173:6820–6830
45. Hazan-Eitan Z, Weinstein Y, Hadad N et al. Induction of FcγRIIA expression in myeloid cells is regulated via activation of CREB by PGE2. *Blood* 2006;108:1758–1766
46. Huang Z-Y, Hunter S, Kim M-K, Indik ZK, Schreiber AD. The effect of phosphatases SHP.1 and SHIP.1 on signalling by ITIM and ITAM containing Fcγ receptors FcγRIIB and FcγRIIA. *J Leukoc Biol* 2003;73:823–829
47. Fossati G, Bucknall RC, Edwards SW. Fcγ receptors in autoimmune diseases. *Eur J Clin Invest* 2001;31:821–831
48. Takai R. Role of Fcγ receptors in autoimmunity. *Nat Rev Immunol* 2002;2:580–592
49. Liu Y, Masuda E, Blank MC et al. Cytokine mediated regulation of activating and inhibitory Fcγ receptors in human monocytes. *J Leukoc Biol* 2005;77:767–776
50. Mina-Osorio P, Ortega E. Signal regulators in FcR-mediated activation of leukocytes. *Trends Immunol* 2004;25:529–535
51. Crocker P, Varki A. Siglecs, sialic acids and innate immunity. *Trends Immunol* 2001;22:337–342
52. Canette C, Aronoff DM, Choe M et al. Differential regulation by leukotrienes and calcium of Fcγ receptor induced phagocytosis. *J Leukoc Biol* 2006;79:1234–1241
53. Caron E, Hall A. Identification of 2 distinct mechanisms of phagocytosis controlled by different Rho GTPases. *Science* 1998;282:1717–1721
54. Chuang FYS, Sasseroti M, Unkeless JC. Convergence of Fc γRIIA and FcγRIIIB signaling pathways in neutrophils. *J Immunol* 2000;169:350–360
- 55a. Garcia-Garcia E, Rosales C. Signal transduction during Fcγ receptor mediated phagocytosis. *J Leukoc Biol* 2002;72:1092–1108
- 55b. Soehnlein O, Kai-Larsen Y et al. Neutrophil primary granule proteins HBP and HNP1-3 boost bacterial phagocytosis by human and murine macrophages *J Clin Invest* 2008; 118:3491–3502
56. Mansfield JP, Shayman JA, Boxer LA. Regulation of PMN leukocyte phagocytosis by myosin light chain kinase after activation of MAP kinase. *Blood* 2000;95:2407–2412
57. Hall AB, Gakidis MAM, Glogauer M et al. Requirements for Vav GEFs and Rho GTPases in FcγR and complement mediated phagocytosis. *Immunity* 2006;24:305–316
58. Suh CI, Stull ND, Li XJ et al. The phosphoinositide binding protein p40Phox activates the NADPHoxidase during FcγIIA receptor induced phagocytosis. *J Exp Med* 2006;203:1915–1925
59. Lowell CA. Rewiring phagocytic signal transduction. *Immunity* 2006;24:243–245
60. Mocsai A, Jakus Z, Vantus T, Berton G et al. Kinase pathways in chemoattractant induced degranulation of neutrophils: role of p38 MAP kinase activated by Src family kinases. *J Immunol* 2000;164:4321–4331

61. Coxon PY, Ranc M, Powell DW et al. Differential MAP kinase stimulation by Fc γ RIIA and Fc γ RIIIB determines the activation phenotype of human neutrophils. *J Immunol* 2000;164:4991–4995
62. Ortiz-Stern A, Rosales C. Fc γ RIIB stimulation promotes β 1 integrin activation in human neutrophils. *J Leukoc Biol* 2005;77:787–799
63. Schroder AK, van der Ohe M, Kolling U et al. PMN leukocytes produce anti-inflammatory IL-1Ra and chemokines but fail to produce pro-inflammatory mediators. *Immunology* 2006;119:317–327
64. Jakus Z, Fodor A, Abram CL, Lowell CA, Mocsai A. Immunoreceptor like signaling by β 2 and β 3 integrins. *Trends Cell Biol* 2007;17(10):493–501
65. Raeder E, Mansfield PJ, Hinkovska-Galcheva V et al. Syk activation initiates downstream signal events during human PMN leukocyte phagocytosis. *J Immunol* 1999;163:6785–6793
66. Greenberg S. Modular components of phagocytosis. *J Leukoc Biol* 1999;66:712–717
67. Witko-Sarsat V, Rieu P, Descamps-Layscha B et al. Neutrophils: functions and pathophysiological aspects. *Lab Invest* 2000;80:617–653
68. Berton G, Mocsai A, Lowell CA. Src and Syk kinases: key regulators of phagocytic cell activation. *Trends Immunol* 2005;26:208–214
69. Willeke T, Schymeinsky J, Prange P et al. A role for Syk kinase in the control of β integrins (CD11/18) in human polymorphonuclear leukocytes. *J Leukoc Biol* 2003;74:260–269
70. Choi OH, Kim J-H, Kinet J-P. Calcium mobilisation via sphingosine kinase in signalling by Fc γ RI antigen receptor. *Nature* 1996;380:634–636
71. Suchard SJ, Mansfield PJ, Boxer LA, Shayman JA. Mitogen activated protein kinase activation during IgG dependent phagocytosis in human neutrophils. *J Immunol* 1997;158:4961–4967
72. Bouchon A, Dietrich J, Colonna M. Inflammatory responses are triggered by Trem-1. *J Immunol* 2000;164:4991–4995
73. Willeke T, Behrens S, Scharffetter-Kochanek K et al. β 2 Integrin mediated signaling involves tyrosine phosphorylation of c-Cbl in neutrophils. *J Leukoc Biol* 2000;68:284–292
74. Guo RF, Riedemann NC, Laudes IJ et al. Altered neutrophil trafficking during sepsis. *J Immunol* 2002;169:307–314
75. Rossie D, Zlotnik A. The biology of chemokines and their receptors. *Annu Rev Immunol* 2001;18:217–242
76. Rodriguez-Frade J, Mellado M, Martinez AC. Chemokine receptor dimerization. *Trends Immunol* 2001;22:612–615
77. Mellado M, Rodriguez-Frade JM, Manes S, Martinez AC. Chemokine signaling and functional responses: receptor dimerization and tyrosine kinase pathway activation. *Annu Rev Immunol* 2001;19:397–421
78. Curnock AP, Logand MK, Ward SG. Chemokine signalling: pivoting and multiple PI3kinases. *Immunology* 2002;105:125–136
79. Ferrandi C, Ardisson V, Ferro P et al. PI3kinase γ inhibition plays a crucial role in inflammation by blocking neutrophil recruitment. *J Pharmacol Exp Ther* 07;322:923–928
80. Stephens S, Ellson C, Hawkins P. Roles of PI3Ks in leukocyte chemotaxis and phagocytosis. *Curr Opin Cell Biol* 2002;14:203–213
81. Rickert P, Weiner OD, Wang F et al. Leukocytes navigate by compass: roles of PI 3-kinase. *Trend Cell Biol* 2000;10:466–467
- 82a. Kinashi T. Intracellular signalling controlling integrin activation in lymphocytes. *Nat Rev Immunol* 2005;5:546–559
- 82b. Thelen M, Stein JV. How chemokines invite leukocytes to dance. *Nat Immunol* 2008;9:953–959
83. Yang M, Sang H, Rahman A et al. Ga16 couples chemoattractant receptors to NF- κ B activation. *J Immunol* 2001;166:6885–6892
84. Ghandour H, Cullere X, Alvarez A et al. Essential role for Rap1 GTPase and CalDEG-GEF in LFA1 but not VLA4 integrin T cell adhesion. *Blood* 2007;110:3682–3690
85. Price LS, Bos JL. RAPL: taking the Rap in immunity. *Nat Immunol* 2004;5:1007–1010

86. Katagiri K, Imamura M, Kinashi T. Spatio-temporal regulation of kinase Mst1 by binding protein RAPL for lymphocyte polarity. *Nat Immunol* 2006;7:919–928
87. Shimonaka M, Katagiri K, Nakayama T et al. Rap1 translates chemokine signals to integrin activation and cell motility. *J Cell Biol* 2003;161:417–427
88. Koretzky GA, Abtahien D, Silverman MA. SLP76 and SLP65: complex regulation of signaling in lymphocytes. *Nat Rev Immunol* 2006;6:67–78
89. Coppola V, Barrick CA, Bobisse S et al. The scaffold protein Cybr is required for cytokine-modulated trafficking of leukocytes. *Mol Cell Biol* 2006;26:5249–5258
90. Garcia-Bernal D, Sotillo-Mallo E, Nombela-Arrieta C et al. DOCK2 is required for chemokine promoted T lymphocyte adhesion. *J Immunol* 2006;177:5215–5225
91. Li HA, Ahamed J, Hernandez-Munai C et al. Chemokine production by a G protein coupled receptor activation in a mast cell line. *J Immunol* 2000;169:7215–7233
92. Yamashiro S, Kamohara H, Wang J-M et al. Phenotypic and functional change of cytokine activated neutrophils. *J Leukoc Biol* 2001;69:698–704
93. Ramos CDL, Fernandes K, Canetti C et al. Neutrophil recruitment in immunized mice depends on MIP2. *Eur J Immunol* 2006;36:2025–2034
94. Ward SG. T lymphocytes on the move. *Trends Immunol* 2006;27:57–108
95. Cronshaw DG, Kouroumalis A, Parry A et al. Phospholipase C dependent, calcium-independent mechanisms are required for directional migration of T lymphocytes in response to the CCR4 ligands CCL17 and CCL22. *J Leukoc Biol* 2006;79:1369–1380
96. Adachi T, Vita R, Sannohe S et al. The functional role of Rho and ROCK in eotaxin signaling of eosinophils. *J Immunol* 2001;167:4609–4615
97. Shulman Z, Pasvolsky R, Woolf E et al. Dock2 regulates chemokine triggered lateral lymphocyte mobility but not endothelial migration. *Blood* 2006;108:2150–2158
98. (a) DeChatelet LR, Shirley PS, McPhail LC et al. Oxidative metabolism of the human eosinophil. *Blood* 1977;50:525–535; (b) Yoon J, Terada A, Kita H. CD66b regulates adhesion and activation of human eosinophils. *J Immunol* 2007;179:8454–8462
99. Rothenberg ME, Hogan SP. The Eosinophil. *Annu Rev Immunol* 2006;24:147–174
100. Bankers-Fulbright JL, Kita H, Gleich GJ, O'Grady SM. Regulation of human eosinophil NADPH oxidase activity: central role for protein kinase C δ . *J Cell Physiol* 2001;189:306–315
101. Adachi T, Choudhury BK, Stafford S et al. The differential role of ERK and p38 MAP kinase in eosinophil functions. *J Immunol* 2000;165:2198–2204
102. Myou S, Leff AR, Myo S et al. Activation of cytosolic phospholipase A2 in eosinophils by PI3kinase through a MAP kinase independent pathway. *J Immunol* 2003;171:4399–4404
103. Logan MR, Odemuyiwa SO, Moqbe R. Understanding exocytosis in immune and inflammatory cells. *J Allergy Clin Immunol* 2003;111:923–932
104. Coffey PJ, Schweizer RC, Dubois GR et al. Analysis of signal transduction pathways in human eosinophils activated by chemoattractant and Th-2 cytokines IL-4 and IL-5. *Blood* 1998;91:2547–2557
105. Urasaki T, Takasaki J, Nagaawa T, Ninomiya H. Pivotal role of 5 lipoxygenase in activation of human eosinophils. *J Leukoc Biol* 2001;69:105–111
106. Odemuyiwa S, Ghahary A, Li Y et al. Human eosinophils regulate T cell subset selection through IDO. *J Immunol* 2004;173: 5909–5913
107. Salanova B, Choi M, Rolle S et al. Beta2 integrins and acquired GPIIb/IIIa receptors cooperate in NF κ B activation of human neutrophils. *J Biol Chem* 2007;282:27960–27969
108. Hilden TJ, Nurmi SM, Fagerholm SC, Gahmberg CG. Interfering with leukocyte integrin activation. *Ann Med* 2006;38:503–511
109. Coppolini MG, Dedhar S. Bidirectional signal transduction by integrin receptors. *Int J Biochem Cell Biol* 2000;32:171–188
110. Defilippi P, DiStefano P, Cabodi S. p130Cas: a versatile scaffold in signaling networks. *Trends Cell Biol* 2006;16:257–263
111. O'Neill GM, Fashena SJ, Golemis EA. Integrin signaling: a new Cas(t) of characters. *Trends Immunol* 2001;22:612–615
112. Sawada Y. Force sensing by p130 Cas. *Cell* 2006;127:1015–1026

113. Mocsai A, Ligeti E, Lowell CA, Berton G. Adhesion dependent degranulation of neutrophils requires src kinases. *J Immunol* 1999;162:1120–1126
114. Thomas RM, Schmedt C, Novelli M et al. C-terminal src kinase control acute inflammation and granulocyte adhesion. *Immunity* 2004;20:181–191
115. Welsh CF, Assoian RK. A growing role for Rho family GTPases as intermediaries in cell cycle progression. *Biochim Biophys Acta* 2000;1471:1421–1429
116. Schwarz MA, Shattil SJ. Signaling networks linking integrins and Rho family GTPases. *Trends Biochem Sci* 2000;25:388–391
117. Liu S, Thomas SM, Woodside DG et al. Binding of paxillin to α 4-integrin modifies integrin dependent biological responses. *Nature* 1999;402:676–679
118. Shattil SJ, Ginsberg MH. Integrin signaling in vascular biology. *J Clin Invest* 1997;100:1–5
119. Tanaka Y, Minami Y, Mine S et al. H-Ras signals to cytoskeletal machinery in induction of integrin mediated adhesion of T cells. *J Immunol* 1999;163:6209–6216
120. Lub M, van Kooyk Y, Figdor CG. Ins and outs LFA-1. *Immunol Today* 1995;17:79–83
121. Chirathaworn C, Kohlmeier JE, Tibbets SA et al. Stimulation through ICAM.1 provides a second signal for T cell activation. *J Immunol* 2002;168:5530–5537
122. van Kooyk Y, Figdor CG. Avidity regulation of integrins: the driving force in leukocyte adhesion. *Curr Opin Cell Biol* 2000;12:42–47
123. Gaglic JL, Greenfield FA, Mattoo A et al. ICAM-1 is critical for activation of CD28 deficient T cells. *J Immunol* 2000;165:6091–6098
124. Morgan MM, Labro CM, van Seventer GA et al. Superantigen induced T cell: B cell conjugation is mediated by LFA-1. *J Immunol* 2001;167:5708–5718
125. Tyagi S, Nicholson-Weller A, Barbashov SF et al. ICAM-1 and beta2 integrins in C1q stimulated superoxide production by human neutrophils. *Arthritis Rheum* 2000;43:2248–2259
126. Mills KH. Induction, function and regulation of IL-17 producing T cells. *Eur J Immunol* 2008;38:2636–2649
127. Afzali B, Lombardi G, Lechler RI, Lord GM. The role of Th17 and regulatory T cells in human organ transplantation and autoimmune disease. *Clin Exp Immunol* 2007;148:32–46
128. Fouser LA, Wright JF, Dunussi-Joannopoulos K, Collins M. Th17 cytokines and their emerging roles in inflammation and autoimmunity. *Immunol Revs* 2008;226:87–102
129. Schmidt-Weber CB, Akdis M, Akdis CA. Th17 cells in the big picture of immunology. *J Allergy Clin Immunol* 2007;120:247–254
130. Roark CL, Simonian PL, Fontenot AP et al. $\lambda\delta$ T cells: an important source of IL-17 *Curr Opin Immunol* 2008;20:353–357
131. Ludwig IS, Geijtenbeek TBH, van Kooyk Y. Two way communication between neutrophils and dendritic cells. *Curr Opin Pharmacol* 2006;6:408–413

Chapter 11

Cell Defence and Survival

Abstract Central to immune defence mechanisms is the role of transcription factor nuclear factor kappa B (NF- κ B). This is a complex biochemical topic with ever more controls revealed. NF- κ B determines the production of proinflammatory cytokines and chemokines. Pharmacologists step in with possible means of control. Other systems involved in defence include the cyclooxygenase 2 (Cox-2) enzyme and peroxisome proliferator-activated receptors. Insulin receptor activation needs to be seen in context. The mTOR system directs uptake of nutrients by cells. mTOR is suppressed by rapamycin, whose usage is now quite considerable in the control of transplant rejection.

11.1 Transcription Factor NF-kappa

Nuclear factor kappa B (NF- κ B) was identified in 1986 as a transcription factor (TF) that binds to the enhancer of the B cell immunoglobulin- κ light chain gene. NF- κ B proteins are cell homologues of viral v-Rel. NF- κ B functions as homo- and heterodimers (p50/p65) (Fig. 11.1a) that share a 300-amino acid DNA binding domain at the amino terminal called the Rel homology domain (RHD) (Fig. 11.1b), which allows nuclear localization and dimerization [1]. These units are held in the cell cytoplasm bound to an inhibitory unit I-kappa B (I κ B). The I κ B proteins have ankyrin repeats that attach to the RHDs. When the I κ B units are phosphorylated on two serine residues, they are ubiquitinated and degraded by the 26S proteasome. Thus, the I κ Bs are removed, and the RelA (p65) RHDs then move rapidly into nuclei to cause activation of target genes [2–4]. In the nuclei, the dimeric Rel homology domains bind to a decameric DNA sequence 5'GGGRNYYCC-3', where R indicates A or G, Y is C or T, and N is any base. The ability of the dimers to recognise slightly differing DNA targets means that a whole range of genes is regulated differentially.

Accordingly NF- κ B is a family of TFs that play a crucial role in the immune and inflammatory responses of cells and in cell survival [5–7]. Various stimuli activate NF- κ B [8, 9]: (a) proinflammatory cytokines interleukin 1 (IL-1) and tumour necrosis factor- α (TNF α), (b) oxidants like H₂O₂, (c) ceramide, (d) protein kinase

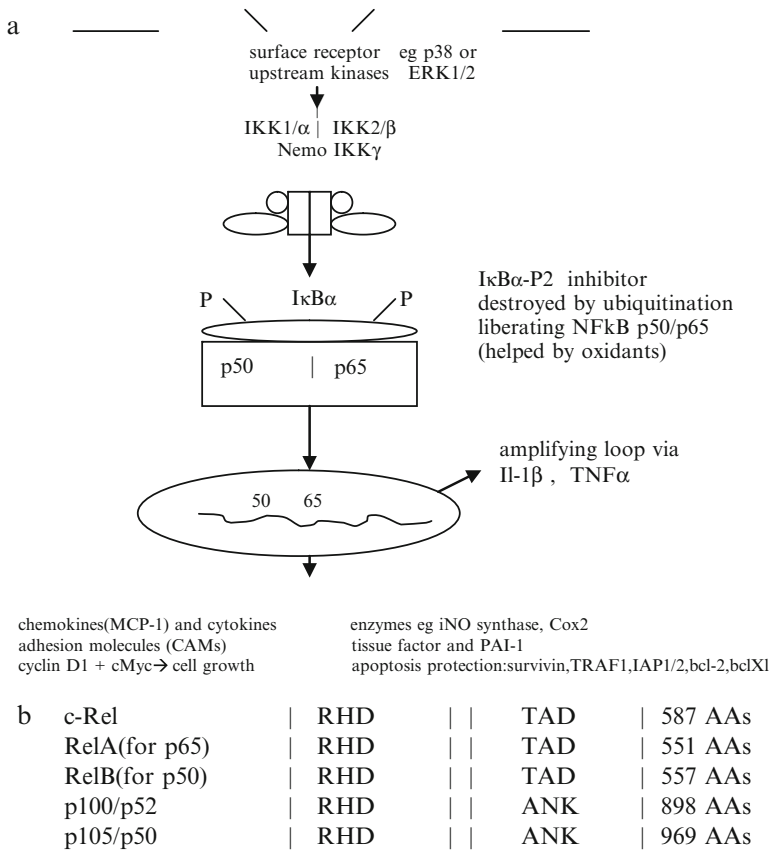


Fig. 11.1a Activation of nuclear factor kappa B (*NF-κB*) by inflammatory stimuli. Microbial products or cytokines that induce *NF-κB* trigger phosphorylation of unit I-kappa B (*IκB*) kinase (*IKK*) -α and -β. The heterodimers *IKKα* or -β then bind to the regulatory *IKKγ*, Nemo, and each heterodimer [1] phosphorylates the *IκB* inhibitory proteins, so that they undergo proteasomal degradation. *NF-κB* p50, p65 is then freed and will move into the nuclei. With regard to the role of ubiquitination [2, 3], it regulates three steps: (a) degradation of the inhibitor *IκB*, (b) processing of the *NF-κB* precursors and (c) *Traf* 6 ubiquitination, which activates the *IKK*. Proteasomes are implicated in *NF-κB* activation: (a) in generation of p50 from the precursor p105 and (b) in the degradation of *IκBα*. Types I and II interferons induce immunoproteasomes in which the reactions are much quicker. Calpain activates *NF-κB* since it degrades the PEST (proline, glutamate, serine, threonine) sequence in the inhibitor *IκBα*. **b** Structures of nuclear factor kappa B (*NF-κB*) family members. All five *NF-κB* members are related through the conserved Rel homology domain (*RHD*), which contains sequences for DNA binding, dimerization, inhibitor association and nuclear localization. The members c-Rel, RelA and RelB contain a C-terminal transactivation domain (*TAD*). The function of the *TADs* is enhanced by phosphorylation and acetylation. The second group (the p105/p50 and p100/p52) arises as precursors p100 and p105 that are partially proteolysed by the 26S proteasome to yield the mature p52 and p50 units. They contain C-terminal sequences with inhibitory ankyrin (*ANK*) repeats, and this means that they can also function as unit I-kappa B (*IκBs*) and retain Rel proteins in the cytoplasm. The five to seven ankyrin repeats of inhibitory *IκB* proteins interact with *NF-κB* dimers and mask their nuclear localization signals (*NLSs*)

B (Akt), (e) protein kinase C (PKC) [10] and (f) viruses. Intracellular oxidants [11, 12] help the I κ B inhibitors to be dissociated from the NF- κ B units. Hence, intracellular reductants like glutathione and thioredoxin have differential roles in the redox regulation of target proteins, and S-nitrosylation can inhibit NF- κ B DNA binding [13]. Yet, within nuclei it is the more reducing environment there that enables NF- κ B RHDs to bind onto the DNA.

As you will now expect, zymosan proinflammatory agent stimulates NF- κ B, causing release of IL-8 so that neutrophils are attracted [14]. That process can be inhibited by nitric oxide (NO) or by IL-10 inhibition of NF- κ B [15]. Examination of synovial tissue in rheumatoid arthritis shows marked expression of NF- κ B1 at sites adjacent to the cartilage-pannus junction. NF- κ B activation upregulates chemokines and adhesion molecules and neutrophils and macrophages are then recruited.

Extra comment about Nemo (I κ B kinase- γ , IKK γ) is appropriate as this is a developing field [16]. Nemo has two coiled domains, CC1 and CC2, and a leucine zipper. Nemo interacts with many proteins involved in NF- κ B activation. It is polyubiquitinated but on differing lysine residues according to the stimulus [17]. Its potential is also influenced by sumoylation and phosphorylation.

11.1.1 The Classical Canonical NF- κ B Pathway

The IL-1 β and TNF α cytokine receptors connect via upstream kinases to the activation of NF- κ B [17, 18]. The two IKK kinases α and β have an activation loop like that of the mitogen-activated protein kinase kinase (MAPKK) proteins, which bears two serines that are phosphorylated in response to IL-1 or TNF α . Cytokine IL-1 can activate either IKK β or the IKK α .

As shown in Fig. 11.1a, serine-threonine kinases IKK α and IKK β form homo- or heterodimers in conjunction with stabilising protein Nemo (the NF- κ B essential modulator), also termed IKK γ [4, 19]. Binding of Nemo to Lys63-polyUb-RIP1 forms the active kinase complex IKK γ , IKK β -P, IKK α -P that phosphorylates the inhibitor unit I κ B α [20], which then dissociates and is destroyed by ubiquitination. Accordingly, NF- κ B as p50/RelA is free to enter nuclei. Oscillations in intranuclear NF- κ B coupled to cycles of phosphorylation of RelA and I κ B are required for NF- κ B-dependent gene expression [21]. As revealed in Table 11.1, NF- κ B activates a range of genes relevant to inflammation and innate immunity. There are genes for the cytokines and chemokines and for regulation of the cell cycle, survival, adhesion and invasiveness. Both apoptotic and antiapoptotic genes are induced. Genes with a role in feedback regulation of the NF- κ B, such as I κ B α and A20, are induced early. *What does A20 protein do?* It inhibits the IKK complex, it inhibits RIP (Fig. 12.5), and it inhibits TAK1 kinase. By inhibiting RIP, A20 stops TNF α -induced NF- κ B activation, a step that requires Richard TAXIBI [21b].

NF- κ B is a key participant in multiple processes affecting the immune system and inflammation. It can interact with other TFs like Interferon Regulatory Factors (IRFs), signal transducers and activators of transcription (STATs) and the p53. Such promiscuity might explain the many roles of NF- κ B in cell functions and fate [22].

Table 11.1 Common genes activated by nuclear factor kappa B (NF- κ B)

Class	Target gene
Immunoreceptors	Immunoglobulin κ light chain
	Interleukin 2 receptor α -chain
	Major histocompatibility complex class I/II
	Platelet-activating factor receptor
	Tissue factor
Cell adhesion molecules (CAMs)	Endothelial leucocyte adhesion molecule 1 (ELAM-1)
	Vascular cell adhesion molecule 1 (VCAM1)
	Intercellular cell adhesion molecule (ICAM1)
Cytokines and growth factors	Tumour necrosis factor- α (TNF α)
	Interleukin 1 β (IL-1 β), IL-2, IL-6, IL-8, IL-12
	Transforming growth factor- β
	Granulocyte colony-stimulating factor (G-CSF)
Chemokines	Monocyte chemotactic peptide 1 (MCP-1)
	Macrophage inflammatory proteins (MIPs) 1 α , 1 β
Acute-phase proteins	C-reactive protein (CRP)
	Lipopolysaccharide-binding protein (LBP)
Others	Nitric oxide synthases (NOSs)
	Cyclooxygenase 2 (Cox-2)
	Phospholipase A2 (PLA2)
	Metalloproteinases

Hydrogen peroxide (from ROS) prolongs nuclear localization of NF- κ B in activated cells [22b]. Fortunately, there is control by heat shock proteins HSP70 and HSP90 which regulate the IKK complex [22c].

NF- κ B plays a crucial role in inflammatory processes. Activation of p38 mitogen-activated protein kinase (MAP) kinase is well recognised to activate NF- κ B [23]. If a single injection of lipopolysaccharide (LPS) is given intraperitoneally to mice, there is transient NF- κ B activation in lung cell types. When the LPS is infused by a pump, there is widespread NF- κ B activation and neutrophilic infiltration that begets lung injury following release of cytokine IL-8 ([MIP2] of mice) (Table 11.1). Since the search is on for means of controlling ARDS (adult respiratory distress syndrome), an inhibitor of NF- κ B could be found to reduce the lung inflammation. As for natural restraints PIAS [24] or MK2 [25] or Cezzare [22b] or CYLD deubiquitinating enzyme [26] block NF- κ B activation. In resting neutrophils, RhoA GTPase inhibits the NF- κ B to prevent TNF α production, and yet in LPS-stimulated neutrophils RhoA activates NF- κ B and supports TNF α formation [27].

11.1.1.1 The Roles of Subunits: subtle feedback control mechanisms

The various homodimers and heterodimers of NF- κ B are kept inactive by association with cytoplasmic inhibitory proteins [28]. They are I κ B α , I κ B β and I κ B ϵ and also the p105 and p100 precursors of p50 and p52. Are there roles for the α -, β -, γ -subunits of the IKK complex? [29]. IKK α is a histone H3 kinase that moves into nuclei to associate with the promoters of NF- κ B-responsive genes [30, 31]. IKK α

contributes to NF- κ B DNA binding on gene promoters; thus, it derepresses IL-8, cIAP2 and more. Yet, it creates a negative feed-back loop for NF- κ B and macrophage activation [32, 32b, 33]. In fact, IKK α resolves inflammation by switching off the canonical pathway. By accelerating turnover of cRel and RelA, IKK α attenuates IKK β driven NF- κ B activation and so inflammation. It can also promote adaptive immunity via the non-canonical pathway [33].

When Nemo/IKK γ shuttles into nuclei, it creates feedback by repression of NF- κ B gene expression. Nemo binds to other proteins like RIP and A20, and it regulates the IKK-related kinases IKK ϵ and TBK1 (TANK binding kinase 1), which regulate interferon regulatory factors IRF3 and IRF7.

As for IKK β , it can increase the activity of hypoxia-inducible factor 2 α (HIF2 α) [33b], so linking hypoxia to innate immunity and inflammation, and it activates a subset of (IFN γ)-stimulated genes. For sure IKK β promotes the canonical pathway of NF- κ B activation (Fig. 11.1a) [29]. IKK β promotes the MAP kinases and cell proliferation (e.g. in skin keratinocytes) [29]. All the actions are tissue specific. Yet, IKK β exerts negative control over the production of IL-1 β [34]. Furthermore in some situations IKK β can suppress M1 host defence macrophages, so that it is anti-inflammatory [34b], but it promotes M2 immune regulation macrophages [34c].

11.1.2 *The Alternative Noncanonical Pathway*

There is an alternative means of NF- κ B activation [35]. Lymphotoxin, BAFF (B cell-activating factor), CD40L or RANKL (receptor activator of NF- κ B ligand) activate NIK (NF- κ B-inducing kinase), and it then acts on IKK α homodimer, whose target is NF- κ B2, p100. Phosphorylation-dependent ubiquitination of the p100 is only partial. The N-terminal p52 is released and joins with RelB to enter the nuclei (Fig. 11.2). This alternative path via p52/RelB enables BAFF (BLys) to activate B lymphocytes. The alternative path essentially provides organogenic chemokines [4] for the development of lymphoid tissue [36] and adaptive immunity. The NF- κ B2 is also required for establishment of thymic central tolerance through an Aire-dependent pathway [37]. AIRE is the autoimmune regulator gene, and NF- κ B2 is required in medullary thymic epithelial cells.

Whenever Rankl stimulates osteoclastogenesis, the NF- κ B2 p100 path is implicated [38]. Acting on receptor rank on bone stromal cells, Rankl stimulates their NFATc1 and c-fos so that osteoclasts are formed. This activation is reinforced by Ca²⁺-calmodulin (CaM) stimulation of NFATc and of CaM kinase IV, which stimulates cAMP response element-binding protein (CREB) on the promoter for osteoclast marker genes. NIK has been shown by the study of mouse knockouts to control the activities of lymphocytes and osteoclasts in inflammatory arthritis [39]. NIK^{-/-} mice have no peripheral lymph nodes, defective T and B cells and impaired Rankl-induced osteoclastogenesis. One might also be interested that when ultra-violet light acts on skin to cause immunosuppression by induction of Treg cells, Rankl on keratinocytes first stimulates Rank on Langerhans cells. Vitamin D₃, which is immunosuppressive, also induces Rankl [40].

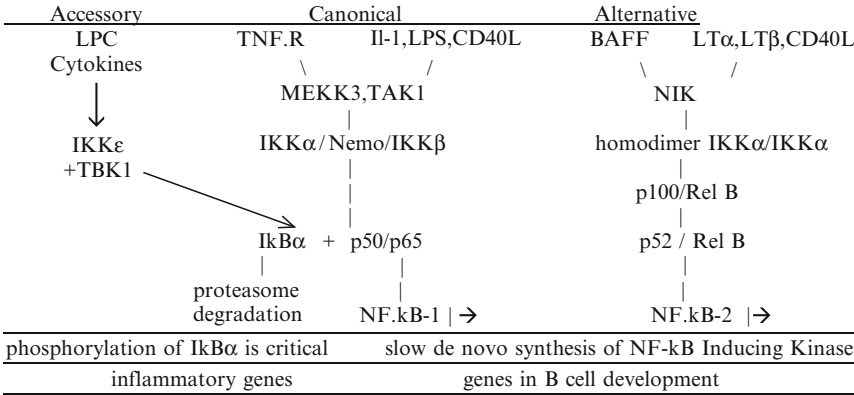


Fig. 11.2 Comparison of the canonical and alternative paths of nuclear factor kappa B (*NF-κB*) activation. Nemo also connects IKK complexes with IKK ε and TBK1 kinases. Monarch-1 suppresses the noncanonical NF-κB2 activation by causing proteasomal degradation of NF-κB-inducing kinase (*NIK*). In T lymphocytes, the NF-κB2/p100 is a late-acting negative-feedback molecule affecting T cell receptor activation of the NF-κB [41]

11.1.3 More about NF-κB Control

All NF-κB activation pathways share the proteasome-mediated step (Fig. 11.1) that causes the degradation of the inhibitory proteins. Posttranslational modifications like phosphorylation [42], acetylation [43] or prolyl isomerization modulate the activity of the p65 (or p50) subunits. Thus, acetylation of multiple lysine residues by p300/CBF acetyltransferases (Fig. 4.8) modifies the behaviour of NF-κB components. Acetylation works as an intranuclear molecular switch [44]. Acetylation of p65 stops inhibition by IκBα and controls nuclear NF-κB. Oxidative stress, via its formation of H₂O₂, which enhances degradation of IκBα, enhances NF-κB activity [22b]; thus, macrophages have increased production of cyclooxygenase 2 (Cox-2), prostaglandin E2 (PGE2), and matrix metalloproteinase 1 (MMP-1) [45].

Activation of NF-κB-inducible genes (Table 11.1) usually takes place by means of the p65/p50 DNA binding heterodimers of the classical pathway. Significant fluctuation in p50 levels can alter the abundance and composition of NF-κB complexes within a cell. When animals or cells are exposed to repeated small doses of LPS, the output of cytokines via NF-κB activation is thwarted, and the animals or cells show *endotoxin tolerance*. The explanation is that p50/p50 homodimers are formed instead of the p65/p50, and the consequence is suppression of NF-κB-inducible genes [46]. p65 has a transactivation domain, whereas p50 does not. p50/p50 homodimers (as produced by IL-10 or by protein kinase A) are inhibitory. Cells that are tolerant to endotoxin still respond to LPS with the degradation of IκB and nuclear translocation of p50 and p65. Yet, they cannot form IL-1β. In such cells, it was found that RelB

represses inflammatory gene expression [47]. Alternatively, in endotoxin tolerance, mobilization of Toll-like receptor 4 (TLR4) into lipid rafts is curtailed [48].

p53 turns out to be a buffer of the innate immune system because p53 works as an antagonist of NF- κ B to dampen chronic inflammation. p53^{null} macrophages show very high release of cytokines IL-1, IL-6 and IL-12 in response to LPS or IFN γ . In fact, p53 downregulates Stat1 and proinflammatory cytokines [49]. In some cells, like leucocytes, I κ B ζ negatively regulates intranuclear NF- κ B activity [50]. We have noted how LPS exposure can attenuate TNF α gene transcription because NF- κ B p50 homodimers bind to the TNF α promoter. In addition, LPS induces Bcl-3 (B cell lymphoma 3), which is an anti-inflammatory regulator [32b,51]. Bcl-3 is only located in cell nuclei, and it associates with p50/p50 homodimers and acts as a negative regulator of TLR signalling [52]. Bcl-3 reduces transcription of proinflammatory cytokines. It mediates some of the anti-inflammatory activities of IL-10 (Sect. 12.16) Bcl-6 is another transcriptional repressor protein. Repression by Bcl-6 is cell-type specific. Bcl-6 negatively regulates the expression of the NF- κ B p105/p50 subunit. In macrophages, it negatively regulates the chemokines monocyte chemotactic peptide 1 (MCP-1), MCP-3 and MIP-1 [53]. Indeed, in Bcl-6^{-/-} mice there is florid T helper lymphocyte Th2-mediated inflammation. Bcl-6 is essential for formation of germinal centres and for a normal antibody response [54]. Bcl-6 suppresses p53 expression in germinal centre B lymphocytes. Bcl-6 interacts with TF Miz-1 to suppress p21 and thus to allow proliferation of germinal centre B cells [55]. Activating mutations or chromosomal translocations that affect Bcl-6 are relevant to B cell lymphomas [56, 57]. The t (3;14) translocation affecting Bcl-6 is part of diffuse large B cell lymphoma (DLBCL).

One should consider the pharmacological means of control of NF- κ B (Table 11.2). Commensal gut bacteria like *Bacteroides* reduce the activity of NF- κ B in gut

Table 11.2 Mediators and drugs that inhibit nuclear factor kappa B (NF- κ B)

Physiological

Interleukins 10 and 13 transforming growth factor- β
 I κ BNS inhibitor, heat shock proteins HSP70 and HSP90, Nemo-binding domain peptide, CYLD
 Cyclic adenosine monophosphate (cAMP), glucocorticoids, oestrogen, melanocortins
 Nitric oxide late in macrophage activation^a S-nitrosylation [9]
 T suppressor lymphocytes (Treg) tumor suppressor p53

Pharmacological

Cyclopentenone prostaglandin 15-deoxy-PGJ2, and the EETs, prostaglandin E2 (PGE2)
 Salicylates, acetylsalicylic acid, sulindac (nonsteroidal anti-inflammatory, NSAID), sulfasalazine, mesalamine
 Antioxidants: vitamin E, N-acetylcysteine, taurine-chloramine, lipoic acid
 Lipoxin A4, pyroldinedithiocarbamate, conjugated linoleic acid (CLA)
 PPAR γ agonists like the thiazolidinediones (TZDs) and PPAR α
 Immunosuppressant cyclosporin 1,25(OH)₂ vitamin D₃ alcohol
 ACE inhibitors, statins-HMG-CoA reductase inhibitors
 Flavonoids, silymarin, green tea polyphenols, curcumin, capsaicin, flavopiridol
 Deoxyspergualin, geldanamycin, retinoids, vitamin D analogues
 Proteasome inhibitors: velcade, lactacystine, epoxomicin, peptide aldehydes

^aEarly in macrophages, nitric oxide induces NF- κ B, but later it inhibits it

mucosal epithelial cells. Actually, *Bacteroides* species activate peroxisome proliferator-activated receptor- γ (PPAR γ) receptors, which take RelA out of cell nuclei of the gastrointestinal (GI) epithelium. Hence, cytokine production via NF- κ B is suppressed [58]. Clearly, inflammatory reactions at the gut mucosa can cause much trouble. Hence, note that local GI macrophages show downregulation of their TREM1 (triggering receptors expressed on myeloid cell 1)-activating receptors. TREM would amplify cytokine-induced responses. This is explained by the local environment, in which there is plentiful IL-10 (suppressive cytokine) and transforming growth factor- β (TGF β) [59].

Enhanced NF- κ B activity occurs in the gut mucosa in inflammatory bowel diseases. In Crohn's disease, there is high expression of the immunoproteasome so that there is enhanced processing of the p105 $\rightarrow$ p50 and enhanced degradation of inhibitor I κ B α . Then p50/c-Rel is important for IFN γ induction of proteasomes via IL-12-driven Th1 cell responses [60].

Phosphorylation of cRel is needed for antiviral responses. Accordingly, TBK1 (Tank binding kinase-1) and IKK ϵ phosphorylate the C-terminal of cRel so that p50/cRel can bind at NF- κ B sites [61]. TBK also phosphorylates IRF3. This means that interferons IFN α / β are induced (Chap. 14) [62].

11.2 How to Inhibit NF- κ B

Anti-inflammatory cytokines IL-10 and IL-13 suppress the activation of NF- κ B. Unlike many cytokines, IL-10 does not activate the NF- κ B. Heat shock proteins inhibit NF- κ B by stopping the degradation of I κ B [22c]. There is accumulating information on drugs and antioxidants like N-acetylcysteine [63], resveratrol of red wine or the polyunsaturated fatty acid (PUFA) conjugated linoleic acid (CLA) [64] that inhibit NF- κ B activation and thus have anti-inflammatory properties.

Those with therapeutic inclinations will be interested in physiological mediators and drugs that can be used to inhibit NF- κ B (Table 11.2). The immunosuppressant cyclosporin stops RelA activation and so reduces IL-2 formation by T lymphocytes [65]. Decoy deoxynucleotides or negative mutants of NF- κ B are used by the biochemist. A recent review of pharmacological aspects of NF- κ B [66] listed inhibitory peptides for NF- κ B that have been used in anti-inflammatory exploration. The cyclopentenone 15-deoxyPGJ2 is anti-inflammatory by virtue of its ability to modulate TFs [67]. It also acts to potentiate nitric oxide (NO), stopping neutrophil ingress.

In this amazing list of diverse agents, note that T regulatory (suppressor) lymphocytes act, in part, by inhibition of NF- κ B. Thus, Li et al. [68] demonstrated how suppressor lymphocytes stop the presentation of CD86 on antigen-presenting cells (APCs). I κ BNS is an inhibitor that binds to nuclear Rel/p65. It accounts for negative selection of thymocytes [69] and T lymphocytes or macrophages [32b].

Dendritic cells (DCs) use NF- κ B for their maturation, and they use NF- κ B to help form Th1 lymphocytes and CD8 cytotoxic T cells. NF- κ B p50-RelA determines the differentiation of DCs and their survival, and their formation of IL-12 depends

on NF- κ B p50-cRel [70]. Not surprisingly, we know of numerous microorganisms that are able to subvert NF- κ B activation to gain entry into the body [71]. Conversely, if there is p38 MAP kinase activation, that will lead to NF- κ B induction of inflammatory genes [72]. NF- κ B supports adaptive immunity and the elimination of microbial invaders.

Glucocorticoids, on that list, are immunosuppressive agents. Their glucocorticoid receptors (GRs) suppress the activity of CREB-binding protein (CBP)/p300 induce corepressors like histone deacetylase 2 (HDAC2) [73]. Similarly, PGE2 is anti-inflammatory by virtue of the fact that it enhances the expression of I κ B α , and ineffectual p50/p50 homodimers form [74]. It turns out that 1,25(OH)₂ vitamin D₃ and 1,24(OH)₂D₂ inhibit TNF α expression by macrophages. What is more, these forms of vitamin D increase the activity of I κ B α ; thus, they inhibit NF- κ B activity [75].

Obviously, using PPAR γ and - α agonists (Fig 4.14) is a way of dealing with inflammation. PPAR α knockout mice show augmented inflammation. In them, there is marked expression of TNF α and IL-1 β and expression of Fas ligand in the course of carrageenan-induced paw oedema or carrageenan-induced pleurisy [76].

11.3 Gene Knockout or Transgenic Experimental Animals

The involvement of effector molecules in physiological or pathological processes can now be elucidated or confirmed by the use of gene knockout (ko) or transgenic (tg) mice, into which an extra gene is inserted. Already, examples have been mentioned. Mouse models that lack almost all the known genes for G protein α -subunits have been generated to give insight into G protein signal pathways. G α q-deficient platelets fail to aggregate in response to thrombin, adenosine diphosphate (ADP) or thromboxane A₂ (TxA₂) due to lack of agonist-induced phospholipase C activation. Mice lacking G α q have increased bleeding times and are protected against thromboembolism. Mice with knockout of gene granulocyte-macrophage colony-stimulating factor (GM-CSF) develop silting of the lungs by “alveolar proteinosis”, which is a clear indication of the cause of this rare condition. Transgenic mice that overexpress phospholipase A₂, which generates leukotrienes and platelet-activating factor (PAF) (Fig. 6.2b), develop exuberant atherosclerosis. They show enhanced oxidation of their plasma low-density lipoprotein (LDL). Mice that lack neutrophil elastase (NE) cannot clear infections with intracellular bacteria [77]. Mice deficient in cathepsin G succumb to *Staphylococcus aureus* [78]. The subtleties of NF- κ B controls are gradually being elucidated by techniques like this (cf. [79]). B lymphocytes with RelA^{-/-} deficiency were killed easily by exposure to TNF α since they had reduced expression of Flice (i.e. caspase 8) and of Bcl-2 antiapoptotic protein. Clearly, NF- κ B enables B cell survival by eliminating the effects of TNF α toxicity. This may remind one that c-Flip, inhibitor of the extrinsic apoptotic pathway, is known to promote NF- κ B signalling and cell survival [80]. RNA interference (RNAi) is a mechanism for sequence-specific posttranscriptional inhibition of gene expression acting on double-stranded RNA molecules. Small interfering RNAs can be applied to mammalian cells. Thus,

Table 11.3 How nuclear factor kappa B (NF- κ B) supports antiapoptosis/cell survival

NF κ B supports:

Inhibitor of apoptosis proteins 1 and 2 and c-FLIP, which inhibit caspase 8

Protein A1 and Bcl-XL, which stop mitochondrial release of cytochrome C

A20 protein, which thwarts (a) IKK, (b) RIP and (c) TAK1 (Fig. 11.2)

XIAP

GADD45 β , which inhibits JNK

FHC, which stops ROS stimulation of JNK

FHC ferritin heavy chain, *GADD45* growth arrest and DNA damage 45, *IKK* unit I-kappa B kinase, *JNK* Jun N-terminal kinase, *ROS* reactive oxygen species, *XIAP* X chromosome inhibitor of apoptosis TAK1 = transforming growth factor β -activated kinase 1.

RNAi was used to show how phospholipase D (PLD-1) colocalises with actin filaments at the adhesive surfaces of macrophages [81]. RNAi has been used to assess the role of p50 NF- κ B1 protein in the maturation of monocyte-derived DCs. siRNA for p50 reduces the transcription of IL-12 p40. Absence of NF- κ B1 p50 or of c-Rel leads to impaired survival of DCs and lack of IL-12 production. IL-12 production in macrophages is blocked when the IFN γ receptor or the TNFRI are removed by gene deletions in mice. NF- κ B2, p52, derived from precursor p100, when eliminated in knockout mice, results in DCs with enhanced RelB activity. Enhanced RelB is accompanied by enhanced MHC class II molecule expression on APCs and an increased ability to induce CD4 lymphocytes (Table 11.3).

11.4 The Control of Cyclooxygenase 2

Various pathways help to stimulate the expression of Cox-2, such as ROS, tumour promoters, growth factors, oncogenes, and cytokines. Her2/Neu related to the epidermal growth factor receptor (EGFR) does this in breast cancer. Also, in breast and colon cancers there is activation of NFAT (nuclear factor of activated T cells), which leads to Cox-2 induction and so formation of PGE₂. Tumours with elevated Cox-2 will be invasive [82]. Indeed, typical metastatic gene products are Cox-2, intercellular cell adhesion molecule 1 (ICAM1), vascular endothelial growth factor (VEGF), and metalloproteinase 9 (see Chap. 18).

Generally, transcription of Cox-2 gene is promoted by PKC, Ras signalling, and especially MAP kinase induction of NF- κ B and C/EBP and factors that stimulate activator protein 1 (AP-1) [83, 84] (Fig. 11.3). This is similar to induction of cytokines. Consider the situation when *Helicobacter pylori* causes chronic gastritis. Study of TFs in gastric epithelial cells showed Cox-2 promoter induction by NF- κ B, AP-1 and C/EBP (NF-IL-6) [85]. In Fig. 11.3, note the canonical TATA motif and several important enhancer elements affected by transactivators. Proinflammatory mediators can activate ribosomal S6 kinase (RSK), which enhances C/EBP β binding to the Cox-2 promoter. Sphingosine-1-P, by stimulation of NF- κ B, also stimulates Cox-2 activity [86].

Cox-2 expression in neutrophils is promoted by ROS induction of NF- κ B in response to cell membrane events [87]. Likewise, when monocytes interact with

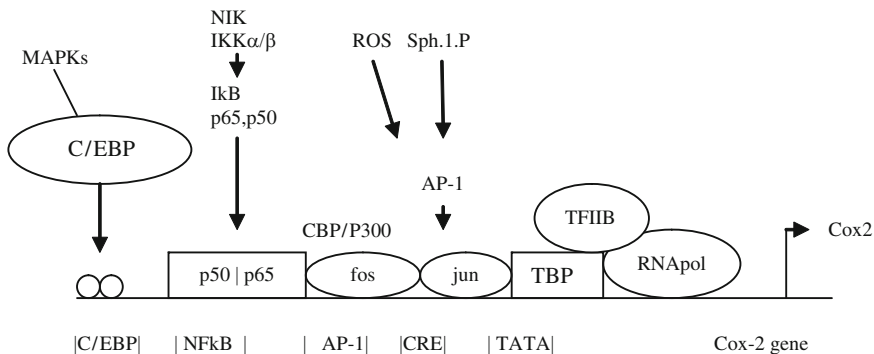


Fig 11.3 Transcription factors affecting the cyclooxygenase 2 (*Cox-2*) promoter. (as in airway smooth muscle cells) C/EBP = CCAAT/enhancer binding protein

platelets or P-selectin-coated surfaces, there is activation of NF- κ B and so Cox-2. In a second phase, IL-1 stabilizes the Cox-2 messenger RNA (mRNA), which it does by silencing of the binding protein ARE; thus, there is enhanced translation [88]. Induction of Cox-2 in macrophages is also biphasic and requires C/EBP β and C/EBP δ TFs [89]. Conserved elements in the macrophage Cox-2 gene promoter as revealed by analysis after exposure to LPS are –CRE2/NF- κ B-C/EBP/AP-1/CRE1/E-box–TATA. The E-box mediates transcriptional repression, whilst the other *cis*-elements are activating. At 6 h after LPS administration, Cox-2 gene transcription was completely dependent on phospho-c-Jun along with the p50 of NF- κ B [90]. One can compare this information with the details of the promoter for Cox-2 in airway smooth muscle cells.

With regard to the discussion in Chap. 4 (Sect. 4.3.1), one can expect that there will be histone acetylation on H3/H4 at the Cox-2 promoter and phosphorylation of H3 histones.

There are different patterns of histone H4 acetylation induced by IL-1 β or by bradykinin. Whereas IL-1-induced transcription utilises NF-IL-6, NF- κ B and CRE, bradykinin uses CRE [91]. Induction of Cox-2 in macrophages is complicated. Normally, any response to NF- κ B activation is rapid. Full Cox-2 expression in macrophages is delayed since there first has to be induction of TNF α by IFN γ [92]. In mesangial cells, TGF β is able to stimulate Cox-2 [93]. When mouse macrophages respond to virus or double-stranded RNA (dsRNA), there is selective induction of Jun N-terminal kinase (JNK) and p38 and therefore activation of NF- κ B that activates Cox-2 [94].

As a lesson, consider how the peptidoglycan of bacterial cell walls stimulates TLR2 receptors; hence, there is NF- κ B activation that activates Cox-2, and PGE2 is produced. This PGE2 then acts on EP2/EP4 surface receptors of the macrophages, so that cAMP is produced and protein kinase A is activated, which then accounts for Ser276 phosphorylation on the p65 of NF- κ B. The consequence is that peptidoglycan elicits the secretion of IL-6 [95]. PGE2 induction of Stat3 is operative in this process [96].

11.5 PPAR γ and PPAR α in Inflammation and Immunity

Transactivation of a particular gene requires a large complex of proteins (Figs. 4.8, 4.19). In their inactive state, PPARs are complexes in the cell cytoplasm along with corepressor proteins. On activation, they dissociate and move into the nuclei, where PPARs form heterodimers with retinoid X receptors (Fig. 4.14). PPAR γ receptors are highly expressed in macrophage-derived foam cells in atheroma. It was found that PPAR γ receptors are involved in the differentiation of monocyte macrophages, and that PPAR γ receptors help to regulate their inflammatory activities [97]. In macrophages, PPAR γ serves as a stimulus for the anti-inflammatory action of carbon monoxide.

PPAR γ will promote the survival of lymphocytes since they suppress ROS by boosting antioxidants, and they attenuate any decline in adenosine triphosphate (ATP) [98]. Yet, fatty acid ligands for PPAR γ can inhibit proliferation of activated T lymphocytes through inhibition of IL-2 formation and induced T cell apoptosis. The PPAR γ associates with NFAT, so the DNA binding and transcriptional activation of the IL-2 promoter is blocked. Also, PPAR γ receptors are expressed on DCs, and PPAR γ ligands inhibit the release of IL-12 [99]. DCs treated with synthetic PPAR γ ligands have reduced ability to stimulate lymphocytes. People are getting excited about the potential use of PPAR γ ligands in conditions like rheumatoid arthritis [100]. PPAR γ agents will decrease synovial production of TNF α , IL-6, IL-8, metalloproteinases [101] and no doubt IL-12 (Fig. 12.12).

Both PPARs and liver X receptors of the nuclear-receptor superfamily function in combination with the GRs to repress inflammatory response genes [102]. NCoR (nuclear receptor corepressor) and SMRT corepressor complexes, which are able to interact with NF- κ B and AP-1, maintain the repression of inflammatory response genes (cf. Fig. 11.4). PPAR α receptors respond to a different set of ligands/drugs. PPAR α receptors are also expressed on monocyte macrophages, and ligands can induce apoptosis of those cells. PPAR α receptors dampen endothelial cell inflammatory responses. In aged mice, oxidative stress-induced NF- κ B is apparent in many tissues. Fortunately, PPAR α agonists restore the cellular redox balance, so there is amelioration of the production of inflammatory cytokines [103] since NF- κ B is inhibited (cf. Fig 11.4). Fenofibrate has been shown to repress IL-17 expression in cultured splenocytes and by Th-17 cells exposed to caecal bacterial antigens.

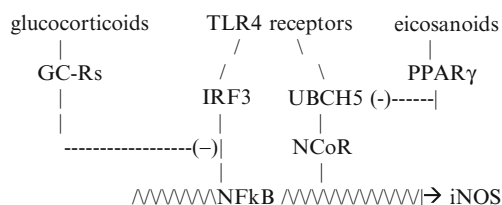


Fig 11.4 Peroxisome proliferator-activated receptor- γ (PPAR γ) with glucocorticoids suppresses inflammatory genes. NCoR nuclear receptor corepressor, UBCH5 ubiquitin-conjugating enzyme 5

Of course, if liver X receptors will suppress inflammatory genes, appropriate agonists will have enormous potential. Cytokine release by monocytes in response to LPS has been investigated [104]. As yet, it is not clear how the process operates.

11.6 Cell Survival: Akt and Glycogen Synthase Kinase 3

Survival of a cell depends on a supply of growth factors from serum and on contact with neighbouring cells or adherence to extracellular matrix proteins [105]. The ERK pathway (Fig. 4.3) has the capacity for survival induction but is negatively regulated by phosphatases in conjunction with the proapoptotic p38/JNK paths. Hence, cancer cells should not emerge. Protein kinase B (Akt) confers the attributes of cell survival [106]. PI3 kinase (PI3K) produces PtdIns(3,4,5)P3 (Fig. 2.6), which binds to Akt so that it can translocate to the cell membrane, where it is phosphorylated by 3-phosphoinositide-dependent kinase 1 (PDK-1). When IL-2 acts on immune cells, Akt ensures their survival. Either a growth factor like PDGF (platelet-derived growth factor) or a cytokine like TNF α will activate Akt, which then associates with and phosphorylates IKK so that NF- κ B is activated. Concurrently, Akt suppresses proapoptotic influences [107]. Akt sequesters the proapoptotic protein Bad, it inhibits caspase 9, and it reduces transcription of Fas ligand (cf. Fig. 8.3). Conversely, since ceramide-activated PKC ζ and protein phosphatase 2A (PP2A) negatively regulate the Akt survival pathway [108], this explains how ceramide often mediates cell apoptosis. Nevertheless, the α 4-subunit of PP2A (and other phosphatases) works to keep apoptosis in check and to maintain cell survival [109].

It will help to be precise about what Akt can contribute. Akt can inactivate (a) death receptor-mediated apoptosis, (b) mitochondrial-dependent apoptosis and (c) p53-induced apoptosis. Akt via Cot serine-threonine kinase activates NF- κ B. NF- κ B supports antioxidant enzymes. Akt phosphorylates glycogen synthase kinase 3 β (GSK3 β), ensuring glucose uptake and metabolism. GSK3 is important in embryonic development. When Akt promotes the survival of adult cells, Akt phosphorylates and inhibits GSK3 [110] and thus enables supportive changes in glucose metabolism. GSK3 β is essential for cell survival, but too much or too little will cause apoptosis. On the one hand, components of the NF- κ B system are phosphorylated by GSK3 to promote NF- κ B activity. Conversely, if p53 binds nuclear GSK3, it is activated and promotes the transcriptional and apoptotic actions of p53 [111]. GSK3 supports formation of proinflammatory cytokines by inducing NF- κ B and CREB interaction with coactivator CBP. GSK3 inhibitors are thus anti-inflammatory [112].

Both growth factor and cytokine survival factors for cells, like insulin-like growth factor 1 (IGF-1) or IL-3 activate Akt, which phosphorylates Bad at three sites, and it then binds 14-3-3 protein, and it (Bad) is sequestered in the cytosol. So, apoptosis cannot occur. When macrophages meet endotoxin, they have to survive. When LPS interacts with TLR4 receptors (Figs. 12.2, 12.5), RIP links TLR4 to PI3K and so to Akt and cell survival [113]. Monocyte survival derives from surface CEACAM1 (CD66a), which connects with PI3K and hence to Akt activation.

Insulin growth factors I and II direct protein anabolism, and they are mitogens. Haemopoietic cells that are destined to die when IL-2 or IL-3 is withdrawn are protected by IGF-1. IGF-1 is antiapoptotic for many cells [114], and in many cancers an autophosphorylated IGF-1R (IGF-1 receptor) mediates antiapoptosis. The biochemical scenario is that when IGF-1 acts on its IGF-1R there is recruitment of a docking protein insulin receptor substrate 2 (IRS-2) (as for insulin) and that leads to activation of PI3K. PI3K activates a PDK-1, and in turn that activates Akt kinase. Naturally, the kinase-regulated signalling pathways are controlled by phosphatases. One is calcineurin, which will dephosphorylate Bad and so reverse the effect of Akt phosphorylation. The other is PTEN (phosphatase as in Fig. 2.6), the product of a tumour suppressor gene [115, 116]. The function of PTEN is to hydrolyze 3-phosphorylated inositol phospholipids [117]. Thus, PTEN acts in opposition to PI3K (Fig. 2.6). In malignancies, both copies of PTEN are deleted or mutated. Loss of PTEN in cells leads to Akt drive, and this is accompanied by resistance of cells to apoptotic stimuli [118]. Hence, cancer is likely to arise.

There are other survival kinases, suppressors of Myc-induced apoptosis, called the *Pim kinases* [119]. Myc induces proliferation but also increased apoptosis of nonmalignant cells. However, defects in control of apoptosis allow Myc to act as an oncogene. Akt or Pim control this. Both Akt and Pim inactivate Bad. Pim kinases are implicated in cancers and lymphomas. Pim kinase was discovered by its ability to stop Myc-induced apoptosis in a mouse model of lymphoma. Cytokines via Jak-Stat action (Sect. 12.9) induce Pim kinases (Pim1, -2, -3).

This section emphasizes the importance of NF- κ B for cell survival. NF- κ B suppresses apoptosis (Fig. 8.2) since NF- κ B supports bcl-2 and bcl-XL, it supports the inhibitor of apoptosis proteins cIAPc and xIAP, and c-FLIP works in its favour [120] it room. Yet, the JNK cascade can work in opposition to the (GADD45) and Xiap proteins, which promote antiapoptosis [120] (Table 11.3). Likewise, the p38 MAP kinase pathway is proapoptotic [121, 122]. Actually, induction of NF- κ B in some cells enables them to stay alive long enough to acquire genetic errors. Hence, NF- κ B activity can promote carcinogenesis [5] and help metastases [123]. Such carcinogenesis is oxygen radical mediated [124]. Yet generally, NF- κ B is acting to suppress tumour cell growth for it aids triggering of p53 and ARF (Fig. 17.5). However, if cells lose their tumour suppressor genes, NF- κ B then promotes oncogenesis [125].

11.7 Insulin Receptors

Insulin is a potent anabolic hormone. Whereas glucose elicits proinflammatory effects, insulin is anti-inflammatory for its action suppresses NF- κ B, AP-1 and early growth response 1 (EGR-1) TFs. Of course, insulin is a principal pancreatic autoantigen relevant to diabetes [126]. Yet, one could be interested in how signalling by the insulin receptors compares with other mechanisms discussed in this text.

Insulin receptor stimulation in insulin-responsive cells leads to translocation of GLUT4 and glucose transport. The insulin receptor is an $\alpha_2\beta_2$ heterotetrameric complex in which two α -subunits and two β -subunits are linked by disulphide bonds. Insulin binds to the extracellular α -subunits, and it thereby transmits a signal across the plasma membrane that activates the intracellular tyrosine kinase of the β -subunit. The one β -subunit phosphorylates its partner on specific tyrosine residues. Accordingly, the receptor tyrosine kinase induces phosphorylation of IRSs on multiple tyrosine residues. These IRS phosphotyrosine residues act as docking sites for $-SH_2$ domain containing proteins, including the p85 regulatory unit of PI3K (Fig. 2.7). Hence, the p110 catalytic unit of PI3K, the lipid kinase unit, will lead to glucose transport [127, 128]. Other proteins that dock at the insulin receptor include adaptor proteins Shc and Grb2 (growth factor receptor-bound protein), leading to Ras-MAP kinase signalling for the mitogenic action of insulin, the SHP2 phosphatase [129] and Cbl (casitas B lineage lymphoma protein).

The PI3K (Fig. 11.5) p110 catalytic unit creates phosphoinositides PI(3,4,5)P₃, PI(3,4)P₂ and PI(3)P at the plasma membrane (Fig. 2.6). They increase GLUT4 translocation but do not actually increase glucose transport. That could be mediated by AS160 (Akt substrate of 160 kDa) [130]. The plasma membrane target for the GLUT4 vesicle is the t-SNARE called syntaxin 4. Inactivation of Glut4 in muscle leads to raised plasma glucose and glucose toxicity that results in insulin resistance [131]. Insulin action is facilitated by the formation of ROS at multiple signalling targets [132], but substantial ROS produced in response to TNF α or dexamethasone creates insulin resistance [133].

There is subtle control by suppressor of cytokine signalling (SOCS) proteins. First, SOCS3 reduces the phosphorylation of IRS-1 and its subsequent association with the p85 of PI3K. Second, the SOCS1 and -6 can inhibit insulin receptor tyrosine kinase activity, thereby reducing the phosphorylation of IRS-1/2 and the downstream events. IRS-1 and IRS-2 are targeted for proteasomal degradation. SOCS1 and -3 are elevated in rodent models of insulin resistance and diabetes.

In adipocytes of obese persons with type 2 diabetes, IRS-2 becomes the main docking protein for PI3K [134]. Local TNF α acting on the adipocytes is a cause of

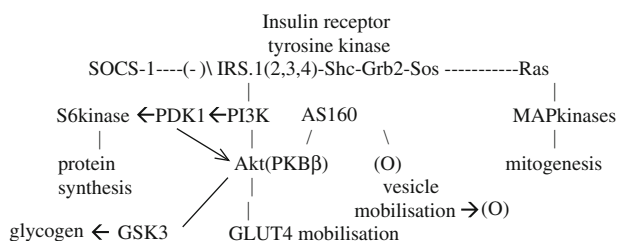


Fig. 11.5 Scheme for insulin receptor activation. *PDK-1* 3-phosphoinositide-dependent kinase 1. Active JNK opposes IRS 1, 2 action. In this scheme, phosphatase Src homology inositol polyphosphate 5-phosphatase 2 (*SHIP2*) regulates the PI3 kinase-dependent signalling. Insulin-like growth factor 1 receptor (*IGF-IR*) also uses insulin receptor substrate 1 (*IRS-1*), which can become intranuclear and thereby activate cell cycle genes. This explains the relevance of IGF-1 to cancers

insulin resistance [135]. Ganglioside GM3 on fat cells corresponds to insulin resistance. TNF α -induced insulin resistance involves activation of inhibitory serine kinases or tyrosine phosphatases, which inhibit the insulin signalling path. It works through H₂O₂ generation. Also, there is serine phosphorylation of IRS-1, rather than IRS-1 tyrosine phosphorylation, that causes reduced insulin receptor kinase activity. In skeletal muscle, TNF α inhibits Akt phosphorylation, causing insulin resistance [136]. Ceramide, by blocking insulin stimulation of Akt, leads to insulin resistance [137]. TNF α can also produce insulin resistance in skeletal muscle by activation of I κ B kinase inhibitor of NF- κ B in a p38-dependent manner [138]. Most particularly there is upregulated JNK which impairs the action of IRS 1/2 [138b].

Crucially, we now realize that adiponectin receptors mediate antidiabetic metabolic effects. Adiponectin promotes glucose uptake, fatty acid oxidation and PPAR α ligand activities. Adiponectin is decreased in obesity, in type 2 diabetes and whenever there is insulin resistance [139]. There is more interest in adipokines from fat stores. The cytokine “resistin” inhibits insulin signalling in liver and muscle in mice [140, 141].

Insulin resistance is essentially due to decreased insulin-stimulated glucose uptake into skeletal muscle. The muscle PI3K (with its p110 catalytic unit and p85 regulatory unit) actually shows increased transcription of the p85 α and of splice variants p55 and p50, and the increase of these subunits must reduce PI3K activity by competing for phosphotyrosine targets [142]. Insulin resistance is accompanied by endothelial cell dysfunction, and there is enhanced aggregability of platelets. Insulin-resistant macrophages are integral to the problem of metabolic syndrome [143]. When they are controlled by knockout of JNK1, insulin resistance is ameliorated, and mice are resistant to high-fat diet-induced obesity [144].

Colca [145] rationalized the complex situation by proposing that insulin resistance is a physiological compensation to excessive oxidative metabolism. A new successful set of antidiabetic drugs, the thiazolidinediones (TZDs) [146], enhances PPAR γ -retinoid X receptor heterodimer formation (Fig. 4.14) and offsets insulin resistance.

11.8 Cross-Talk between Insulin Signalling and the Angiotensin II System

Insulin and angiotensin II (Ang II) play pivotal roles in the metabolic and circulatory systems. Failure in their proper actions leads to diabetes mellitus and hypertension, respectively [147]. There is cross-talk at multiple levels between these systems that affects the action of antihypertensive drugs used in the control of cardiac hypertrophy and energy acquisition by heart muscle. Thus, insulin actually promotes a significant increase of AT1 receptor protein expression in vascular smooth muscle cells (cf. Fig. 5.10).

The insulin signalling system described stems from a balance between tyrosine phosphorylation of IRS-1 and the negative effect of IRS-1 serine phosphorylation.

Too much of the latter occurs in insulin resistance (Fig. 11.5). In fact, PI3K, ERK and JNK as activated by AngII catalyse the serine phosphorylation of the insulin receptor and the IRS-1, and indeed of PI3K, impairing insulin-promoted activation toward Akt. Hence, in contrast to the effect of insulin, IRS-1/2-associated PI3K activity, and thereby endothelial nitric oxide synthase (eNOS) activity, is inhibited by Ang II in a dose-dependent manner [148]. So, insulin-induced vasodilatation is thwarted by Ang II. Furthermore, Ang II induces SOCS3 activity to impair signal transduction through the Jak2/Stat5 signal pathway [149].

11.9 Understanding mTOR

mTOR is mammalian target of rapamycin. It is an integrator of cellular energy status, nutrient and growth factor signals and the coordinator of cell growth and cell cycle progression [150]. TOR directs cell surface expression of various nutrient transporters. Amino acids also stimulate mTOR via the class 3 PI3Ks [151]. Since insulin and IGF-1 are anabolic, Fig. 11.6 shows how those receptors connect via IRS proteins to activation of PI3K and hence Akt. In its turn, Akt phosphorylates and inhibits substrates like the Foxo (Forkhead TFs), Bad, GSK3 and tuberin or hamartin. Tuberin, characteristic of benign tumours called hamartomas as seen in tuberose sclerosis [152, 153], inhibits TOR, but tuberin is inhibited by Akt. This means that Akt activity stimulates glucose uptake and cell growth and proliferation, and apoptosis is inhibited. Actually, mTOR exerts negative feedback on PDGF receptors [154]. The TOR pathway requires nutrients like the amino acid leucine, and then Tor complex 1 (mTORC1) activates S6 kinase and promotes the eukaryotic translation initiation factor 4E (eIF4E) [155]. Additionally, mTORC2 regulates polarization of the actin cytoskeleton. Furthermore, should cell ATP levels fall, the energy-sensing kinase AMPK inhibits hepatic gluconeogenesis (Fig. 1.1) because it promotes TORC2 phosphorylation, which blocks its nuclear accumulation [156].

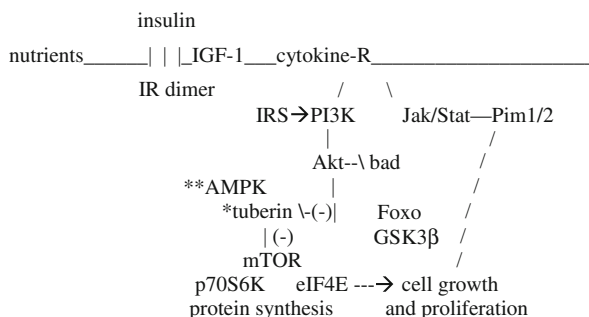


Fig 11.6 Model of the PI3 kinase-Akt-tuberose sclerosis (*TSC*)-mammalian target of rapamycin (*mTOR*) pathway. *Tuberin (*TSC2*) or hamartin (*TSC1*) are tumour suppressors. **AMPK, which is activated by energy deprivation, stimulates *TSC2*/TOR complex 2 (*Torc2*). The eukaryotic translation initiation factors (*eIFs*) are versatile scaffolds for translation initiation complexes [139]

AMPK is a fuel sensor. In muscle AMPK is activated by exercise [157]. It is a key metabolic regulator in liver, heart and muscle. AMPK is activated by cell cAMP, and it will stimulate fatty acid β -oxidation. That means that if hepatic AMPK is inactivated, as occurs in diabetes, there is fatty acid synthesis and development of hyperlipidaemia. In T cells, AMPK controls metabolic steps required for cell proliferation [158].

The tuberose sclerosis 1 and 2 (TSC1-TSC2) protein complex heterodimers integrate cues from growth factors, the cell cycle and nutrients to regulate the activity of mTOR, p70 S6 kinase, 4E-BP1 $\rightarrow$ eIF4E and ribosomal S6 protein [159]. As you will predict, hypertrophy of cells is achieved by augmentation of their RNA and protein content. Formation of mRNA is determined (a) by growth factor Ras-ERK-Mnk-1 $\rightarrow$ eIF4E and (b) by the path mTOR $\rightarrow$ p70 S6 kinase.

Note that these molecules in Fig. 11.6 do not form a linear signal pathway, but they act as agonists and antagonists of each other's activities. Stimulation of mTOR and S6 kinase by amino acids requires participation of a class 3 PI3K called hvp34 (human vacuolar protein sorting 34) [160]. PI3Ks promote glycolysis and thus production of ATP. As in other situations, PTEN controls PI3K activity. Loss of PTEN in mice leads to hyperactive signalling to TOR and disturbance of the control of haemopoietic stem cells and a liability to leukaemia [161]. Understandably, mTOR must be implicated in carcinogenesis [162].

GSK3 β inhibitors are anti-inflammatory, witness that inhibitors like thiazolidinediones (TZDS) attenuate carrageenan-induced lung injury [163].

In T cells, Akt is activated maximally when there is coligation of the TCR and CD28 (Fig. 13.5). Moreover, T cells have AMPK α 1, which is rapidly activated with TCR triggering via the adapter molecules LAT and SLP76 (Fig. 13.4) [164]. When amino acids are supplied, mTOR phosphorylates components of the translational apparatus like the eukaryotic initiation factor eIF4E and p70 S6 kinase. Cytokines also induce PIM1/PIM2 kinases, which help maintain a high rate of glycolysis and protein translation [165].

Rapamycin is a useful immunosuppressant for allograft transplants. Yet, rapamycin does not phenocopy the effects of nutrient or growth factor deprivation. Actually, it fails to prevent the early stages of T lymphocyte activation, including cell cycle entry and upregulation of cell surface activation markers. Outstandingly, rapamycin, by its inhibition of mTOR and thereby of the phosphorylation of S6 kinase and eIF, prevents cell hypertrophy of a remaining kidney after unilateral nephrectomy [166].

S6 kinase is integral to multiple pathways in which cells are rendered unresponsive to insulin, as during chronic insulin exposure, elevation of free fatty acids and perhaps TNF α exposure. A feedback leads to IRS1/2 serine phosphorylation and hence downregulation. Accordingly, there is insulin resistance and impaired cell survival.

Chronic localised activation of mTOR results from genetic loss of certain tumour suppressor genes like TSC1, PTEN in Cowden's disease and 1KB1 (Peutz-Jegher's syndrome) [167].

11.10 Alveolar Macrophage Survival

Armed as we are now with so much new knowledge, we can tackle the question of how alveolar macrophages (AMs) survive so well, even though they are exposed continually to chemical pollutants, ROS, inflammatory mediators and microbial invaders. In fact, AMs have two survival pathways: (a) the PI3K → Akt pathway and (b) ERK activity that leads to inhibition of proapoptotic proteins but good antiapoptosis. The Hunninghake group [168] investigated how it is that ERK activity leads to sustained protein translation initiation via eIF4E or hypophosphorylated (active) eIF2 α . Actually, ERK decreases proapoptotic JNK activity by stabilizing MPK7 dual phosphatase. When JNK activity is low, there is higher activity of phosphatase PP1 α . Accordingly, there is hypophosphorylation of translation initiation factor eIF2 α , so there is ongoing protein translation that means survival of the AMs.

References

1. Perkins MD. The Rel/NF κ B family: friend and foe. *Trends Biochem Sci* 2000;25:434–440
2. Fanton E, Traincard F, Levy SG et al. Nemo oligomerization in the dynamic assembly of the I κ B kinase core complex. *FASEB J* 2007;27:2540–2551
- 3a. Mukhopadhyay D, Riezman H. Proteasome independent functions of ubiquitin in endocytosis and signaling. *Science* 2007;315:201–205
- 3b. Wullaert A, Heyninck K, Janssens S, Beyaert R. Ubiquitin: tool and target for intracellular NF- κ B inhibitors. *Trends Immunol* 2006;27:533–540
- 4a. Sun S-C, Ley SC. New insights into NF- κ B regulation and function. *Trends Immunol* 2008;29:469–479
- 4b. Pereisa SG, Oakley F. Nuclear Factor Kappa B: regulation and function. *Int J Biochem Cell Biol* 2008;40:1425–1430
5. Kumar A. Nuclear factor κ B: its role in health and disease. *J Mol Med* 2004;82:434–438
6. Barnes PJ. Nuclear factor kappa-B. *Int J Biochem* 1997;29:415–435
7. Bonizzi G, Karin M. The two NF- κ B activation pathways and their role in innate and adaptive immunity. *Trends Immunol* 2004;25:280–289
8. Mercurio F, Manning AM. NF κ B as a primary regulator of the stress response. *Oncogene* 1999;18:6163–6171
9. Bauerle PA, Henkel T. Function and activation of NF κ B in the immune system. *Annu Rev Immunol* 1994;12:141–179
10. Catley MC, Cambridge LM, Nasuhara Y et al. Inhibitors of PKC prevent activated transcription: downstream of NF κ B DNA binding. *J Biol Chem* 2004;279:18457–18466
11. Sec CK, Parker L. Antioxidant and redox regulation of gene transcription. *FASEB J* 1996;10:709–720
12. Kamata H, Hirata H. Redox regulation of cellular signalling. *Cell Signal* 1999;11:1–14
13. Marshall HE, Hess DT, Stamler JS. S-Nitrosylation: physiological regulation of NF κ B. *Proc Natl Acad Sci U S A* 2004;101:8841–8842
14. Friedland JS, Constantin D, Shaw TC, Stylianou E. Regulation of Il-8 gene expression after phagocytosis of zymosan by human monocytic cells. *J Leukoc Biol* 2001;70:600–605
15. Natarajan R, Gupta S, Fisher BJ et al. Nitric oxide suppresses Il-8 transcription by inhibiting c-Jun terminal kinase induced AP-1 activation. *Exp Cell Res* 2001;266:203–212

16. Sebban H, Yamaoka S, Gourtois G. Post-translational modifications of Nemo and its partners in NF- κ B signaling. *Trends Cell Biol* 2006;16(11):569–577
17. Woronicz JD, Gao X, Cao Z, Rothe M, Goeddel DV. NF κ B activation and complex formation with I κ B kinase and NIK. *Science* 1997;278:806–809
18. Israel A. The IKK complex: an integrator of all signals that activate NF- κ B. *Trends Cell Biol* 2000;10:129–132
19. Ling L, Cao Z, Goeddel DV. NF κ B inducing kinase activates IKK α by phosphorylation of Ser176. *Proc Natl Acad Sci U S A* 1998;95:2791–2797
20. Delhase M, Hayakawa M, Chen Y, Karin M. Positive and negative regulation of I κ B kinase activity through IKK β subunit phosphorylation. *Science* 1999;284:309–313
- 21a. Nelson DE, Ihekwa AEC, Ellitoo M et al. Oscillations in NF κ B signaling control the dynamics of gene expression. *Science* 2004;306:704–708
- 21b. Grey S T. The Ying and Yang of NF- κ B activation. *Immunol Cell Biol* 2008;86:299–300
- 22a. Wietek C, O'Neill LA. Diversity and regulation in the NF-kappaB system. *Trends Biochem Sci* 2007;32(7):311–319
- 22b. Enesa K, Ito K, Luong LA et al. Hydrogen peroxide Prolongs nucleus localization of NF- κ B in activated cells by suppressing negative regulatory mechanisms *J Biol Chem* 2008;283:18582–18590
- 22c. Salminen A, Paimela T, Surronen T, Kaarniranta K. Innate immunity meets with cellular stress at the IKK complex: regulation of the IKK complex by HSP10 and HSP 9d. *Immunol Cells* 2008;117:9–15
23. Olson CM, Hedrick MN, Izadi H et al. p38 MAP kinase controls NF-kappaB transcriptional activation and TNF α production. *Infect Immun* 2007;75:270–277
24. Liu B, Yang Y, Chermishof V et al. Proinflammatory IKK α phosphorylation of PIAS1 to restrict inflammation and immunity. *Cell* 2007;129:903–914
25. Gorska MM, Liang Q, Stafford SJ et al. MK2 controls the level of negative feedback in the NFkappaB pathway. *J Exp Med* 2007;204:1637–1652
26. Kolls JK. Caught between CYLD and Charybdis. *Immunity* 2007;27:187–189
27. Fessler MB, Arndt PG, Just I et al. Dual role for RhoA in suppression and induction of cytokines in the human neutrophil. *Blood* 2007;109:1248–1256
28. Gloire G, Dejardin E, Piette J. Extending the nuclear roles of I κ B kinase subunits. *Biochem Pharmacol* 2006;72:1081–1089
29. Hacker H, Karin M. Regulation and function of IKK and IKK-related kinases. *Sci STKE* 2006;Oct 17;2006(357):re13
30. Israel A. A regulator (IKK α) branches out. *Nature* 2003;423:596–597
31. Gloire G, Horion J, El Mjiyad N et al. Promoter dependent effect of IKK α on NF-kappaB/p65 DNA binding. *J Biol Chem* 2007;282:21308–21318
- 32a. Lawrence T, Bebie M. IKK α in regulation of inflammation and adaptive immunity. *Biochem Soc Trans* 2007;35(2):270–272
- 32b. Ghosh S, Hayden MS. New regulators of NF- κ B in inflammation. *Nat Revs Immunol* 2008;8:837–848
- 33a. Lawrence T, Bebie M, Liu GY, Nizet V, Karin M. IKK α limits macrophage NF κ B activation and contributes to resolution of inflammation. *Nature* 2005;434:1138–1143
- 33b. Rius J, Guma M, Schachtrup C et al. NF- κ B links immunity to the hypoxic response through transcriptional regulation of HIF-1 α . *Nature* 2008;453:807–811
34. Greten FR, Arkan MC, Bollrath J et al. NF- κ B is a negative regulator of IL-1 β secretion as revealed by genetic and pharmacological inhibition of IKK β . *Cell* 2007;130:918–931
- 34b. Fong C, Bebie M, Didierlaurent A et al. An anti-inflammatory role for IKK β through the inhibition of classical macrophage activation. *J Exp Med* 2008;205:1269–1276
- 34c. Timmer AM, Nizet V. IKK β /NF- κ B and the miscreant macrophage. *J Exp Med* 2008;205:1255–1259
35. Muller JR, Siebenlist U. Lymphotoxin receptor induces sequential activation of distinct NF κ B factors via separate signaling paths. *J Biol Chem* 2003;278:12006–12012

36. Cupedo T, Mebius RE. Cellular interactions in lymph node development. *J Immunol* 2005;174:21–25
37. Zhu M, Chin RK, Christiansen PA et al. NF- κ B2 for establishment of central tolerance through an Aire dependent pathway. *J Clin Invest* 2006;116:2964–2971
38. Novak DV, Yin L, Hagen-Stapleton A et al. The I κ B function of NF κ B2 p100 controls stimulated osteoclastogenesis. *J Exp Med* 2003;198:771–781
39. Aya K, Alhawaari M, Hagen-Stapleton A et al. NF- κ B inducing kinase controls lymphocyte and osteoclast activities in inflammatory arthritis. *J Clin Invest* 2005;115:17–22
40. Yamaguchi T, Sakaguchi S. Skin controls immune regulators. *Nat Med* 2006;12:1358–1359
41. Legarda-Addison D, Ting AT. Negative regulating of T cell receptor signaling by NF κ B2/p100. *J Immunol* 2007;178:7767–7778
42. Neumann M, Naumann M. Alternative regulation of NF κ B. *FASEB J* 2007;21:2642–2644
43. Saha R, Jana M, Pahan K. MAPkinase p38 regulates transcriptional activity of NF- κ B in primary human astrocytes via acetylation of p65. *J Immunol* 2007;179:7101–7109
44. Chen L-F, Greene WC. Regulation of NF κ B transcription factor complex by acetylation. *J Mol Med* 2003;81:549–557
45. Lu Y, Wahl LM. Oxidative stress augments production of MMP1, Cox2 and PGE2 through enhancement of NF κ B in LPS activated human monocytes. *J Immunol* 2005;175:5423–5429
46. Qu J, Zhang J, Pan J et al. Endotoxin tolerance inhibits lipopolysaccharide initiated acute pulmonary inflammation and lung injury in rats by the mechanism of NF κ B. *Scand J Immunol* 2003;58:613–619
47. Yoza BK, Hu JY-Q, Cousart SL et al. Induction of Rel B participates in endotoxin tolerance. *J Immunol* 2006;177:4080–4085
48. Cuschieri J, Billigren J, Maier RV. Endotoxin tolerance attenuates LPS induced TLR4 mobilization to lipid rafts. *J Leukoc Biol* 2006;80:1289–1297
49. Zheng S-J, Lamhamedi-Cherradi S-E, Wang P, Yu L, Chen YH. Tumor suppressor p53 inhibits autoimmune inflammation and macrophage function. *Diabetes* 2005;54:1423–1428
50. Totzke G, Essman F, Pohlmann S et al. I κ B- ζ as a regulator of NF- κ B activity. *J Biol Chem* 2006;281:12645–12654
51. Wessells J, Baer M, Young HA et al. Bcl-3 and NF κ B p50 attenuate lipopolysaccharide induced inflammatory responses in macrophages. *J Biol Chem* 2004;279:49995–50003
52. Carmody RJ. Negative regulation of TLR signaling by NF κ B p50 blockade ubiquitination. *Science* 2007;317:675–678
53. Toney LM, Cattoretti G, Graf JA et al. Bcl-6 regulates chemokine gene transcription in macrophages. *Nat Immun* 2000;1:214–220
54. Dent AL, Shaffer AL, Yu X et al. Control of inflammation, cytokine expression and germinal centre formation by Bcl-6. *Science* 1997;276:589–592
55. Phan RT, Saito M, Basso K et al. Bcl-6 interacts with transcription factor Miz-1 to suppress the CDKI p21 and cell cycle arrest in germinal centre B cells. *Nat Immunol* 2005;6:1054–1058
56. Seto M. Genetic and epigenetic factors involved in B cell lymphomagenesis. *Cancer Sci* 2004;95:704–710
57. Pasqualucci L, Migliazza A, Basso K et al. Mutations of the Bcl-6 protooncogene disrupt its negative autoregulation in diffuse large B cell lymphoma. *Blood* 2003;101:2914–2919
58. Beg AA. Compartmentalising NF- κ B in the gut. *Nat Immun* 2004;5:14–16
59. Schenk M, Bouchen A, Birrer S et al. Macrophages expressing triggering receptor expressed on myeloid cells-1 are underrepresented in the human intestine. *J Immunol* 2005;174:517–524
60. Visekruna A, Joeris T, Seidel D et al. Proteasome mediated degradation of I κ B α and processing of p105 in Crohns disease and ulcerative colitis. *J Clin Invest* 2006;116:3195–3203
61. Harris J, Oliere S, Sharma S et al. Nuclear accumulation of c-Rel following C-terminal phosphorylation by TBK1/IKK ϵ . *J Immunol* 2006;177:2527–2535

62. Kaisho T, Tanaka T. Turning NF- κ B and IRFs on and off in DC. *Trends Immunol* 2008; 29:329–337
63. Khachigian LM, Collins T, Fries JW. N-Acetylcysteine blocks mesangial VCAM-1 and NF- κ B expression in vivo. *Am J Pathol* 1997;151:1225–1229
64. Loscher CE, Draper E, Leavy O et al. Conjugated linoleic acid suppresses NF- κ B activation and IL-12 production in dendritic cells. *J Immunol* 2005;175:4990–4998
65. Nishiyama S, Manabe N, Kuhota Y et al. Cyclosporin inhibits the early phase of NF- κ B/RelA activation induced by CD238 costimulation signaling to reduce IL-2 expression in human peripheral T cells. *Int Immunopharmacol* 2005;5:699–710
66. D'Acquisto F, Ianaro L. From willow bark to peptides: the ever widening spectrum of NF- κ B inhibitors. *Curr Opin Pharmacol* 2006;6:387–392
67. Kim E-H, Singh Y-J. 15Deoxy-PGJ2 as potential endogenous regulator of redox-sensitive transcription factors. *Biochem Pharmacol* 2007;72:1516–1528
68. Li J, Liu Z, Jiang S et al. T suppressor lymphocytes inhibit NF- κ B mediated transcription of CD86 gene in antigen presenting cells. *J Immunol* 1999;163:6386–6371
69. Fiorini E, Schmitz I, Morrison WE et al. Peptide induced negative selection of thymocytes activates transcription of an NF- κ B inhibitor. *Mol Cell* 2002;9:637–648
70. Quaaaz F, Arron J, Zheng Y et al. Dendritic cells require distinct NF- κ B subunits. *Immunity* 2002;16:257–270
71. Tato CM, Hunter CA. Subversion and utilization of the NF- κ B pathway during infection. *Infect Immunol* 2002;70:3311–3317
72. Sacani S, Pontano S, Natoli G. p38 dependent marking of inflammatory genes for increased NF- κ B recruitment. *Nat Immun* 2002;3:69–75
73. Adcock IM, Ito K, Barnes PJ. Glucocorticoids: effects on gene transcription. *Proc Am Thorac Soc* 2004;1:247–254
74. Gomez PF, Pillinger MH, Attur M et al. Resolution of inflammation: prostaglandin E2 dissociates nuclear trafficking of NF- κ B subunits. *J Immunol* 2005;175:6924–6930
75. Cohen-Lahav M, Douvdevani A, Chaimovitz C, Shany S. The anti-inflammatory activity of 1,25 dihydroxyvitamin D3 in macrophages. *J Steroid Biochem* 2007;103:558–562
76. Cuzzocrea S, Mazzon E, Di Paola R et al. The role of PPAR- α in the regulation of inflammation. *J Leukoc Biol* 2006;79:999–1010
77. Belaaouaj A et al. Mice lacking neutrophil elastase reveal impaired host defence against gram-negative bacterial sepsis. *Nat Med* 1998;4:615–618
78. Reeves EP et al. Killing activity of neutrophils is mediated through activation of proteases by potassium flux. *Nature* 2002;416:291–297
79. Perkins ND. Oncogenes, tumour suppressors and p52NF- κ B. *Oncogene* 2003;22: 7553–7556
80. Kataoka T et al. Caspase 8 inhibitor FLIP promotes activation of NF- κ B and ERK signaling pathways. *Curr Biol* 2000;10:640–648
81. Iyer SS, Agrawal RS, Thompson CR et al. PLD-1 regulates phagocyte adhesion. *J Immunol* 2006;176:3686–3696
82. Yiu GK, Toker A. NFAT induces breast cancer cell invasion by promoting the induction of Cox2. *J Biol Chem* 2006;281:12210–12217
83. Pettus BJ, Bielawski S, Porcelli AM et al. The sphingosine kinase: sphingosine-1-phosphate pathway mediates Cox2 induction in response to TNF α . *FASEB J* 2003;17:1411–1421
84. Vega A, Chacon P, Alba G et al. Modulation of IgE dependent Cox2 gene expression by reactive oxygen species in human neutrophils. *J Leukoc Biol* 2006;80:152–163
85. Seo JH, Kim H, Kim KH. Cyclooxygenase 2 expression by transcription factors in *H. pylori* infected gastric epithelial cells. *Ann N Y Acad Sci* 2002;973:477–480
86. Ki SH, Choi MJ, Lee CH, Kim SG. G α 12 regulates Cox-2 induction by sphingosine-1-phosphate. *J Biol Chem* 2007;282:1938–1947
87. Dixon DA, Tolley ND, Bemis-Standoli K et al. Expression of Cox2 in platelet-monocyte interactions involving adhesion and cytokine signaling. *J Clin Invest* 2006; 116:2727–2732

88. Kititoshi S, Nishikowa T, Sonoda K et al. Reactive oxygen species from mitochondria induce Cox-2 gene expression in human mesangial cells. *Diabetes* 2003;52:2570–2576
89. Caivano M, Gorgoni B, Cohen P et al. The induction of Cox2 in macrophages is biphasic. *J Biol Chem* 2001;276:48693–48701
90. Kang Y-J, Wingerd BA, Arakawa T, Smith WL. Cox2 gene transcription in a macrophage model of inflammation. *J Immunol* 2006;177:8111–8122
91. Nie M, Pang L, Inoue H, Knox AJ. Transcriptional regulation of Cox-2 by bradykinin and interleukin 1 beta in human airway smooth muscle cells: involvement of different promoter elements, transcription factors and histone H4 acetylation. *Mol Cell Biol* 2003;13:9233–9244
92. Vila-del Sol V, Fresno M. Involvement of TNF α and NFkB in the transcriptional control of Cox-2 expression by IFN γ in macrophages. *J Immunol* 2005;174:2825–2833
93. Rodriguez-Barbero A, Dorado F, Velasco S et al. TGF β 1 induces Cox2 expression and PGE2 synthesis in human mesangial cells. *Kidney Int* 2006;70:901–909
94. Steer SA, Moran JM, Christmann BS et al. MAPK in regulation of ds-RNA and virus induced Cox2 expression by macrophages. *J Immunol* 2006;177:3413–3420
95. Chen B-C, Liao C-C, Hsu M-J et al. Peptidoglycan induced Il-6 production in Raw 264.7 macrophages is mediated by Cox-2. *J Immunol* 2006;177:681–693
96. Han C, Demetris AJ, Stolz DB et al. Modulation of Stat3 activation by the cPLA2 and Cox2 controlled PGE2 signaling pathway. *J Biol Chem* 2006;281:24831–24846
97. Clark RB. The role of PPARs in inflammation and immunity. *J Leukoc Biol* 2002;71:388–400
98. Jo S-H, Yang C, Miao Q et al. PPAR γ promotes lymphocyte survival via actions on cell metabolic activities. *J Immunol* 2006;177:3737–3745
99. Faveeuw C, Fougeray S, Angeli V et al. PPAR γ activators inhibit interleukin 12 production in murine dendritic cells. *FEBS Lett* 2000;486:261–266
100. Fahmi H, Pelletier J-P, Martel-Pelletier J. PPAR γ ligands as modulators of inflammatory and catabolic responses in arthritis. *Arthritis Rheum* 2002;29:3–12
101. Yamasaki S, Nakashima T, Kawakami A et al. Functional changes in rheumatoid fibroblast synovial cells through activation of PPAR γ signaling pathway. *J Immunol* 2002;169:1145–1150
102. Glass CK, Ogawa S. Combinatorial roles of nuclear receptors in inflammation and immunity. *Nat Rev Immunol* 2006;6:44–55
103. Poynter ME, Davies RA. PPAR γ activation modulates cellular redox status, represses nuclear factor-B signaling, and reduces inflammatory cytokine production in ageing. *J Biol Chem* 1998;273:32833–32841
104. Myhre AE, Agren J et al. Liver X receptor regulator of cytokine release. *Shock* 2008;29:465–474
105. Yamada KM, Clar K. Survival in three dimensions. *Nature* 2002;419:790–791
106. Brazil DP, Hemmings BA. 10 years of protein kinase B signaling. *Trends Biochem Sci* 2001;26:657–664
107. Franke TF, Kaplan DR, Cantley LC. PI 3-kinase downstream AKTion blocks apoptosis. *Cell* 1997;88:435–437
108. van Blitterswijk WJ, van der Luit AH, Veldman RJ et al. Ceramide: second messenger or modulator of membrane structure. *Biochem J* 2003;369:199–211
109. Kong M, Fox CJ, Mu J et al. The PP2A associated protein α 4 is an essential inhibitor of apoptosis. *Science* 2004;306:695–698
110. Hoeflich KP, Luo J, Rubie EA et al. Requirement for glycogen synthase kinase 3 in cell survival and NFkB activation. *Nature* 2000;406:86–90
111. Jope RS, Johnson GVW. The glamour and gloom of glycogen synthase kinase-3. *Trends Biochem Sci* 2004;29:95–101
112. Martin M, Rehane K, Jore RS, Michalek SM. Toll-like receptor and cytokine production is differentially regulated by GSK3. *Nat Immun* 2005;6:777–784
113. Vivarelli MS, McDonald D, Miller M et al. RIP links TLR4 to Akt and is essential for cell survival in response to LPS stimulation. *J Exp Med* 2004;200:399–404

114. O'Connor R, Fennelly C, Krause D. Regulation of survival signals from the IGF-1 receptor. *Biochem Soc Trans* 2000;28:47–51
115. Wang X, Gjorloff-Wingren A, Saxen M et al. The tumor suppressor PTEN regulates T cell survival and antigen receptor signaling. *J Immunol* 2000;164:1939–1943
116. Choi Y, Zhang J, Murga C et al. PTEN suppresses the PI3K/Akt pathway and induces growth inhibition and apoptosis of myeloma cells. *Oncogene* 2002;21:5289–5300
117. Stambolic V, Mak T, Woodgett JR. Modulation of cellular apoptotic potential: contributions to oncogenesis. *Oncogene* 1999;18:6094–6103
118. Stambolic V. PTEN: a new twist on β -catenin. *TIPS* 2002;23:104–106
119. Cibull TL, Jones TD, Li L et al. Overexpression of Pim.1 during progression of prostate adenocarcinoma. *J Clin Pathol* 2006;59:285–288
120. Kyriakis JM. Life or death decisions. *Nature* 2001;414:265–266
121. Ono K, Han J. The p38 signal transduction pathways. *Cell Signal* 2000;12:1–13
122. Li S-P, Junttila MR, Han J et al. The p38 MAP kinase pathway suppresses cell survival by inducing dephosphorylation of MAP/ERK1,2. *Cancer Res* 2003;63:3473–3477
123. Mayo MW, Baldwin AS. The transcription factor NF κ B: control of oncogenes and cancer therapy resistance. *Biochim Biophys Acta* 2000;1470:M55–M62
124. Klaumig JE, Kamendulis LM. The role of oxidative stress in carcinogenesis. *Annu Rev Pharmacol Toxicol* 2004;44:239–267
125. Perkins ND. NF- κ B: tumour promoter/suppressor. *Trends Cell Biol* 2004;16:64–69
126. You S, Chatenoud L. Proinsulin: a unique autoantigen triggering auto-immune diabetes. *J Clin Invest* 2006;116:3108–3110
127. White MF. The IRS signaling system: a network of docking proteins that mediate insulin and cytokine action. *Recent Prog Horm Res* 1998;53:119–138
128. Pessin JE, Saltiel AR. Signaling pathways in insulin action: molecular targets of insulin resistance. *J Clin Invest* 2000;106:165–169
129. Goldstein BJ, Ahmad F, Ding W et al. Regulation of the insulin signaling pathway by protein tyrosine phosphatases. *Mol Cell Biochem* 1998;182:91–99
130. Watson RT, Pessin JE. Bridging the GAP between insulin signaling and GLUT4 translocation. *Trends Biochem Sci* 2006;31:215–222
131. Kim JK, Zisman A, Fillmore JJ et al. Glucose toxicity and development of diabetes in mice with muscle specific inactivation of Glut4. *J Clin Invest* 2001;108:153–160
132. Goldstein BJ, Kalankar M, Wu Y. Insulin antigen is facilitated by insulin stimulated ROS with multiple potential signaling targets. *Diabetes* 2005;54:311–321
133. Houstis N, Rosen ED, Lander ES. ROS have a causal role in multiple forms of insulin resistance. *Nature* 2006;440:944–946
134. Rondinone CM, Wang M, Lonnroth P et al. IRS-1 is reduced and IRS-2 is the docking protein for PI3kinase in adipocytes from subjects with NIDDM. *Proc Natl Acad Sci U S A* 1997;94:4171–4175
135. Brindley DN, Wong C-N, Mei J et al. TNF α and ceramide in insulin resistance. *Lipids* 1999;34(Suppl):S85–86
136. Plomgaard P, Bouzahari K, Krogh-Madsen R et al. TNF α induces skeletal muscle insulin resistance via inhibition of Akt phosphorylation. *Diabetes* 2005;54:2939–2945
137. Stratford S, Hoehn KL, Liu F, Sumners SA. Regulation of insulin action by ceramide. *J Biol Chem* 2004;279:36608–36615
- 138a. de Alvaro C, Tervel T, Hernandez R, Lorenzo M. TNF α produces insulin resistance in skeletal muscle by activation of inhibitor kappa-B kinase. *J Biol Chem* 2004;279:17070–17078
- 138b. Yang R, Trevillyan JM-Jun N-terminal kinase pathways is diabetes. *Int J Biochem Cell Biol* 2008;40:2702–2706
139. Yamaudi T, Kaman J, Ito Y et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003;423:762–768
140. Yudkin JS, Eringa E, Stehouwer CDA. Vasocrine signalling from perivascular fat: mechanism linking insulin resistance to vascular disease. *Lancet* 2005;365:1817–1820
141. Arner P. Resistin. *Diabetologia* 2005;48:2203–2205

142. Bandyopadhyay GK, Yu JG, Ofrecio J, Olefsky JM. Increased p85/55/50 expression and decreased PI3kinase activity in insulin-resistant human skeletal muscle. *Diabetes* 2005;54:2351–2359
143. Liang C-P, Hans S, Senokuchi T, Tall AR. The macrophage at the crossroads of insulin resistance and atherosclerosis. *Circ Res* 2007;100:1546–1555
144. Solinas G, Viku C, Neels JG et al. JNK1 in hematopoietically derived cells contributes to diet induced inflammation and insulin resistance. *Cell Metab* 2007;6:386–397
145. Colca JR. Insulin sensitizers may prevent metabolic inflammation. *Biochem Pharmacol* 2006;72:125–131
146. Stumvoll M, Haring H-U. Glitazones: clinical effects and molecular mechanisms. *Ann Med* 2002;34:217–223
147. Velloso LA, Folli F, Perego L, Saad MJA. The multi-faceted cross-talk between the insulin and angiotensin II signal systems. *Diabetes Metab Res Rev* 2006;22:98–107
148. Velloso LA, Folli F, Sun XJ et al. Crosstalk between the insulin and angiotensin signaling systems. *Proc Natl Acad Sci U S A* 1996;93:12490–12495
149. Calegari VS, Alves M, Picardi PK et al. SOCS3 provides a novel interface in crosstalk between angiotensin II and insulin signaling systems. *Endocrinology* 2005;146:579–588
150. Fingar DC, Blenis J. Target of rapamycin (TOR). *Oncogene* 2004;23:3151–3171
151. Nobukuni T, Joaquin M, Roccio M et al. Amino acids mediate mTOR signaling through activation of class 3 PI3kinase. *Proc Natl Acad Sci U S A* 2005;102:14238–14243
152. Manning BD. Balancing Akt with S6K: implications for both metabolic diseases and tumorigenesis. *J Cell Biol* 2004;167:399–403
153. Crino B, Nathanson KL, Henske EP. The tuberous sclerosis complex. *N Engl J Med* 2006;355:1345–1356
154. Zhang H, Bajraszewski N, Wu E et al. PDGF-receptors are critical for PI3kinase/Akt activation: negative regulation by mTOR. *J Clin Invest* 2007;117:730–738
155. Martin DE, Hall MN. The expanding TOR network. *Curr Opin Cell Biol* 2005;17:158–166
156. Koo S-H, Flechner L, Qi L et al. The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. *Nature* 2005;437:1109–1113
157. Hardie DG, Sakamoto K. AMPK: a key sensor of fuel and energy status in skeletal muscle. *Physiology* 2006;21:48–60
158. Jones RG, Thompson CB. Signal transduction fuels T cell activation. *Immunity* 2007;27:173–178
159. Hinnebusch AG. eIF3: a versatile scaffold for translation initiation complexes. *Trends Biochem Sci* 2006;31(10):553–562
160. Nobukuni T, Kozman SC, Thomas G. Hsp34 enters a growing game: mTOR complex 1/ S6K1 signaling. *Curr Opin Cell Biol* 2007;19:135–141
161. Cheung AM, Mak TW. PTEN in the haematopoietic system and its therapeutic indications. *Trends Mol Med* 2006;12:503–505
162. Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. *Cancer Cell* 2007;12:9–22
163. Cuzzocrea S, Crisafulli C, Mazzon E et al. Inhibition of GSK-3 β attenuates development of carrageenan-induced lung injury. *Br J Pharmacol* 2006;155(5):965–970
164. Tamas P, Hawley SA, Clarke RG et al. Regulation of the energy sensor AMP-activated protein kinase by antigen receptor and Ca²⁺ in T lymphocytes. *J Exp Med* 2006;203:1665–1672
165. Fox CJ, Hammerman PS, Thompson CB. The Pim-kinases control rapamycin resistant T cell survival and activation. *J Exp Med* 2005;201:259–266
166. Chen J-K, Chen J, Neilson EG, Harris RC. Role of mammalian target of rapamycin signaling in compensatory renal hypertrophy. *J Am Soc Nephrol* 2005;16:1384–1313
167. Petroulakis E, Mamone Y, Le Bacquer O et al. mTOR signaling: implications for cancer. *Br J Cancer* 2006;94:195–199
168. Monick MM, Powers LS, Gross TJ et al. Active ERK contributes to protein translation by preventing JNK-dependent of protein phosphatase-1. *J Immunol* 2006;177:1636–1645

Chapter 12

Signalling in Immune Reactions

Abstract Regarding signalling in immune reactions, by now there is sufficient background for logical understanding of the immune response and how macrophages work. Macrophages and dendritic cells bear Toll-like receptors (TLRs) whose signal transduction pathways link to the formation of proinflammatory cytokines and chemokines. There are also intracellular sensors for pathogens called nucleotide oligomerisation domain (NOD)-like receptors. NLRs occur as “inflammasomes” that account for production of the important cytokine interleukin 1 (IL-1). The cytokines and their functions are delineated. The activation of macrophages and dendritic cells is presented, for example, in response to IL-1 and TNF α . The processes must not get out of control, and IL-10 exerts a moderating influence that is not completely understood. Attempting to understand rheumatoid arthritis highlights cutting-edge aspects of immunology.

12.1 The Immune Response

Microbial invaders bear pathogen-associated molecular patterns (PAMPs). As part of the innate nonspecific immune response, these PAMPs are recognised by pattern recognition receptors (PRRs) on host dendritic cells (DCs) and macrophages. Indeed, the innate immune response utilizes phagocytic cells like macrophages and neutrophils, natural killer (NK) cells, or cells that release inflammatory mediators such as basophils, mast cells and eosinophils. The innate humoral defence is comprised of the complement system, the antiviral action of interferons and the acute-phase proteins. Soon, the acquired antigen-specific adaptive immune response gives support in the form of cell-mediated immunity (CMI) by CD4 T lymphocytes and CD8 T lymphocytes, which are cytotoxic T cells, and there is a humoral immune response as antigen-specific CD4 T helper cells aid B lymphocytes and associated plasma cells to produce antibodies (Table 12.1) [1].

Table 12.1 Immune defence mechanisms

Innate immunity	Antigen-specific adaptive immunity
Cellular (a) The phagocytes: Macrophages, monocytes Dendritic cells, neutrophils (b) Natural killer cells and natural killer T (NKT) cells (c) Basophils, mast cells, eosinophils	<i>Cell-mediated immunity (CMI)</i> CD8 cytotoxic T Lymphocytes CD4 helper T lymphocytes
Humoral (a) Complement system Mannose-binding protein (MBP) Mannose receptors Scavenger receptors (b) Interferons (c) Acute-phase proteins	<i>Humoral antibody-forming system</i> Antigen-presenting cells CD4 helper lymphocytes CD8 suppressor lymphocytes B lymphocytes → plasma cells that synthesise antibodies

12.1.1 Dendritic Cells

The dendritic cells (DCs) are antigen-presenting cells (APCs) that induce primary immune responses much more efficiently than macrophages. There are type I DC-1s that induce Th1 lymphocytes and DC-2s that induce Th2 lymphocytes. When DC-1s secrete interleukin 12 (IL-12), proinflammatory Th1 lymphocytes form; together with macrophages, they can mount a delayed-type hypersensitivity (DTH) reaction toward invaders [2]. Glutamate released by DCs helps to promote T cell proliferation and Th1 cells. Conversely, in an anti-inflammatory environment created by local prostaglandin E2 (PGE2) or IL-10, DC-2s induce the Th2 lymphocytes with their particular set of cytokines IL-4/-5/-13 (Fig. 12.4). Released prostacyclin PGI2 inhibits formation of proinflammatory cytokines. DCs have an array of PRR molecules that recognise the outer membrane lipids and proteins of bacteria so that an innate immune response can be triggered. Amongst these are several types of Toll-like receptor (TLR) [3, 4] (Tables 12.2 and 12.3). The scavenger receptor PSOX (CXC chemokine ligand 16), which helps the phagocytosis of bacteria, is another PRR. It enables uptake of pathogens and chemotaxis of T and natural killer T cells (NKT cells) [5]. Tissue-resident *immature DCs* (iDCs) quickly engender innate immunity, for example, by creating IL-2 and type I interferon (IFN), which stimulates NK cells [6]. The iDCs occur in the respiratory tract, lungs, gut, spleen and liver. When they become migratory *mature DCs* (mDCs), soon to be sited in lymph nodes, they induce the acquired immune responses. *Tolerogenic DCs* induce Treg suppressor cells. When IL-10 or dexamethasone are used to condition DCs, an internal change via GILZ (glucocorticoid-induced leucine zipper) means that those DCs release IL-10, but not IFN γ , and they do not induce T cell proliferation. In Table 12.2, take a look at the PRRs found on DCs and macrophages for they account for the uptake of particular types of antigen.

Table 12.2 Innate pattern recognition receptors (PRRs) on macrophages

Toll receptors 1–10	See Table 12.3
CD14	For LPS or peptidoglycan
CR2 (CD21) or CR3	iC3b opsonized particles
FcγRs	IgG antibodies
Scavenger receptors	LPS, lipoteichoic acid, bacteria
Marco	For bacteria, oxidised LDL
PSOX	For bacteria
Mer	For lipids, for apoptotic cells
Mannose receptors	For mannan, for collagens
FMLP receptors	N-formylated bacterial peptides
Dectin-1, DC-SIGN	<i>c-type lectin (e.g. for β-glucan)</i>
Integrin-α3β1	Fibronectin
CD44	hyaluronic acid receptor

FMLP

N-formyl-methionyl-leucyl-phenylalanine, LDL low-density lipoprotein, LPS lipopolysaccharide, PSOX CXC chemokine ligand 16

Table 12.3 Toll receptors and their ligands

Toll-like receptor (TLR)	Ligand
TLR2	Lipoproteins, mycoplasma lipopeptide Peptidoglycan zymosan
TLR3 ^a	Double-stranded RNA viruses
TLR4 + CD14 + MD2	Lipopolysaccharide (LPS) lipoteichoic acid Heat shock proteins fibronectin
TLR5	Flagellin
TLR6	Lipoproteins, peptidoglycan
TLR7/8 ^a	Imidazolequinoline antivirals
TLR9 ^a	Bacterial DNA, CpG-DNA
TLR10	Toxoplasma membrane antigen

^a Cytosolic

12.1.2 Macrophage Types: M1 Microbicidal, M2 Regulatory or M2'' Would Healing

Antimicrobial M1 macrophages will be activated by IFNγ, lipopolysaccharide (LPS), TNFα, GM-CSF or the GPCR gene [7]. They produce proinflammatory cytokines, high levels of IL-12 and IL-23, and ROS and inducible nitric oxide synthase (iNOS). The necessary acidification of their phagosomes is determined by the CFTR Cl⁻ channel. Conversely for encapsulation or killing of parasites there are alternative M2 macrophages, with chitinase, which has been induced by IL-4 or IL-13[8], or by IL-10, by glucocorticoid (GC) or by secosteroid (vitamin D). They produce no IL-12/23 but large amounts of IL-10 and IL-1 receptor antagonist. They show high surface scavenger, mannose and galactose-type receptors. M2'' would

healing cells use arginase so that ornithine and polyamines are produced. Thus M2 can be anti-inflammatory (regulatory) or M2'' pro-fibrotic (wound healing) cells according to the situation [8b]. Even this classification could become expanded in future [8c]. Activin A is noted to function as a Th2 cytokine [9] in the activation of alternative M2 macrophages, as does IL-21. They are inhibited by IL-27.

The regulatory anti-inflammatory M2 macrophage often arises from stimulation by immune complexes, and it produces immunosuppressive IL-10. It might also be induced by prostaglandins, glucocorticoids (GC), apoptotic cells or even IL-10. Its surface CCR1 attracts eosinophils and Th2 lymphocytes. This Mφ expresses LIGHT, and it stimulates Th2 T cells, which then produce IL-4 [10].

Interestingly, in the fat of lean mice there are the wound healing M2'' macrophages. In such mice IL-10 protects the adipocytes from TNFα-induced insulin resistance [11]. However if mice become obese their macrophages become M1 proinflammatory types which display TNFα and iNOS activity.

12.1.3 Toll-Like Receptors

The extracellular portion of the TLRs consists of leucine-rich repeats (LRRs) that are separated by cysteine-rich motifs. LRRs are noted for their ability to mediate protein interactions (Table 12.4). The cytoplasmic domain of each Toll receptor has a close similarity to the IL-1 receptor and is called a TIR. The ligands for the TLRs are listed in Table 12.3. The interaction with the bacterial PAMPs is enhanced by heparans, hyaluronic acid or fibronectin fragments or by heat shock protein HSP70. TLR4 will activate cell PI3 kinase (PI3K), and the NF-κB is activated (Fig. 12.1). TLR4 also activates p38 MAPK so that scavenger receptors are upgraded for use in phagocytosis [16].

Antigen meets iDCs first, and they enter the lymph nodes; there they become mDCs, which perform antigen presentation for the T lymphocytes. According to the type of PAMP, the DCs may have become polarised so that DC-1s induce formation of Th1 lymphocytes, DC-2s promote production of Th2 cells, or in response to

Table 12.4 Toll-like receptors (TLRs) on immune cells

Cell type	TLRs	Type of response
Monocytes	1, 2, 4, 5, 6, 8	Not 3, but occurs in mice
Myeloid DCs	3, 8	IL-12, TNFα, IL-6
Macrophages	All TLRs 1–9	Phagosomal killing
Plasmacytoid DCs	7, 9	Type I IFNs
CD8 DCs	1, 2, 3, 6, 9	IL-12
CD11b DCs	1, 2, 3, 5, 6, 7, 9	IL-10
Mast cells	2, 4, 6, 8	Cytokines and chemokines
T lymphs	3, 5, 9(not -2, -4)	Downregulated by TGFβ
B lymphocytes	1, 2, 4, 6, 7, 9, 10	Antibody formation

DC dendritic cell, IFN interferon, IL interleukin, TGFβ transforming growth factor-β, TNFα tumour necrosis factor-α

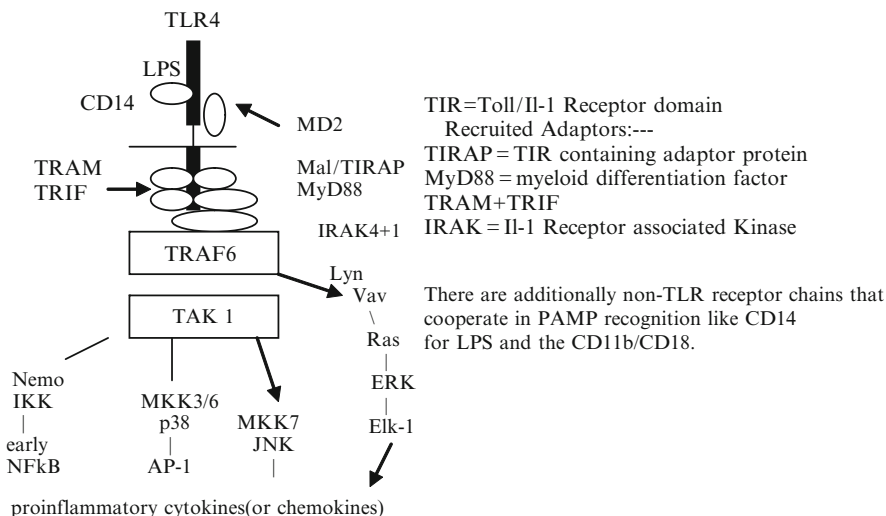


Fig. 12.1 Bacterial product stimulation of Toll-like receptors (*TLRs*). The MyD88-dependent path MyD88/Tirap and *IRAK4/TRAF6* is for TLR4/2 signalling. The MyD88-independent path via TRAM-TRIF is for TLR4/3 signalling (not shown here).

In the response to TLR4, an attached surface recognition molecule called MD2 is required for ligand action [12]. Note how there is homology of the TLR receptors with the IL-1 receptor (Fig. 12.10). TLR4 activation utilises the scaffolds MyD88/Tirap [12–14]. They phosphorylate IRAK, enabling TRAF6 to activate the MKKK that is called TAK1 (TGFβ-activated kinase) through ubiquitination, and hence the canonical IκB kinases (IKKα/β), so that NF-κB is activated. So also are the p38 and JNK kinases and the ERK. IRAK4 is essential for NF-κB activation in both TLR and T cell receptor signalling [15]. The association of Rac1/Nox4 with the TLR receptor produces ROS that facilitate the signalling process. Tollip blocks activation of IRAK4/1 and so thwarts NF-κB. VIP blocks TLR2/4 up-regulation on monocytes.

ERK extracellular signal-related kinase, *IκB* unit I-kappa B, *IL* interleukin, *IRAK4* IL-1 receptor-associated kinase 4, *JNK* Jun N-terminal kinase, *MyD88* myeloid differentiation marker 88, *NF-κB* nuclear factor kappa B, *Nox4* nicotinamide adenine dinucleotide phosphate oxidase, *ROS* reactive oxygen species, *TAK1* TGFβ-activated kinase, *TGFβ* transforming growth factor-β, *Tirap* Toll IL-10 receptor domain-containing adaptor protein, *TLR* Toll-like receptor, *TRAF6* tumour necrosis factor-α receptor-associated factor 6, TRAM TRIF-related adaptor molecule, *TRIF* Toll receptor-associated activator of interferon, *VIP* vasoactive intestinal peptide

In order to achieve TLR4 activation Mal must recruit IRAK4 and IRAK1 to MyD88. The IRAK4 will phosphorylate IRAK1, and the kinases then dissociate from MyD88. Next IRAK4 phosphorylates pellino which is an E3-ligase that causes K63-polyubiquitination of IRAK1. That then serves as a platform to recruit the Nemo/IKK1/IKK2 complex. In turn IRAK1 will cause K63-polyubiquitination of TRAF6 which serves as a platform to recruit TAK1 into close proximity with IKK2 in the complex, so activating IKK2! Accordingly NF-κB activation proceeds.

self-antigen, T regulatory cells develop. Bacteria or parasites that carry phosphorylcholine on their outer membranes incite DC-2s. Most pathogens, like streptococci or *Mycobacterium tuberculosis*, provoke Th1 T cells. One TLR4 mediate responses to LPS, and microbial products that incite TLRs 2 and TLRs 4 explain how interaction of DCs with gram-positive and gram-negative bacteria lead to production of Th1

lymphocytes. As shown in Table 12.4, the associated formation of cytokines determines the immune response. Understanding of the mode of signalling by TLRs, the human homologues of the *Drosophila* receptor Toll, shows how they are crucial to immune defence [17]. TLRs cause the activation of DCs, and they are implicated in presentation of antigens. Unfortunately, unchecked TLR4 activation could result in autoimmune disease. The intracellular domains of the TLRs are similar to those of the IL-1 receptor (IL-1R) [13, 17] (Fig. 12.10).

Signalling by TLRs is at the interface between innate and adaptive immunity [18, 19]. TLR action is integrated with phagocytosis [20]. The several TLRs use different adaptor molecules. In LPS signalling that is CD14 independent, TLR4 uses MyD88 with MAL (MyD88 adapter-like) leading to early NF- κ B activation. MyD88 means myeloid differentiation marker 88. MAL is the same as TIRAP (TIR domain-containing adaptor protein) [14]. TIRAP contains a PIP2 binding domain, so that it can be recruited to the membrane. In the MyD88-independent pathway, the TLR4 signals through TRIF (Toll receptor-associated activator of IFN) [21] and TRAM (TRIF-related adaptor molecule). TRAM was demonstrated to associate with TRIF and TIRAP/Mal and so with TLR4, but not with TLR3 (Fig. 12.2). Newly recognised adaptor SARM is a negative regulator of NF- κ B and IRF3.

One has to have a clear view of the five names of the Toll-like adaptor protein family. There is MyD88 and MAL, also called TIRAP, then TRIF and TRAM, also called TICAM-2. MyD88 is essential for the host response to many pathogens.

TLR4 can activate NF- κ B and MAPKs in MyD88-deficient cells, albeit with delayed kinetics [22]. The delayed responses fail to induce gene expression of inflammatory cytokines but are sufficient for DC maturation and for expression of IFN β and IFN-inducible genes. So, we realise that TRAM/TRIF adaptor molecules lead to (a) late activation of NF- κ B via Nemo activation of NF- κ B that is supported

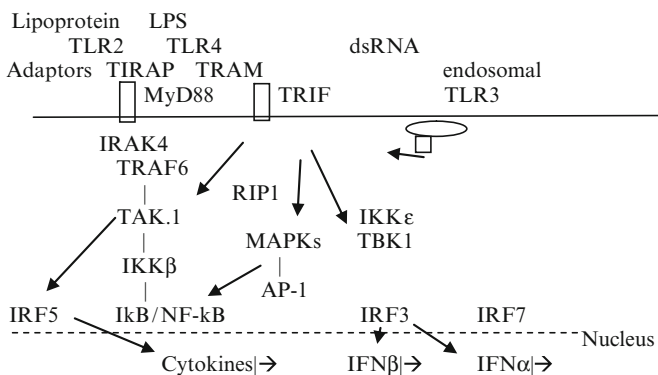


Fig. 12.2 A composite view of the Toll-like receptor (TLR) signalling pathways. TLR3/4 for viruses stimulate the myeloid differentiation marker 88 (*MyD88*)-independent, Toll receptor-associated activator of interferon (*TRIF*)-dependent pathway. One could add virus-induced TLR7/9 pathways to the right of this scheme. Cytokine transforming growth factor- β (*TGF β*) inhibits the TLR-MyD88-dependent path and CD14 expression

by RIP1 (receptor-interacting protein 1) and (b) activation of IRF3 and hence formation of IFN α/β [23a, 23b]. The latter path means that virus infection (via TLR3 receptors and IRF3/7) will induce members of the IFN α gene family that will be activators of NK cells, supporters of memory T cells, promoters of IFN γ secretion and agents for isotype switching of B cells as they produce antibodies [24]. TLR3 leads to TRIF $\rightarrow$ IKK ϵ /TBK1 $\rightarrow$ IRF3.

When bacterial products like LPS, lipopeptide or unmethylated CpG motifs in bacterial DNA (CpG-DNA) act on TLRs of DCs, if cot kinase (Tp1–2) is involved, DCs produce more IL-12, and the response is skewed toward Th1 cells [25]. The MAP3K (MAP kinase kinase kinase) Cot kinase is also required for TLR4 activation of the MAPK cascade in LPS-stimulated macrophages. Agonists of TLRs evoke cytokines from monocytic cells, albeit these receptors tend to become downregulated [25].

One has to know which TLRs appear on which sort of immune cell and how that determines their responses (Table 12.3). Signals from the TLRs initiate the innate immune responses to infections since they are predominantly on APCs like the DCs and monocyte macrophages.

As noted in Table 12.3, TLR7 incites responses to viruses, and TLR9 enables CpG-DNA to activate DCs to release IL-12. When flagellin acts on TLR5 at the gut mucosa, release of IL-8 attracts neutrophils and Rantes, macrophage inflammatory proteins (MIPs) 1 α and 1 β attracts DCs and NK cells. In the gut of mice, there are proximal colon TLR2 receptors for peptidoglycan and lipopeptides and TLR4 receptors in the distal colon. Yet, actually commensal gut bacteria lead to downregulation of TLRs and quiescence of the mucosal NF- κ B. TLRs on macrophages lead to release of IL-8, which attracts neutrophils, and of Rantes, MIP-1 α and MIP-1 β , which attract DCs and NK cells. Via IRF3 $\rightarrow$ IFN β , chemokines IP10, Mig and I-Tac will be released [26]. RIP1 in macrophages helps (TAK1)-dependent activation of PI3K [27], which will help to keep them alive. Signals via TLRs activate PI3K and in turn glycogen synthase kinase 3 β (GSK3 β), which modulates the expression of cytokines. In fact, PI3K is generally a negative regulator of TLR signalling. So, there is inhibition of the expression of proinflammatory TNF α and of IL-12.

12.1.4 Negative Regulation of Toll-Like Receptors

Cooperativity between TLRs, which actually occurs within the endosomes, means that an immune response is tailored to the particular microbial invasion. All TLRs, with the exception of TLR3, provoke proinflammatory cytokines, whilst viral double-stranded RNA (dsRNA) acting on TLR3 $\rightarrow$ TRIF $\rightarrow$ TLR3 and single-stranded RNA (ssRNA) or CpG-DNA acting on TLRs 7–9, and so MyD88 $\rightarrow$ IRF7 lead to production of type I interferons [28]. There is a possibility of overproduction of IL-12 with IL-23 administration [28], but it has been demonstrated that involvement of TLR $\rightarrow$ PI3K, and thence Akt, causes inhibition of GSK3, with the result that activation of NF- κ B is dampened. Accordingly, IL-12 production fails, and there is formation of anti-inflammatory IL-10 [29]. IL-10 acts through the intermediary of (Stat3).

Negative regulators of TLR signalling have been identified [30, 31]. First, there are soluble extracellular decoy receptors for TLR2 and TLR4. Then, following TLR stimulation, there is upregulation of transmembrane suppressor ST2 of IRAK-M of SOCS1 which targets Mal for degradation [32]; and A20. A20 was found as a TNF α -induced zinc finger protein that suppresses TNF α -mediated NF- κ B activation. A20 blocks LPS-induced TLR signalling by its deubiquitination of (TRAF6) [33] and inhibition of recruitment of RIP receptor interacting protein-1. A20 can regulate MEKK1 (Table 12.12). A20 reduces both MyD88-dependent and the independent signal pathways. In macrophages, the LPS signal to NF- κ B1 p105 conscripts arrestin2, leading to negative regulation [34]. The binding of β -AR to the Traf-N domain within TRAF6 prevents autoubiquitination and so prevents downstream activation of NF- κ B and (AP-1). Activation of (NADPH) oxidase can also result in negative feedback of TLR signalling because NADPHox activates the negative regulator TRAF4, which inhibits TRAF6 and thereby NF- κ B or IFN β activation [35].

C5a negatively regulates TLR4-induced immune responses [36] and so does ligation of PECAM-1. On many cells, molecule RP105 (radioprotective 105) is expressed. It is a TLR homologue and thus is a negative regulator of TLRs [37]. It has been found that IRF4^{-/-} mice show enhanced production of proinflammatory cytokines by macrophages, and thus it has been realised that when TLR4 connects with IRF4 there is negative feedback at MyD88. So, IRF4 is a negative regulator of TLRs, in particular of TLR9 [38].

The subject of negative regulation of macrophages is developing under the title of *inflammation suppressor genes*. The TAM (Tyr3, Axl, Mer) receptors inhibit inflammation in macrophages and DCs. TAM signalling inhibits TLR induction of proinflammatory cytokines and is important for the restraint of chronic inflammation [39].

There is a summary of negative regulation of receptors in Sect. 12.4.1 (Table 12.8) and a recent article by Luke O'Neill [39b] emphasizes phosphatases (SHP1/2) as regulators and the role of ubiquitination.

12.1.5 Inside Sensors for Outside Pathogens: Nucleotide Oligomerisation Domain-Like Receptors

Mucopeptides like muramyl dipeptide (MDP) and other PAMPs of intracellular bacteria are recognised by NOD (nucleotide oligomerisation domain) proteins, which are able to activate NF- κ B via the recruitment of RIP2 [40, 41]. TLRs encounter the NOD-like receptors (NLRs) in the endosomes of phagocytic cells [42, 43]. NOD1 and NOD2 can activate NF- κ B. A NOD is a cytosolic PRR. The NODs have at the N-terminal a caspase recruitment domain (CARD), followed by a nucleotide-binding domain (NBD), also called NACHT (for the domains NAIP, CIITA, HET-E and TP1), and at the C-terminal a series of LRRs (leucine rich repeats), as does a TLR [41]. The LRR is involved in ligand binding, and in autoregulation. Peptidoglycan will activate NOD [43, 44] and thus stimulate

NF- κ B, forming cytokines or α -defensins and chemokines and antiviral proteins [45]. In Crohn's disease patients, NOD might be mutated [43]. A NOD1 detects diaminopimelic acid as in peptidoglycan. A NOD2 reacts to MDP [44]. When MDP primes murine macrophages, IFN γ increases expression of NOD2, so nitric oxide (NO) is induced [45].

Fungal infection will stimulate DCs via their NF- κ B to form inflammatory cytokines. In that case, NOD2 interacts with RIP2, which directly activates NF- κ B, and CARD9 of the caspase-recruitment domain family activates Jun N-terminal kinase (JNK) and p38 MAPK.

The NACHT family proteins [46] are like TLRs for they also have C-terminal spans of LRRs. The NACHT refers to the domains NAIP, CIITA, HET-E and TP1 [46]. NOD1 protein is formed, as stated, by (CARD-NBD-LRRs), and NOD2 protein has the domain sequence (CARD-CARD-NBD-LRR). The NACHT family also includes a larger family of PYRIN proteins [47] with the sequence PYRIN-NACHT-LLR. Either form will use CARD (actually RICK/RIP2/CARD [48]) for caspase 1 activation (Fig. 12.3), which is involved in the release of IL-1 and IL-18 in response to peptidoglycan. TREM (triggering receptors expressed on myeloid cell) amplifies these NLR signals [49a]. Conversely, NTAL (non-T cell activation linker) negates Trem-induced cytokine production [49b].

NLRs are the largest family of intracellular PRRs, with more than 24 members. In fact, their permutations account for the activation of inflammasomes by a great variety of bacteria and their toxins [50]. How *Pseudomonas aeruginosa* provokes caspase 1 activation to secrete interleukins has been described by Franchi et al. [43].

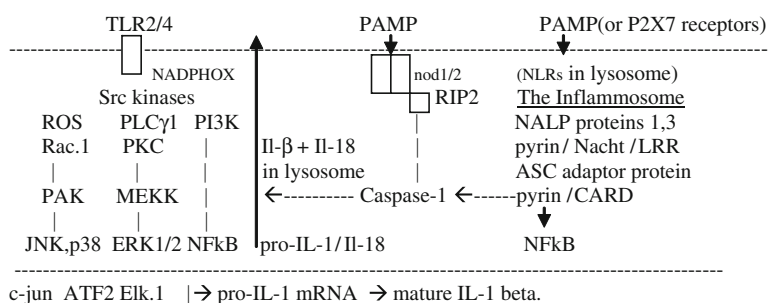


Fig. 12.3 Signal transduction pathways for caspase 1 induced pro-IL-1/IL-18 secretion. Inhibitors of JNK or of p38 stop IL-1 secretion. So do small inhibitors COPs (CARD only protein) and POPS (PYD-only proteins [47b]). NOD1 enhances pro-IL-1 processing [46]. RIP2 is an essential adaptor [47]. Pyrin inhibits processing of IL-1 β , possibly via NF- κ B modulation. This is NALP2. Lysosomal processing may vary in cell types. Note how adaptor ASC links to caspase 1 via its modular domains: an N-terminal pyrin and a C-terminal CARD of the inflammasome. ASC apoptosis-associated speck-like protein containing a CARD, CARD caspase recruitment domain, COPs coatomer protein complexes, IL interleukin, ICE IL-1 converting enzyme (i.e. procaspase 1), NF- κ B nuclear factor kappa B, Nod1 nucleotide oligomerisation domain 1, RIP2 receptor-interacting protein 2, ROS reactive oxygen species. Production of interleukin1 (IL-1) involves cross-talk between TLRs and NALP3 [39b]. NALP3 is a key component of the caspase-1 containing inflammasome. Its activation involves P2X7 receptors and ROS from NADPHox.

12.1.6 Production of Interleukin 1

Procaspase 1, which converts the 33-kDa pro-IL-1 β to the secreted 17-kDa mature protein, is within secretory lysosomes, in which adenosine triphosphate ATP is utilised in the activation process by phosphatidylcholine-specific phospholipase C (PLC) and phospholipase A2 (PLA2) [51]. IL-1 β release from macrophages actually requires two signals: (a) an inflammatory signal via TLRs to induce the pro-IL-1 β and (b) ATP to induce the inflammasome. In some cell types, IL-2 facilitates the formation of IL-1. Adjuvants activate caspase 1 and so IL-1 β and IL-18 release.

12.1.6.1 Interleukin 18 (IFN γ -Inducing Factor)

Interleukin 18 (IFN γ -inducing factor, IGIF) comes from various sources and promotes formation of both Th1 and Th2 cytokines. During stress, under the influence of ACTH, pro-IL-18 in the adrenals is converted by caspase 1 to active IL-18, which is released into the plasma [55]. IL-18 is IGIF, which functions along with IL-12 or IL-15. When IL-18 acts via its receptor (akin to IL-1 in Fig. 12.10) and MyD88 and via IRAK 1:2 and TRAF6, there is activation of NF- κ B and stimulation of the IFN γ promoter (Fig. 13.10). MyD88/IRAK also lead to p38 MAPK activation and so to cytokine and chemokine formation. IGIF together with IL-12 help the formation of Th1 cells as in rheumatoid arthritis (RA) joints. Systemic effects of IL-18 are rather surprising. IL-18 acting alone will induce Th2 cells. IL-18 induces basophils to release IL-4. It upregulates Fas ligand. It regulates NO synthesis and thereby controls adhesion molecules. The ability of IL-18 to upregulate ICAM-1 is thwarted by adenosine and its agonists (cf. Sect. 6.4). IL-18 also determines energy intake and insulin sensitivity and weight gain. IL-18 binding proteins prevent receptor activation.

12.1.6.2 NLRs in Inflammasomes

For LPS to activate caspase 1, there must be apoptosis-associated speck-like protein containing a CARD (ASC). The ASC molecule has Pyrin at its N-terminus and CARD at the C-terminus. The NLRs termed NALP1 and NALP3 (cryopyrin) and Ipaf (for flagellin [56]) are involved in ASC-determined, caspase 1-activating complexes called *inflammasomes* (Fig. 12.3). Proteins with Pyrin domains can bind to procaspase 1 and to the IKK complex that activates NF- κ B. Mutations in the CIAS-1 gene, which encodes cryopyrin, cause hereditary “periodic fever” syndromes, that is, the Muckle-Wells syndrome and familial cold autoinflammatory syndrome. The NALP3 inflammasome can be activated by bacterial mRNA, for bacteria such as *Listeria* and *Staphylococcus aureus*, bacterial toxins, ATP acting on P2X7 receptors and uric acid crystals. If ever macrophages lack NALP3, ASC or caspase 1, they cannot secrete IL-1 β in response to such stimuli. Thus, the inflammasome is a *sensor of danger signals* that might threaten a cell. Urate crystals in gout or calcium pyrophosphate

crystals in pseudogout are detected by the cryopyrin inflammasome, which then activates IL-1 β and the inflammatory cascade [57]. NALP3 protein activation leads to recruitment and activation of ASC and caspase 1 via PYD-PYD and CARD-CARD homotypic interactions, leading to IL-1 β / IL-18 formation. The consequent activation of NF- κ B will enhance the transcription of neutrophil-recruiting chemokines. Key components of the inflammasome have been demonstrated in skin keratinocytes during a contact sensitizer (trinitrochlorobenzene)-induced reaction.

12.1.6.3 Interleukin 12

IL-12 has already received mention since it is important for formation of Th1 lymphocytes. It consists of a p35 plus p40 heterodimer, making p70, which acts on IL-12R β 1 and - β 2 receptors. A microbial priming signal to CD8 α^+ mDCs or to pDCs (when viral) acts via CD40L/CD40 to induce the IL-12 heterodimer. cRel with NF- κ B p50 is needed for formation of p35 in DCs or p40 in macrophages. Accordingly, in rheumatoid synovium TLRs 2–4 and TLRs 7 and 8 lead to IL-12 production. When TNF α and IFN γ promote formation of IL-12, that occurs via the intermediary of p38 MAPK. As explained in Sect. 12.1.2, a positive-feedback loop involving IRF3 gives rise to type I IFN, and that induces IL-12 p70 secretion. Support is also given by IFN γ , TNF α , IL-4 and IL-13. Control at the IL-12 promoter is by means of the p300 histone acetyl transferase (HAT) (Fig. 4.8) interacting serially with Sp-1/AP-1/Ets-2/PU1/NF- κ B/C-EBP until histone deacetylases (HDACs) intervene [58, 59]. Thus, histone acetylation/deacetylation is important for the control of the IL-12 p40 promoter [60]. LPS-induced acetylation of histones opens the chromatin structure to allow access of Rel to the IL-6 and IL-12 promoters. However, there is a negative regulator of TLR4, called activating transcription factor 3 (ATF3), that causes HDAC deacetylation of histones, closing the chromatin [61]. Furthermore, strong extracellular signal-related kinase (ERK) signalling in macrophages, albeit not in DCs, curtails IL-12 formation [62].

Production of IL-12 is stopped if cellular (cAMP) becomes elevated or when IL-10 or (TGF β) are acting. Furthermore, PI3K exerts a crucial negative regulatory function during induction of the Th1 immune response by suppressing the production of IL-12 by DCs.

Now, we know of IL-12 p40 that acts as a homodimer. It can be a chemoattractant for macrophages or for DCs. IL-12 p40 homodimer is even produced by the respiratory epithelium in asthma [63]. In allograft rejection, IL-12 p40 homodimer activates CD8 T cells. Stimulation of single TLRs (rather than the synergy of TLRs) will evoke low levels of IL-12 p40.

12.2 Cytokines and the Immune Response

In most immune responses, uncommitted Tho cells produce a broad range of cytokines, and both Th1 and Th2 lymphocytes are formed. Th1 cells promote CMI, whereas cytokines from Th2 cells help formation of antibodies. Crucially, the amount of

IL-12 formed by DCs determines Th1 lymphocytes and negates Th2 polarization. As indicated in (Fig. 13.9), IL-12, IL-18 and IL-27 veer development towards Th1 cells, whereas the absence of IL-12 promotes the Th2 response. IL-12 production requires sustained TLR stimulation over time. As stressed in Chap. 13, IFN γ induces transcription factor (TF) T-bet, which works through cofactor Ets-1 to develop Th1 cells [64]. Protein kinase C θ PKC θ is critical for the formation of Th2 cells. Until recently, it was not realised that IL-4 supports differentiation of Th1 cells in addition to its definitive role in Th2 lymphocyte development (Table 12.5) [65].

Intracellular events within DCs that determine their polarization of T helper cells have been studied. LPS and flagellin that incite TLR4 and TLR5, respectively, on

Table 12.5 Cytokines in immunity

Cytokine	Origin	Actions
IL-1	Macrophages	Fever, T cell activation, acute-phase proteins
IL-2	T lymphocytes	Growth factor for T, B, NK and Treg cells
IL-12	DCs, M ϕ s, B cells	Induces CD4 T cells, activates NK cells
IL-18	Macrophages	Induces IFN γ and FasL, Th1 and Th2 cells
IFN γ	NKT, NK, Th.1, CD8	Th1 differentiation: activation of M ϕ s, PMNs, NK cells Increased MHC antigen expression
TNF α	Macrophages, NK cells	Activates PMNs, M ϕ s, endothelial cells Local inflammation, fever
IL-4	NKT and Th2 cells	Differentiation of Th2 cells (and Th1) Inhibits the IL-12 receptor β 2 Formation of antibodies, including IgE
IL-5	Th2 lymphocytes	Eosinophilia, allergy
IL-6	Th2, fibroblasts, M ϕ s	B cell growth factor, acute-phase proteins, proinflammatory
IL-8	Macrophages, most cells	Chemokine for PMNs and T cells
IL-9	Mast cells, Th2 cells	Helminth defence: stem cell development
IL-10	Monocytes, Th2, B cells	Activates B cells; immunomodulatory Downregulates M ϕ s, T and NK cells
IL-11	Bone marrow stroma	Haemopoietin, acute-phase proteins, in part suppressive
IL-13	Th2 lymphocytes	Promotes allergic response, fibrosis
IL-15	M ϕ s, various cells	Apex of T cell stimulation, B and NK cells
IL-16	CD4 + CD8 T cells	Chemoattractant: CD4 is the receptor
IL-17	CD4 + CD8 Th17 cells	Induces proinflammatory mediators in A-I disease
IFN α	Macrophages	Antiviral, activates NK cells, MHC class I
IFN β	Fibroblasts	Antiviral, activates NK cells, MHC class I
IL-21		Development of NK, B and Th17 cells
IL-22	(IL-10 like)	Reduces IL-4 from Th2, monocytes unaffected
IL-23		IL-12 like: induces IL-17 helper T cells and TNF α
IL-25	IL-17 family	Releases Th2 cytokines, eosinophilia
IL-26	(IL-10 like)	Produced by Th17 cells
IL-27		Immunosuppressive via IL-10 formation: inhibits Th17 cells
IL-32	activated T cells	Pro-inflammatory cytokines and chemokines
IL-32		activates Th2 cells

DC dendritic cell, *IFN* interferon, *IgE* immunoglobulin E, *IL* interleukin, *MHC* major histocompatibility complex, *NK* natural killer, *NKT* natural killer T cells, *PMN* polymorphonuclear leucocyte, *TNF α* tumour necrosis factor- α

murine DCs determine phosphorylation of p38 MAPK and JNK 1:2 kinases, stimulating IL-2 production and a Th1 lymphocyte response [66]. On the other hand, a TLR2 agonist (Pam3cys) and schistosome egg antigens induce ERK1/2 phosphorylation, which results in stabilization of c-fos (which suppresses IL-12), and there is then Th2 polarization [67]. A strong stimulus can induce negative-feedback circuits that reduce the amount of ERK phosphorylation [68]. There is also modulation from PI3K (influenced by cytokines and growth factors), which lowers the threshold for ERK phosphorylation [69].

Those rare patients who have mutations in IL-12 receptor subunits develop severe infections with mycobacteria and *Salmonella* spp. Actually, IL-12 is a potent stimulus for NK cells and CD8 T cells to produce IFN γ . IL-27 also promotes CD4/CD8 T cells and NK cell activities.

12.3 Activation of Macrophages

Macrophages express several types of PRR (Table 12.2) (i.e. mannose receptors, scavenger and leucine-rich receptors, CD14 molecules and Toll receptors). In response to interferon- γ , resting macrophages are activated to become inflammatory macrophages and then cytotoxic macrophages. The last have unusual ribosomal RNA, and they produce proteases and TNF α . As is the case for neutrophils, there are various activating agents. The macrophage-activating factors (MAFs) are IFN γ , TNF α and GM-CSF [70, 70b, 71]. Other recognised activators of macrophages include high-mobility group B1 (HMGB1) from chromatin of necrotic cells, S100 calgranulin proteins and heat shock proteins and purine metabolites like ATP. IL-10 is a deactivator. IL-10 tends to be associated with anti-inflammatory type 2 macrophages (Sect. 12.1.2) [8b,8c,72].

Table 12.6 Products of macrophage activation

1. Inflammatory mediators
PAF and the eicosanoids (i.e. prostaglandins, thromboxanes, leukotrienes)
Complement components
Reactive oxygen species like superoxide anions
Nitric oxide via iNOS
2. Enzymes: neutral and acid lysosomal hydrolases
Angiotensin converting enzyme (ACE)
3. Other proteins
Tissue factor thromboplastin
Fibronectin, osteopontin, plasminogen activator and inhibitor
Apolipoprotein E
MHC class II antigens
4. <i>Chemokines</i> : MCP-1, MIP-1 α and MIP-1 β , Oncostatin M.
<i>Cytokines</i> : IL-1 β , TNF α , IFN γ
<i>Growth Factors</i> : PDGF and TGF β

IFN γ interferon- γ , *IL* interleukin, *iNOS* inducible nitric oxide synthase, *MCP-1* monocyte chemotactic peptide 1, *MHC* major histocompatibility complex, *MIP* macrophage inflammatory protein, *PAF* platelet-activating factor, *PDGF* platelet-derived growth factor, *TGF β* transforming growth factor- β

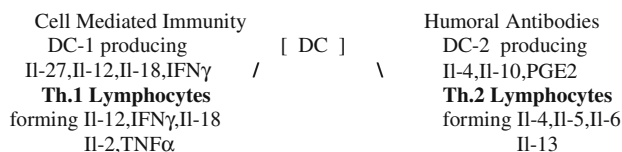


Fig. 12.4 Role of cytokines in the balance of Th1/Th2 lymphocytes

The object of IFN γ activation of macrophages is to limit the growth of microbial invaders [71]. The effects of IFN γ are (a) to downregulate transferrin receptors, decreasing intracellular iron; (b) to promote the formation of leukotrienes; (c) to induce 2,3-indoleamine dioxygenase (IDO) so that there is depletion of intracellular tryptophan, depriving microorganisms; and (d) formation of superoxide anions and NO $\cdot$ for microbial killing [73]. GITR-L, by reverse signalling, will activate ERK and thereby the NF- κ B proinflammatory response [74].

Vitamin D plays a supportive role in H₂O₂ formation by macrophages under the influence of IFN γ , which is a reminder that when tuberculosis is not responding to therapy, there could be vitamin D deficiency [75]. *Mycobacterium tuberculosis*, acting via TLR2 receptors (and TLR9), induces vitamin D receptors to upregulate the antimicrobial peptide cathelicidin, which helps kill that agent [76]. In TB, the inability of phagosome-to-lysosome fusion arises from a secreted PI3 phosphatase, which reduces levels of PI(3)P on the phagosomal membranes.

Peptidoglycan and bacterial cell wall constituents like lipoprotein signal via TLR2 [77, 78]. When *M. tuberculosis* encounters macrophages, TLR2 receptors are stimulated; accordingly, NF- κ B leads to TNF α release, which stimulates iNOS (Chap. 3). *Mycobacterium tuberculosis* also release IFN γ from NK cells.

Nitric oxide is damaging to proteins and to tissues (Chap. 3). Its immediate local action can be to cause apoptosis of macrophages. Yet, sustained NO $\cdot$ formation by macrophages leads to an increase of inhibitors of apoptotic protein (IAPs) and a dampening of caspase activity. Thus when iNOS is boosted, macrophages can survive; therefore, they can continue with killing of microbial agents. NO is a crucial determinant of lung inflammation, characterized by neutrophil sequestration, alveolar macrophage activation, micro-vascular permeability and alveolar oedema.

Release of C5a in sepsis has profound effects. C5a acting on macrophages or neutrophils incites sphingosine kinase [79], so that there is Ca²⁺ influx and thus degranulation that releases cytokines TNF α , IL-6 and IL-8. Although macrophage activation by IFN γ has been emphasised in delayed hypersensitivity reactions, actually CD40–CD154 interactions between NK cells and macrophages are critical for macrophage activation during sepsis. This last process is not IFN γ dependent [80]. Of course, there is formation of ROS for TLR4 stimulates the Nox4 (NADP oxidase 4), and this helps NF- κ B activation [81]. Downregulating influences that help cell survival are indicated in Table 12.8. RIP (cf Fig. 12.6) links TLR4 receptors to Akt; accordingly, it helps macrophage survival after LPS stimulation [82].

Not surprisingly, leukotrienes B₄/D₄ are mitogenic for macrophages, even in the absence of other growth factors [83].

12.3.1 Control of Macrophages

A need to control activated macrophages is quite evident in multiorgan failure (MOF), which is often a sequel to endotoxaemia or septicaemia [84]. At this time, primed macrophages form ONOO⁻ peroxynitrite, and this highly reactive compound damages tissues. Naturally, the microvascular damage creates a low oxygen tension, and hence catabolism of adenine nucleotides leads to production of adenosine [85]. Adenosine A_{2A} receptors on cells are sensors of tissue damage. Within immune cells, adenosine leads to AMP elevation and thereby a dampening of responses, so this is an example of feedback control. Expression of adhesion molecules and β 2-integrins and phagocyte degranulation and formation of oxygen radicals are stopped. Pharmacologists have sought to dampen macrophage responses by using drugs like pentoxifylline that elevate intracellular cAMP. Epac1, exchange protein directly activated by cAMP, appears as monocytes differentiate into macrophages, and in them cAMP can inhibit FcR-mediated phagocytosis via the protein kinase A/Rap1 paths [86]. Also Epac1, acting via Rap, regulates monocyte adhesion and chemotaxis [87]. C-cbl is the natural negative regulator of macrophage activation and cytokine production (Table 12.7) [88].

In discussing the role of Fc γ receptors in phagocytosis (Fig. 10.6), it was emphasized that ITIM bearing Fc γ IIb predominate. They tone down release of cytokines, at the same time enabling efficient clearance of invading bacteria. The coopted SHIP phosphatases converting PIP₃ to PIP₂ act as negative regulators of cell activation and of proliferation of haemopoietic cells [89]. On tissue macrophages, the receptor RON of the Src tyrosine kinases (Fig. 12.5) or the Tyro3 family receptors like Tyro 3, Axl and Mer [90] also help to curtail NF- κ B activation and thus the release of NO⁻ and cytokines, so their display is anti-inflammatory. Indeed, murine Mer knockouts display enhanced sensitivity to endotoxin shock. The accepted function of Mer is to promote macrophage engulfment of apoptotic cells. It is possible that compromised

Table 12.7 Biochemical pathways in macrophage activation

1. A receptor connects with one or more G proteins
2. There is activation of phospholipase A₂, releasing arachidonate for the formation of eicosanoids (Fig. 6.2)
3. There is activation of phospholipase C and thus activation of PKC
 4. The cascade Ras $\rightarrow$ Raf1 $\rightarrow$ MAPKK $\rightarrow$ MAPK is promoted
The MAPK, JNK and p38 MAPK paths are all activated (Fig. 4.3)
Ceramide can activate Raf1 and so the JNK stress pathway
5. Early response genes like AP-1 (c-fos/c-jun) and c-myc are expressed
6. There is activation of NF- κ B, albeit ceramide might inhibit this

AP-1 activator protein 1, **JNK** Jun N-terminal kinase, **MAPK** mitogen-activated protein kinase, **MAPKK** mitogen-activated protein kinase kinase, **NF- κ B** nuclear factor kappa B, **PKC** protein kinase C

[77, 78]. Yet, CD14 are GPI-linked, have short tails and thus are unable to transmit a signal. As alone in Figs. 12.2, 12.5, LPS acts via the TLR4 receptors [16, 77, 78], so MAPK, p38 and JNK pathways are all activated (Fig. 12.5) and thereby many genes are activated, including those for iNOS and Cox-2.

Before we tackle the details of macrophage activation, we have to realise that LPS elicits a proinflammatory phase that lasts 0.5–2 h, during which TNF α is produced. During this time, any possible overactivity of JNK kinases and p38 MAPK is restrained by phosphatase MKP-1 (via glucocorticoid [101a]). Then, after 2 h the macrophages become anti-inflammatory, and they produce IL-10. At this stage MKP-1 has declined, and accordingly levels of JNK and p38 MAPK are high. This knowledge helps to emphasize the important role of the specific phosphatases.

Figure 12.5 indicates that LPS activates the ERK/JNK and the p38 cascades. This is also the case when monocytes are coping with microorganisms. The use of inhibitors has confirmed that the p38 pathway helps to generate cytokines and iNOS.

The TLR4 receptor activates PI3K and thereby 3-PDK-1, and thus PKC ζ , which activates a phosphatidylcholine-dependent PLC, liberating ceramide in the membrane [101,101b]. This ceramide helps TLR4 receptor assembly within lipid rafts [102]. In response to LPS, ceramide formation is transient and weak, and it is unlikely to inhibit NF- κ B or to reduce the induction of iNOS and Cox-2 enzymes [103]. Yet, in patients with septic shock ceramide will be elevated in white cells in proportion to the plasma TNF α , and white cells then become ineffectual.

The TLR4 pathway acting through PKC ϵ promotes formation of IL-12, albeit the ERK pathway acts as a dampener for this. The brake is lifted when PKC ϵ activates the phosphatase MKP-1. So, there are subtle controls, exemplified further by the fact that, whereas LPS stimulates TNF α release via conventional PKC α/β , PKC ζ leads to the release of modulating IL-10 [104]. IL-10 is also released when the p38 signal acts via Sp-1 on the IL-10 promoter (cf. Sect. 12.15). Likewise, Akt involvement helps [105]. Release of IL-10 in sepsis exerts a moderating influence that helps patient survival [106].

12.4.1 Negative Regulators of LPS Activation and TLR Receptor Signalling

There is an amazing number of negative regulators of LPS activation of TLR4 and therefore of NF- κ B [107, 108]. They act in concert rather than individually. They are summarized in Table 12.8.

β -Arrestin (Fig. 1.2) interacts with TRAF6 and prevents its oligomerization following LPS or IL-1 β stimulation [110]. Thus, it is another negative regulator. Also, complement protein C1q can stop LPS-induced proinflammation by monocytes since it induces p50-p50 homodimers with reduced NF- κ B binding, and it creates CREB phosphorylation. The consequence is IL-10 production [111].

Table 12.8 Molecular inhibitors of LPS activation

-
1. TRIAD3 or SIGIRR control TLR4. CD44 is a negative regulator of TLR signalling [109] RP105 stops LPS binding to MD2. IRAK-M thwarts the IRAK-TRAF6 enhanceosome. ST2 orphan receptor interacts with MyD88 and with TIRAP-SARM stops NF- κ B activation
 2. A20 protein regulates MEKK1 agent. ATF3 is a TLR-negative regulator. FADD prevents MyD88 $\rightarrow$ IRAK and so NF- κ B activation. A1 (homologue of Bcl-2) inhibits NF- κ B. TRAIL-R inhibits NF- κ B. SOCS1 targets Mal and regulates TLR4 and TLR2 signalling. SHP-2 negatively regulates IFN β production via TLR3/4-mediated signalling. (Yet curiously SHP-1 increases IFN β production [109b]!) LRG47 induced by IFN β production stops MyD88/NF- κ B/p38-mediated TNF α synthesis
 3. Receptor RON and TAM receptors Ax1, Mer and tyro3
 4. Dok1 and Dok2 inhibit the MAPK cascade; so can β -arrestin
 5. SHIP1 negates LPS $\rightarrow$ Akt activation. SHIP1 negates I κ B α degradation
 6. Complement protein C1q inhibits LPS activation of monocytes
-

See text for definitions of abbreviations.

12.4.2 Interaction IFN γ /TLR Signalling in Macrophages

There is synergy in signalling of IFN γ and the TLRs into macrophages [70b,112]. (a) IFN γ enhances TLR ligand binding and the activation sequence leading to NF- κ B since Stat1 induces the NF- κ B promoter. (b) TLRs can boost TFs in the IFN γ response. Thus, Stat1 is boosted and so is the ICSBP that leads to IL-12 production. (c) IFN γ induces the formation of IFN β , which is the delayed arm of TLR signalling.

12.5 Dendritic Cells

DCs are specialised APCs that originate from CD34⁺ stem cells of the bone marrow and are distributed throughout all tissues. DCs convey the signals that control all other immune cells. The iDCs that ingest antigen migrate to the lymph nodes, and there they become mDC-1 cells or DC-2 plasmacytoid DCs. pDCs produce IFN α to combat viral infections. One has to emphasize that mouse and human DC forms are not directly comparable [113], but their functions are similar.

The explanation for the status of the iDCs is that cAMP is inhibiting the activity of Src kinases via protein kinase A-mediated signalling (Sect. 6.3), which causes CREB phosphorylation. cAMP blocks the formation of IL-12 and TNF α , and it promotes the production of IL-10 [114]. In fact, ATP that is released from dying cells can act on DCs via their P2Y11 receptors, and that process elevates their cAMP, so that they remain semimature. If ADP is encountered, that incites mobilisation of Ca²⁺ ions and ERK activation [115]. ATP and ADP inhibit the release of IL-12. We now know that in iDCs caspase 3 trims the AP-1 adapter complex, whereas caspase 3 is low in mDCs and full-length AP-1 retains DM chaperone protein for major histocompatibility complex (MHC) II in the endosomes, enabling correct antigen presentation [116].

DC maturation occurs in response to various stimuli, like (a) TLRs for pathogens; (b) superoxide anions [117]; (c) mediators $\text{TNF}\alpha$, IL-1, PGE₂; (d) $\text{TNF}\alpha$ receptors for CD40, Fas or OX40L; (e) Fc receptors for immune complexes or antibodies [118]; (f) P2Y₁₁ receptors for ATP; and (g) sensors for necrotic cells (i.e. heat shock proteins) [119]. The mDCs carry co-stimulatory molecules that enable optimal T cell activation. Not surprisingly, the strength and persistence of antigen stimulation determines the response of DCs. A weak transient stimulation via CD40 causes monocytic DCs to migrate into lymph nodes. A strong persistent CD40 stimulation elicits cytokine secretion [120]. Mere transient activation of the ERK of associated T helper lymphocytes will engender Th2 cells, whilst persistent ERK activation will create Th1 lymphocytes.

When cultured spleen cells are examined, one finds large iDCs and smaller mDCs, which have genes for antigen processing, for mitochondrial and ribosomal proteins, and for proteins that organise the cytoskeleton. Since our interest is in signal transduction, note that the iDCs bear microdomains in which are floating empty MHC II molecules. However, when antigen is presented, the numbers of peptide-MHC heterodimers increase. Peptide-loaded MHC II molecules are moved to the cell surface via newly generated tubular endosomes, so that MHC II molecules with bound neopeptide can be presented [121]. The mDCs have developed lipid rafts that contain PKC δ , Lyn tyrosine kinase and actin.

When DCs arise from monocytes, RelB is upregulated. That happens also as macrophages are formed. When GM-CSF or IL-4 is used to produce iDCs, RelB, c-Rel and p50 are all expressed (cf. Sect. 11.1). In mDCs, an increased expression of NF- κ B goes with the transcription of Rel genes. The p50 Rel protein is linked to the production of IL-12. As indicated (Sect. 12.1.6.3), it would be disastrous if DCs were to form too much IL-12. In fact, TLR4/2 engender a PI3K-mediated negative feedback through GSK3 [29]. Furthermore, PI3K induces Akt, which inhibits p38 MAPK, so that activation of IL-12 genes is suppressed.

DCs upgrade their scavenger receptors and like molecules (Table 12.3) when they engage in phagocytosis. Class A scavenger receptors like Marco aid the uptake of bacteria or of oxidised low-density lipoprotein (LDL). Tip-DCs [122] that produce $\text{TNF}\alpha$ and iNOS are important for defence against bacterial infections. Look again at Fig. 12.1 and consider that cell to be a DC. When stimulated, important chemokines will be expressed: (a) Rantes and MIP-1 α/β will attract monocytes and iDCs, (b) IL-8 will attract neutrophils and (c) IP10, Mig or I-Tac will attract activated T and NK cells. It is described how neutrophils cluster with iDCs [123] (Sect. 10.8). So, one realises how microbial stimulation of DCs sparks a local inflammatory reaction.

ROS at relatively low concentrations are second messengers for various physiological stimuli like DC-T lymphocyte interactions [124]. So, during antigen-specific contact of DCs with T cells, oxidative stress facilitates signalling. When LPS stimulates DCs, ROS are generated (Fig. 12.5) [125]. Pathogen stimulation of the TLRs on DCs leads to activation of their NADPHox [126]. Correspondingly, when Kupffer cells present antigen, there is a display of MHC II and co-stimulatory molecules, and ROS are formed. The process is stopped by antioxidants or by inhibitors of NADPHox [127].

IL-10 suppresses NF- κ B since I κ B kinase is diminished. IL-10 also thwarts the PI3K/Akt pathway. Hence, it downregulates DCs [128]. Another suppressor agent for DCs is TGF β , which downregulates expression of the CD1 glycoprotein that helps to present lipid and glycolipid antigens [129]. We know now that IL-10, TGF β and GCs like dexamethasone suppress the antigen-presenting function of DCs by downregulating B7 costimulatory molecules like CD80 and CD86; so does PPAR γ . There is then no activation of T lymphocytes. Inhibitory agents work on GILZ, which prevents formation of IFN γ by the DCs but allows IL-10 release [130]. It also has become apparent that CD45 phosphatase negatively regulates production of TNF α and IL-6 in DCs [131]. Anti-inflammatory molecules like IL-10, corticosteroids and vitamin D₃ or cytokines IL-4 and IL-13 induce mannose receptors on DCs. They facilitate endocytosis, and they make iDCs tolerogenic. *Tolerogenic DCs* express ILT3/4 inhibitory receptors, and they create Treg cells [132]. They utilise Karap-12/Dap12 to boost IDO _ activity [133].

12.6 Signalling by TNF α

In a bacteraemia or septicaemia caused by gram-negative Enterobacteriaceae and in gram-positive bacterial shock, the macrophages are incited to release TNF α at an early stage. This reproduces most of the disastrous features that lead to death of the victim [134], albeit we now know that there is late release of HMGB1 to complete the picture. HMGB1 is a DNA binding cytokine that causes cell necrosis [135]. HMGB1 counts as an *alarmin* [136], and its release is carefully controlled via hsp72 [137]. All TLRs can signal via MyD88 to the promoter of the gene encoding TNF α , albeit some (i.e. TLRs 1, 2, 4, 6) also require the use of TIRAP. One ought to concentrate on the receptors for TNF α . There is a p55 TNFRI that incorporates a death domain (DD) and a p75 TNFRII that has no death domain but can create apoptosis [138, 139].

TNFRI signals by recruiting to its death domain the supporting structure TNF receptor-associated death domain (TRADD), binds TRAF2 and RIP as complex I, and using ubiquitination this activates NF- κ B and AP-1. Whether TNF α action results in apoptosis depends on the cell type, the receptor engaged (TNFRI/RII) and interplay of other regulators like JAB. TNF α causes apoptosis when, as shown in Fig. 12.6, it causes TRADD to associate with Fas-associated death domain (FADD) and there is activation of caspases 8 and 10 [146] (Sect. 8.8.1), as well as JNK. Actually, FADD activation of caspase 8 prevents cell necrosis, which is a ceramide/ROS-mediated event. Next, there is formation of complex II containing TRADD, RIP, FADD and caspase 8, and there will be apoptosis. FADD will even associate with the TNFRII to cause apoptosis. T lymphocytes of the elderly show enhanced TRADD-FADD and so are liable to apoptosis [147]. If TRAF2 activates Ask-1 (apoptosis signaling regulating kinase), it activates JNK and so apoptosis, but the activity of Ask-1 depends on the redox status of thioredoxin. Generally,

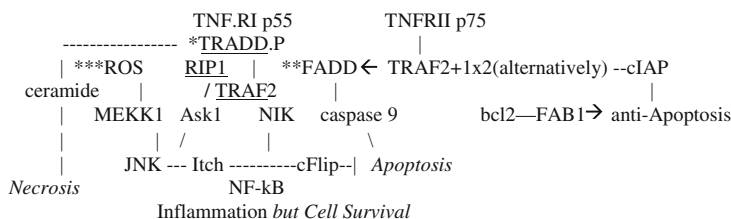


Fig. 12.6 Diagram of TNF α receptors I and II. (a) Only the TNFRI incorporates the TRADD, which is a platform and assembler of signal complex I—FADD/TRADx2/TRAF2/RIP. TNFRI is inhibited (*) by an SODD. (b) When TRADD is internalised with FADD, forming complex II [140, 141], there will be apoptosis that should be inhibited (**) by c-FLIP. (c) Early and transient activation of JNK is mediated by TRAF2, while delayed and persistent JNK activation results from ***ROS activation of MAP3K and MAP2K, and the JNK then induces apoptosis. Only when NF- κ B inhibits ROS formation and thus JNK activation can there be cell survival. NF- κ B inhibits the sustained phase of TNF α -induced JNK activation [142]. Hence, cell survival is the usual outcome. (d) RIP1 kinase is an important regulator of ROS scavenging enzymes and endogenous antioxidants [143]. (e) TNFRII (p75) is mainly expressed by immune cells and endothelial cells. Binding of TNF α to TNFRII can lead to formation of inhibitor of apoptosis proteins (IAPs). TNFRII in T lymphocytes is a costimulator of IL-2 production, and it helps CD4 and CD8 cells survive. (f) As drawn, TNFRII sustains antiapoptosis, but agonists of TNFRII often deplete TRAF2 and TRAF2-associated protective factors so that NF- κ B is not activated; thus, there will be apoptosis [144, 145]. There is a constant interplay between survival and death complexes at these particular receptors. *c-FLIP* FADD-like IL-1 converting inhibitory protein, *FADD* Fas-associated death domain, *JNK* Jun N-terminal kinase, *NF- κ B* nuclear factor kappa B, *RIP* receptor-interacting protein, *ROS* reactive oxygen species, *SODD* silencer of death domain, *TNF α* tumour necrosis factor- α , *TRADD* TNFR-associated death domain, *TRAF2* TNF α receptor-associated factor 2 TRADD is necessary for TNF-induced apoptosis and necrosis, as well as for initiation of inflammatory processes. The RIP1 death domain has a domain has a high affinity for the death domain of TRADD. TRADD recruits TRAF2 which then ubiquitinates RIP1 [147b]. TRAF2 can also play a role in activation of p38 MAPK, JNK and NF- κ B [147c]

c-Flip (FADD-like IL-1-converting inhibitory protein) opposes apoptosis as in Fig. 12.6. Nevertheless, JNK can activate Itch-P, (HECT-type E3 ubiquitin ligase) which ubiquitinates and degrades c-FLIP when bound to FADD, and apoptosis ensues [142].

So, there is a complicated interplay of complexes that determine cell survival or death. Also, the interaction of RIP1 with TRADD could be responsible for a signalling switch between antiapoptotic NF- κ B activation and death induction [147b].

Generally, TNF α does not cause apoptosis of cells. Even when GM-CSF is withdrawn, TNF α in conjunction with TLRs 2 ensures survival of macrophages [148]. If the TRADD recruits TRAF2 (with TRAF1) to the TNFRI [149], there is activation of NF- κ B and protection from apoptosis since there is NF- κ B inhibition of ROS formation and of JNK activation [150]. Furthermore, TNF α can stimulate ERK, which phosphorylates the p55 TNF receptor (TNFR), so that antiapoptotic bcl-2 is formed. Via the TNFRII, there is formation of cIAP2 inhibitor of apoptosis [151]. TRAF1 and TRAF2 work in conjunction with cIAP1/2 to stop apoptosis initiated from the TNFRI [152,152b].

In certain cells like the rheumatoid synoviocytes, the agent JAB1 causes regulated TRAF2 ubiquitination, and that accounts for its recruitment to TNFR1, so that antiapoptosis ensues [153]. Using small interfering RNA (siRNA), one can knock down JAB1, and then TNF α leads to apoptosis. If there is S-nitrosylation of NF- κ B, TNF α then causes apoptosis. The structure of FLIP is akin to that of caspase 8 (Flice), yet without the catalytic sequences, and thus it acts as a decoy. c-FLIP stops apoptosis by inhibiting caspase 8 and by activating survival signals [154]. c-Flip is expressed in cells that need to stop the action of Fas ligand and Fas receptor action and it controls the TNF Receptor (Fig. 12.6).

So, the situation is complex because there is a tightly connected regulatory network [139]. There are still points about the choice between apoptosis and antiapoptosis (Fig. 12.6) that require full clarification. Integral to this are the explanations of the role of ROS [155] and of the role of RIP kinases, which are sensors of cellular stress [156–158]. RIP kinases support prosurvival and inflammatory responses since RIP can activate IKK and so NF- κ B. However, RIP has a C-terminal death domain kinase. When RIP1, TRAF2 and FADD work together to induce ROS, then cell necrosis will occur [159]. To ensure antiapoptosis of T cells, the T cell receptor (TCR) must stimulate NF- κ B to induce A20, a zinc finger antiapoptotic protein [159]. Surprisingly, TNF α -induced ceramide production (Fig. 7.3) is not obligatory for TNF α -induced cell necrosis. Cell necrosis occurs (a) if ASase (acid sphingomyelinase) forms ceramide and so cathepsin D and (b) RIP1 creates ROS that causes DNA damage, and poly(ADP-ribose)polymerase (PARP) action results in ATP depletion. One path does not appear in Fig. 12.6. When TNF α is required to induce Cox-2 to produce PGE2 and thromboxanes (Fig. 6.2), this induction occurs by means of the sphingosine kinase-to-sphingosine-1-phosphate pathway [160]. Although TNF α is proinflammatory initially and must exert antimicrobial activity [161], at a later stage the effects of TNF α can become anti-inflammatory. TNF α -treated murine macrophages produced less IL-12 and IL-23 in response to either IFN γ or LPS [162]. Undoubtedly, TNF α can so modulate the TCRs of T lymphocytes that autoimmune disease is averted.

12.7 Macrophage Migration Inhibitory Factor

Messenger RNA for macrophage migration inhibitory factor (MIF) is plentiful where there are monocyte macrophages. MIF ensures that macrophages that have been attracted to a site stay in that vicinity, and that they are geared for action against microbial agents. MIF induces TLR4 receptors. MIF originates from macrophages, T cells and synovial cells, and it is produced by epithelia in contact with the environment. Endocrine cells are also a source, and it is plentiful in eosinophils. As MIF is stored in intracellular pools, it does not need de novo protein synthesis before it is secreted. Actually, MIF lacks an amino-terminal leader sequence, signifying that it is released from cells by a nonconventional protein secretion pathway.

MIF overrides the suppressive effects of physiological amounts of GCs on the proinflammatory cytokines. The anterior pituitary produces MIF that is released by

low concentrations of CRF. This MIF counteracts the anti-inflammatory effect of GCs. Thus, MIF opposes GC inhibition of T cell proliferation or inhibition of macrophage production of $\text{TNF}\alpha$. MIF ensures the expression of receptors for IL-1 β and for $\text{TNF}\alpha$ [163]. MIF evokes release of $\text{TNF}\alpha$, PGE2 and metalloproteinases. When $\text{TNF}\alpha$ causes apoptosis of neutrophils, large amounts of MIF are released. Gene-targeted MIF deficiency in lupus-prone MRL/lpr mice attenuates glomerulonephritis and skin lesions. MIF is an important effector of organ injury in systemic lupus erythematosus (SLE) [164].

A MIF allele is relevant to the genesis of juvenile arthritis. MIF induces the secretion of $\text{TNF}\alpha$; in turn, $\text{TNF}\alpha$ induces MIF production. Indeed, MIF is a pivotal regulator of innate immunity. Almost 40 years ago, MIF was discovered to be part of the delayed hypersensitivity reaction. When MIF acts on its receptors on macrophages, the ERK1/2 cascade is activated, which activates Ets TFs, and so the TLR4 gene is induced. By means of phosphorylation of ERK1/2, MIF can also act to induce cytoplasmic PLA2 (Fig. 6.2b), and the released arachidonate stimulates Cox-2, so that PGE2 and the thromboxanes are formed. MIF counteracts the GC-mediated induction of inhibitor of NF- κ B, and it overrides GC-mediated inhibition of PLA2 activity. It also inhibits p53-dependent apoptosis of macrophages [165, 166].

MIF antagonises the growth arrest and proapoptotic action of p53. MIF has the unusual ability to stimulate sustained ERK1/2 MAPK activation, a feature that is akin to transformation by oncogenic Ras. MIF also increases the effects of hypoxia-inducing factor 1 α (HIF1 α) and delays tumour cell senescence. It is possible that MIF provides a link between chronic inflammation and malignant progression (chap. 18). MIF sustains MAPK, and it will activate the Akt pathway. It is controlled by MAPK phosphatase-1 (MKP1) [101a].

12.8 Cytokines and Growth Factors

It should be mentioned that there is posttranscriptional control over cytokines like $\text{TNF}\alpha$ and GM-CSF since their mRNAs have AREs (adenine and uridine-rich elements), for which ARE-binding proteins determine the level of protein expression [167]. These RNA binding proteins have names like TIA-1 and TIAR, and they can regulate expression of $\text{TNF}\alpha$. Interestingly AUF, the regulator of $\text{TNF}\alpha$ mRNA decay, is targeted by auto-antibodies in patients with systemic rheumatic diseases [167].

12.8.1 *Receptors for Cytokines and Cellular Activation*

Unlike the typical growth factor receptors, receptors for cytokines have no intrinsic tyrosine kinase activity. They rely on cytoplasmic kinases like the JAKs (Janus (gateway) phosphotyrosine kinases) to initiate intracellular signalling (Fig. 12.7).

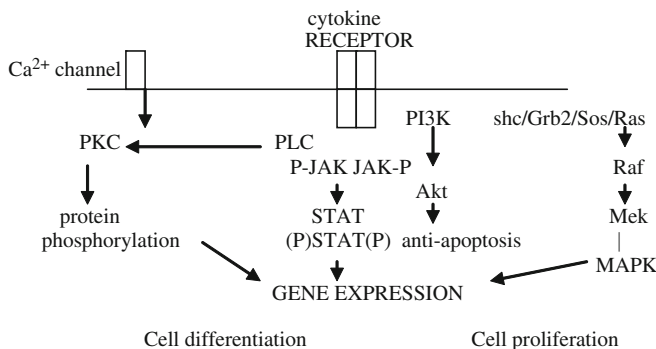


Fig. 12.7 Scheme of cytokine-induced signalling pathways

12.8.2 *IL2Receptor and IL-2*

For all this to be meaningful, one has to look at examples. The IL-2 receptor, which is important for T cell proliferation in response to IL-2 (T cell growth factor), consists of an α -chain p55 (called Tac antigen), a β -chain that has motifs that signal for cell proliferation and the common γ -chain (Fig. 12.9). The associated nonreceptor tyrosine kinases Lck, Fyn and Syk together with adaptor protein Shc convey the signal to Ras, and so they lead to MAPK activation. In the process of IL-2 signalling, DUSP5 dual-specific phosphatase 5 controls MAPK activity (cf Fig. 4.7) [169].

Formation of IL-2 following T lymphocyte TCR and CD28 costimulation receives brief mention in relation to Fig. 13.5. The IL-2 gene is activated at 45 min after TCR stimulation. The enhancer region positioned 5' of the promoter of the IL-2 gene contains several DNA binding motifs. So, in Fig. 13.5 one notes binding sites for nuclear factor of activated T cells (NFAT), AP-1, NF- κ B and Oct-1. Actually, the CD28 response element (CD28RE) is a composite binding site for a complex of NFAT and AP-1. TCR engagement alone will activate NFAT, but little AP-1. For proper assembly of AP-1, CD28 signalling is needed, and the CD28RE is activated. The cAMP response element modulator (CREM) promotes deacetylation of histones, and so condensation of the chromatin, and it thereby downregulates IL-2 production [170].

Of course, one would expect precise control over IL-2 formation. In activated T cells, Blimp-1 expression restricts the formation of IL-2 [171].

It turns out that IL-2 inhibits IL-17 production and Th17 T cell polarisation. IL-2 acting via Stat5 inhibits the orphan nuclear receptor ROR γ that is required for Th17 cells [172]. In mice, IL-6 acting along with TGF β to stimulate Stat3 stimulates Th17 cell formation [173], but in humans action on ROR γ is determined by IL-6 acting with TGF β and IL-21.

Physicians are interested in the potential of immunosuppressive drugs. GCs, cyclosporin A (CSA) and tacrolimus (FK506) stop T cell activation. CSA and FK506

Table 12.9 Synopsis of growth factors and cytokines (CGHIKLM)**C Chemokines**

- α C-X-C chemokines (e.g. IL-8, MIP 2)
- β C-X chemokines (e.g. monocyte chemoattractant proteins, MCPs)
- Macrophage inflammatory proteins (MIPs), Rantes and eotaxin
- C chemokine lymphotactin; Cys-X-X-X chemokine fractalkine

G Growth Factors

- Epidermal growth factor (EGF), fibroblast growth factors (FGF α and FGF β)
- Nerve growth factor (NGF), platelet-derived growth factor (PDGF)
- Transforming growth factor- α and - β , endothelial growth factor receptor family
- Insulin receptor family, hepatocyte growth factor (HGF) receptor family
- Erythropoietin-producing hepatocyte kinase (Eph) family
- Vascular endothelial growth factor (VEGF) family, neurotrophin receptor family

H Haemopoietic Growth Factors

- Erythropoietin (EPO), IL-3
- Colony-stimulating factors (CSF): granulocyte-macrophage (GM-CSF), granulocyte (G-CSF) or macrophage (M-CSF)

I Interferons (IFNs): IFN α , IFN β or IFN γ **K (killing) Tumour Necrosis Factors (TNFs)**

- TNF α , TNF β (lymphotoxin)

L Lymphokine: Interleukins

- IL-1 lymphocyte activating factor; IL-2 T cell growth factor
- Others (IL-3, IL-4/5/6) (**Table 9.1**)

M Macrophage Activating Factors (MAFs): IFN γ , GM-CSF, TNF α

Each of these mediators has its own cell receptor that leads to signalling in its own unique way. There are 12 subclasses of growth factor receptor. Some utilise non-tyrosine kinase receptors.

When discussing PDGF as a growth factor (Fig. 5.2), it was emphasised that phosphotyrosine kinases (PTKs) are part of the receptor.

We need now to create a general schema of cytokine-induced signalling pathways. Essentially, there will be (a) activation of protein kinase C, (b) PI3 kinase activation of Akt to create antiapoptosis, (c) Jak $\rightarrow$ Stat activation-inducing genes, (d) MAP kinase pathway activation via Ras.

Jak Janus phosphotyrosine kinase, *MAP* mitogen-activating protein, *Stat* signal transducer and activator of transcription

each bind to a cytosolic immunophilin, and that complex inhibits the Ca²⁺-calmodulin-dependent phosphatase called calcineurin. So, there is inhibition of the activation and nuclear translocation of NFAT. These drugs also interact with the CRE (Fig. 4.8), inhibiting the transcription of IL-2. In fact, CSA inhibits the early phase of NF- κ B activation arising via costimulation of the CD28, reducing IL-2 expression. FK506 stops the expression of IL-2 and IL-7 receptors on T cells. HDAC inhibitors target the CD154 (CD40) pathway on T cells so that formation of IL-2 wanes [174]. The agent rapamycin interacts with mTOR (mammalian target of rapamycin) (Fig. 11.6), which is on the signal pathway for IL-2 and IL-4. mTOR is needed for T cells to progress in the G1/S-phase. The Mannich base NC1153 blocks IL-2 activation of Jak3 and downstream Stat5a/6 [175]. The purine synthesis inhibitors like MMF and azathioprine halt the cell cycle. There is a cyclohexane derivative TAK242 that inhibits TLR4-mediated cytokine production [176]. Added to this is the finding that adenosine agonists suppress monocyte macrophages and more [177].

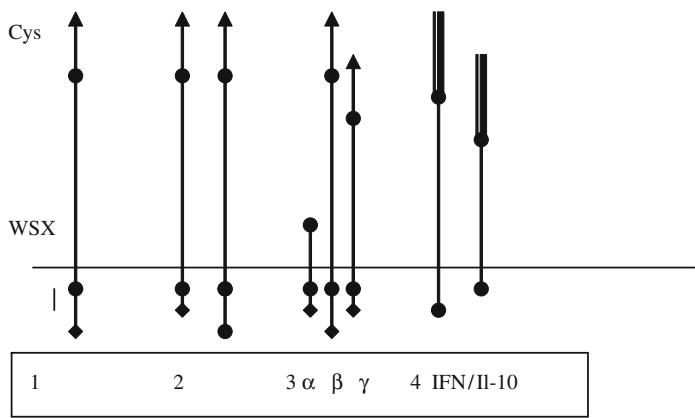


Fig. 12.8 Cytokine receptor super family members. The extracellular segments of the haemopoietin receptor family have two pairs of conserved cysteine residues linked via disulphide bonds and then a C-terminal WSXWS motif (Trp-Ser-Pro-Trp-Ser) close to the cell membrane. Such is the cytokine receptor module. Some receptors are capped by immunoglobulin domains (e.g. IL-6R, IL-11R, G-CSF-R). Others bear fibronectin domain type III modules close to the plasma membrane. For structure detail see Cavaillon 2007 [168a].

Table 12.10 Haemopoietin receptor superfamily

1. Units: receptors for erythropoietin and G-CSF
The receptor is a single chain that must undergo homodimerization after ligand binding, so that there can be signal transduction
2. Units: receptors for IL-3, IL-5, GM-CSF or the IL-6 family
There is an α -chain for ligand binding and the common β -chain (gp140/130) for signal transduction; IL-3R is for formation of myelomonocytic cells
3. Units: receptors for IL-2, IL-4, IL-7, IL-9, IL-15, IL-21
The α -subunit is for ligand binding, and there are two signalling units, β and γ . The common γ -chain is shared among these cytokine receptors
4. There are two ligand-specific chains in the case of interferons (IFN) α/β and γ and the IL-10R

Not so much is known of chemokine receptors. It has been clarified that dimerization of chemokine receptors regulates G protein-coupled receptors. Homo- and heterodimerization adds a degree of complexity to such controls

G-CSF granulocyte colony-stimulating factor, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *IL* interleukin

12.8.3 Transpresentation of IL-15

Although both IL-2 and IL-15 have three-unit receptors, there are significant differences in the modus operandi of IL-15 compared with IL-2. Autocrine secretion of IL-15 from cells that are expressing IL-15R α receptors will cause an enhancement of the effects of IL-15 in a local environment. In graft-versus-host disease, that can cause local tissue destruction. Yet, IL-15 protects T cells from IL-2-induced activation-induced cell death (AICD). DC-derived IL-15 is a mediator of inflammatory

at position 700 that is phosphorylated during activation. While not sufficient for activation, phosphorylation of serine can amplify Stat transcriptional activation. Dual phosphorylation of tyrosine and serine enables the Stats to integrate signals from a variety of extracellular stimuli. Phosphorylated Stats dimerize and translocate into nuclei, where they bind to specific DNA elements. An overview (Table 12.11) of how the cytokine receptors connect with Jak-Stats will help. Yet, be aware that specificity is determined by the receptor. There is no particular Jak for a particular Stat. Tyrosine phosphatases can dephosphorylate Jaks, Stats and cytokines receptors. The phosphatase PTP-BL is a regulator of STAT signalling.

Note how IL-12 is activated by Stat4. IL-12 is released early in microbial infection, and it causes differentiation of Th1 helper lymphocytes and NK cells [181], which then release IFN γ . IL-12 also activates Jak2 and hence Stat5, and so it induces cell proliferation [182]. Since Stats 1 and 2 mediate the action of interferons, they support innate immunity. Acquired specific immunity is supported by Stat4, which promotes IL-12 formation, and by Stat6, which promotes IL-4. Conversely, Stat3 is the TF for the anti-inflammatory IL-10. In DCs, Stat3 is critical for induction of antigen-specific T cell tolerance. A Jak3 antagonist CP-690,550 is available.

We refer to SOCS proteins, which negatively regulate cytokine receptors. (a) SOCS can directly inhibit JAK kinases by binding to the receptor or the JAK activation loop. (b) SOCS compete with SH2 domain molecules for binding to the receptor. (c) They target the receptor for proteasomal degradation via SOCS box recruitment of an E3 ubiquitin ligase.

A SOCS has a box at its C-terminus, a central SH2 domain, and an N-terminal KIR (kinase inhibitory region), which explains how SOCs 1/3 inhibit JAK tyrosine kinase activity [183]. Socs1 modulate IFN γ and IL-4 signalling. Socs1 will inhibit IFN α and γ receptors and NF- κ B. It can regulate DCs and M ϕ s and the development of T cells. Socs regulate early Th1/Th2 cell differentiation (cf. Fig. 13.9).

Table 12.11 Jak-Stat connections

Cytokine receptor	Connecting Jak	STAT protein used
IFN γ	Jak1, Jak2	STAT1
IFN α/β	Jak1 Tyk2	STAT1/2
IL-6R	Jak1, Jak2, Tyk2	STAT3
IL-10	Jak1 Tyk2	STAT3
IL-12	Jak2, Tyk2	STAT4
IL-2	Jak1, Jak3	STAT5
IL-3	Jak2	STAT5
EPO	Jak2	STAT5
IL-4	Jak1, Jak3	STAT6
IL-13	Jak1, Jak2, Tyk 2	STAT6
IL-23	Jak2, Tyk 2	STAT3/4 IL-27
Jak2, Tyk 2	STAT1/3	
Tyk2 140 kDa is akin to Jak3 120 kDa		
IL-23 and IL-27 are like IL-12		

Jak2 mutations for erythropoietin (EPO) are the common cause of polycythaemia vera/*IFN* interferon, *Jak* Janus phosphotyrosine kinase, *STAT* signal transducer and activator of transcription

Socs3 modulates gp130(IL-6) signalling. Socs3 levels correlate inversely with production of IL-2. Socs3 maintains T helper cells in a quiescent state [184]. Socs3 is also a feature of M1 macrophages.

12.10 Activation of Macrophages by IL-1 and TNF α

IL-1 and TNF α are proinflammatory cytokines. They activate genes for the other cytokines, NOS, collagenase, stromelysin, leucocyte-endothelial cell adhesion molecules and some acute-phase proteins. When attempts were started to unravel the signalling by IL-1, the problem was that its effects could vary with the tissue. IL-1 and TNF α operate via the stress-activated kinases p38 and JNK [185, 186] and use signalling akin to TLRs (Fig. 12.1) [185b]. TNFRs have been pictured already in Fig. 12.6. Many problems in understanding have been overcome. The outstanding function of TNF α is regulation of inflammatory cells with preservation of antiapoptosis. In vivo TNF α induction of apoptosis is rare and cell dependent.

There is current discussion on how oxidation is implicated in activating TFs that induce genes [187, 188]. In many cells, NADPHox is the source of ROS. In lymphoid and epithelial cells, IL-1 activates 5-lipoxygenase, and so ROS are produced [189]. TNF α often induces Cox-2 expression (Fig. 11.3). Cox-2 is induced via NF- κ B by various inflammatory and mitogenic stimuli, so that prostanooids and thromboxane A2 are produced. The TNFR1 impulse through TRAF2 will activate NF- κ B-inducing kinase (NIK) and so the NF- κ B. The TNFR1 also activates PKC α $\rightarrow$ cSrc $\rightarrow$, which phosphorylates two tyrosine residues (Tyr188, Tyr199), thereby activating IKK/NF κ B and Sc Cox-2 [190].

In Chap. 11, it was stated that H₂O₂ activates NF- κ B, but actually the response is cell type dependent. In an inflammatory milieu, there will be 10 μ M H₂O₂ along with cytokines like TNF α , and initially proinflammatory cytokines and adhesion molecules will be induced. Yet, with time, low-concentration H₂O₂ will induce anti-inflammatory genes like haem oxygenase and IL-6 and A20 protein, which curtails the action of the TNF α [191].

12.11 TRAF Adapter Proteins

After binding of TNF α to its receptors (Fig. 12.10), TNFR1 translocates to membrane lipid rafts that are rich in cholesterol and sphingolipids [197]. TRAF1/2 adaptor proteins were identified as signal transducers of the TNFR1/II (Fig. 12.10), and then TRAFs 3–6 were found to operate in other situations. TRAF proteins have a novel TRAF coiled-coiled domain at the C-terminus that is followed by a conserved TRAF-C domain. This TRAF domain mediates self-association and upstream interactions with receptors and adaptors [198] TNFRs do not have any kinase motif in their cytoplasmic tails. Instead, the TRAFs connect with relevant kinases [199]. TRAF2, TRAF5 and

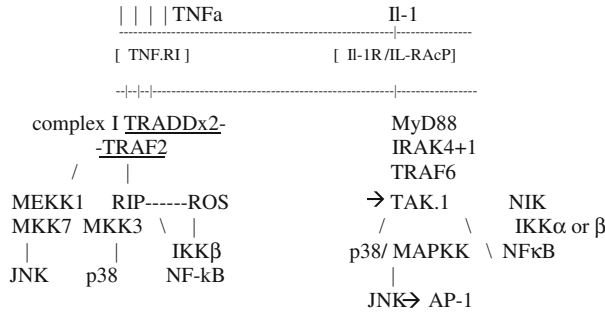


Fig. 12.10 Signalling by tumour necrosis factor- α ($TNF\alpha$) and by interleukin 1 ($IL-1$). The p55 TNFR1 leads to activation of nuclear factor kappa B ($NF-\kappa B$) and Jun N-terminal kinase (JNK). *TRADD* platform TNF receptor (TNFR)-associated death domain, *TRAF* TNFR-associated factor, *NIK* $NF-\kappa B$ -inducing kinase, *IL-1* receptor-associated kinase (*IRAK*) is controlled by *IRAK-M*. *IRAK4* is used for *IL-1R* (or *TLR* receptor) induced *TAK-1* dependent $NF-\kappa B$ activation. Transforming growth factor- β -associated kinase 1 (*TAK1*) is a MAP3K kinase used in innate and adaptive immune responses. *TAK1* is regulated by the phosphatase *PP6*. There are *TAK1*-dependent and *TAK1*-independent (*MEKK3* instead) means of *IL-1* activation of $NF-\kappa B$ [192]. Receptor-interacting protein 1 (*RIP1*) kinase binds to *TRAF2* and mediates unit I-kappa B kinase (*IKK*) activation. That occurs because *Traf2* catalyses K63 polyubiquitination of itself, and *Traf2* is released into the cytosol. There is *Traf2*-mediated K63 polyubiquitination of *RIP*. This means that recognition by *TAB2* and *TAB3* and kinase *TAK1* activates the *IKK* complex and so $NF-\kappa B$ [193]. *TAK1* helps $NF-\kappa B$, *p38* and *JNK* activation [194]. *IL-1* activation of *TAK1* is helped by heat shock protein 27 (*HSP27*), as shown by small interfering RNA (*siRNA*) studies [195]. In Fig. 12.10, it is evident how *MKK3* will activate *p38* mitogen-activated protein kinase (*MAPK*), and *MKK7* can activate *JNK*. Actually, several parallel pathways lead to *JNK* [155], but apoptosis induction is rare. Interestingly, *IRAK4*-inactive knock-in mice show impaired *JNK*, but the residual Toll-like receptor (*TLR*)/*IL-1R* signals allow formation of some cytokines. Hence, this manipulation should be beneficial in sepsis or chronic inflammatory responses [196]

TRAF6 mediate the activation of $NF-\kappa B$ and $JNK \rightarrow AP-1$. Both *TRAF2* and *TRAF6* are involved in K63 polyubiquitination [199]. *TRAF6* functions as a RING domain ubiquitin ligase [179]. Gene deletion experiments clarify that loss of *TRAF2* results in defective *JNK* signalling. *TRAF2* positively regulates the canonical and negatively regulates the noncanonical $NF-\kappa B$ pathways [200] (Sects. 11.1.1 and 11.1.2). As seen in Fig. 12.10, *TRAF6* aids signalling through the *IL-1R/IL-RACp* and via Toll receptors, *CD40*, the nerve growth factor (*NGF*) receptor and *RANK* (receptor activator of $NF-\kappa B$) [201]. Curiously, knockout of *TRAF6* creates multiorgan inflammatory disease, which tells us that *TRAF6* must suppress the $PI3K \rightarrow Akt$ path. Thus, *TRAF6* is presumed to play a part in peripheral tolerance [202].

TRAF proteins can activate members of the MAPK kinase kinases, *MAPK3* and *MAPK4* (Table 12.12). *MAPK3* include *MEKK1*, *TAK1* and *ASK-1*. *MAPK4* include germinal centre kinase (*GCK*) and *GCK*-related kinase (*GCKR*). They are on the path to *JNK* activation, and they are implicated in B lymphocyte maturation.

TRAF3 is an adapter molecule for *TRIF*-mediated *IRF3* activation, and it is crucial for the recruitment of type I IFNs (Figs. 12.2, 14.4) and the activation of *IL-10* [203].

Table 12.12 The complete MAP kinases

MAP kinases	Stress path	p38 Kinases
MKKK: MEKK1, MEKK2, MEKK3, Tpl-2	MEKK1-4	Tak1, Ask-1, PAK
Raf1, B-Raf, A-Raf, Mos-TAO, MLK3	Tak1, Tpl-2, Ask-1	MK2
MKK: MEK1:2	MKK 4/7	MKK3/6
MAPK: ERK 1:2	JNK 1, 2, 3	p38 α , β , γ , σ

Ask-1 apoptosis signal-regulating kinase 1, *ERK* extracellular signal-related kinase, *JNK* Jun N-terminal kinase 3, *MAP* mitogen-activated protein, *MAPK* mitogen-activated protein kinase, *MLK3* mixed lineage kinase 3, *PAK* p21-activated kinase, *Tak1* transforming growth factor- β -associated kinase 1 MK2 is MAPKAP kinase 2

12.12 CD40 Signalling

CD40 controls the maturation of APCs, their ability to upregulate co-stimulatory molecules and their formation of IL-12. In fact, CD40 triggers p38 MAPK so that there is production of IL-12 p40. Persistent CD40 stimulation on DCs means persistent ERK stimulation and so production of cytokines and a Th1 response [204]. One will recall that p38 MAPK is always essential for formation of cytokines [205]. CD40 is co-stimulatory for T cells [206].

B lymphocytes in lymph nodes are activated when they acquire antigen on their receptors from germinal centre follicular DCs, together with a second signal from CD40 ligand of T helper cells interacting with their CD40 molecules [205]. Indeed, the CD40 receptor is a key regulator of the 'differentiation branch point' between production of memory B cells or plasma cells. CD40 stimulation induces IL-6, so it supports immunoglobulin M (IgM) production [207]. The CD40 receptor connects with TRAF 2/3/5/6 [208], albeit TRAF3 does not seem important since in TRAF3^{-/-} mice CD40 still upregulates CD23. CD40 does not stimulate ERK, but p38 MAPK is upregulated. TRAFs 1 and 2 cooperate in the activation of B cells [209]. CD40 connects with PI3K, and thereby p27 (cell cycle inhibitor) is lowered and B cell proliferation occurs. CD40 via PI3K activates Akt. CD40 through TRAF6 activates NIK and so the IKK complex and hence NF- κ B. Also, CD40 through TRAFs 2/3 activates JNK and NF- κ B. This stresses the importance of CD40 for immunoglobulin production. The antiapoptotic Bcl-2 is induced. In fact, CD40 induces B lymphocytes to produce TNF α , and the binding of TNF to the TNFR2 receptor (CD120b) contributes to the effects of CD40 on B cells so that an amplification loop is created. There is a negative regulator of CD40 (and BAFF, B cell-activating factor) signalling in the form of adaptor molecule Act-1 [210], which brakes the NF- κ B2.

12.13 Other TNFR Superfamily Molecules

There are over 20 members of the TNFR superfamily of type I transmembrane proteins and almost as many ligands, which are type II TM proteins [211]. The ligands have names like Rank, Trance, Trail (TNF-related apoptosis-inducing ligand), Tweak,

osteoprotegerin (OPG) ligand, HVEM (herpes virus entry mediator), BAFF or Light or APRIL. These ligands are concerned with T cell costimulation, B cell proliferation and aiding the release of cytokines. Thus, CD30s will provide survival and proliferation signals at the peak of a primary lymphocyte response. CD30L on APCs leads to Th2 cell formation [212]. Light is a ligand that acts as a costimulatory molecule [213] for the TCR by interaction with HVEM, a herpes simplex virus glycoprotein D entry receptor. 4-1BB is another T cell costimulatory regulator [214, 215] that helps CD8 and CD4 T lymphocytes. OX40L is present on APCs like DCs, macrophages and activated B cells. IL-18 links the innate and adaptive immune responses by stimulating OX40 on DCs. OX40 is a coreceptor for T cells, which helps generate memory T cells. OX40 signalling uses TRAF2 and thereby stimulation of NF- κ B and JNK. The cells that are generated show increased survival [216]. There is enhanced bcl-2/bcl-XL, elevated protein kinase B (Akt) and reduced Fas. Early during antigen priming, OX40 signals check T cell proliferation [217].

GITR is at low levels in resting T cells but is upregulated in activated T cells and on Treg cells. GITR co-stimulates the suppressive activity of Treg cells [218, 219]. It also enhances inflammatory activity of macrophages [220].

12.14 RAGE Receptors and Cytokine Production

RAGEs, meaning receptors for advanced glycosylation end products (AGEs), are for the clearance of glycosylated proteins and lipids in the course of ageing, diabetes or uraemia. RAGEs on macrophages or lymphocytes activate NF- κ B and so account for proinflammatory effects like release of cytokines IL-1 β , IL-2 or IFN γ and chemokines. All its ligands are not identified as yet, but they include the S100 calgranulins that are released by leucocytes into the extracellular space [221], leucocyte integrins and the cytokine HMGB1 (and amyloid β -peptide). Yet, when amyloid peptide activates monocytes via RAGE, it is sufficient for ERK1/2 to activate gene Egr-1, which activates AP-1 (c-fos/ jun). Actually, Egr-1 activates some 300 genes. ROS act as intermediaries in both forms of activation [222].

RAGE and proinflammatory ligands like N- ϵ -carboxymethyllysine are found on bronchial epithelium and on macrophages, whilst S100B and S-100A12 occur in the lungs on polymorphonuclear leucocytes (PMNs) and DCs. These findings point to action in lung inflammatory diseases that lead to fibrosis [223]. Importantly, chronic inflammation can drive tumour development, helped by signals downstream of RAGEs [224]. Interestingly, the RAGE also mediates the transfer of neutrophils across intestinal epithelium [225].

In contradistinction, there are AGE receptors that lead to endocytosis of AGEs, so that there is nullification of an inflammatory response by recipient cells. Such is the case with mesangial cells [226, 227]. However, the principal action of AGEs acting on mesangial cells is to generate intracellular ROS, which up-regulate AT-1 receptors for angiotensin II (Ang II), and Ang II can then induce TGF β -Smad2 phosphorylation (Chap. 14) so that fibronectin is synthesised [228].

12.15 IL-10 Immunomodulatory Cytokine

IL-10 is a cytokine synthesis inhibitory factor that arises from monocyte macrophages that are stimulated by TNF α or from Th0, Th1 or Th2 lymphocytes that have been induced by IL-12 or by IL-6. Since IL-10 prevents macrophages forming proinflammatory cytokines and it down-regulates iNOS and Cox-2, it dampens or aborts Th1 cell-mediated DTH reactions. It suppresses formation of IL-12 [229]. Both DCs and macrophages have high expression of IL-10 receptors. In addition, IL-10 down-regulates oxidative metabolism in neutrophils so that they are deactivated. IL-10 upregulates IL-1R antagonist and upregulates haemoxygenase1, which has anti-inflammatory, antiapoptotic and antiproliferative actions.

As well as macrophages and T cells, B lymphocytes and DCs form IL-10 as can type I Tregs Th 17 cells and CD8 T cells [230a]. Accordingly, multiple cell types regulate local immune responses. IL-10 inhibits the activation of NF- κ B in monocytes and some subsets of T cells, but IL-10 promotes NF- κ B and AP-1 activities in CD8 T cells. There are, of course, naturally occurring Treg cells, and IL-10 produces Treg cells, which curb Th1 and Th2 responses. IL-10 ensures pathogen clearance without tissue damage [230b]. Since there seem to be multiple mechanisms involved, it has not been clear precisely how IL-10 exerts its anti-inflammatory action [231b, 232]. TLR-activated macrophages and DCs rapidly produce IL-10. T cell production of IL-10 is required to control chronic but not to affect acute inflammation. (a) IL-10 induces prostaglandin dehydrogenase so that there is catabolism of inflammatory prostaglandins like PGE2 [231]. (b) IL-10, by its association with the adaptor molecule insulin receptor substrate 2 (IRS-2), activates PI3K and thereby Akt; thus, it promotes cell survival. It is a differentiation factor for cytotoxic T lymphocytes. (c) IL-10 induces SOCS3 and SOCS1 [233], and they suppress Th1 lymphocytes. IL-10 is known to destabilize cytokine mRNA [234]. The use of Jak1 or Stat3 knockout mice has shown that Socs 1/3 are necessary for IL-10-mediated inhibition of TNF α /IL-6 production. (d) The IL-10R via the Jak1/Stat3 pathway suppresses TNF α output from macrophages [231]. IL-10-induced TNF α mRNA destabilisation is mediated via IL-10 suppression of p38 MAPK [235]. Stat3 is the only Stat that is used for the anti-inflammatory effects of IL-10, as in the sequence IL-10 $\rightarrow$ IL-10R $\rightarrow$ Jak1 $\rightarrow$ Stat3 $\rightarrow$ inhibition of proinflammatory genes [236]. Yet, Stat3 action is cell-type specific [231]. (e) The basic leucine zipper c-Maf TF (Fig. 4.13) will promote release of IL-4 and IL-10 by Th2 cells, and it stops formation of IL-12 [237]. (f) LPS activates various signal cascades, leading to the production of cytokines by macrophages. IL-10 inhibits both the NF- κ B and the p38 MAPK pathways. There are many IL-10-inducible genes [229, 232]. In particular, Bcl-3 associates with the p50 subunit of NF- κ B, which accounts for Bcl-3 being a negative regulator of NF- κ B. Incidentally, I κ B protein Bcl-3 negatively regulates the IL-10 gene in macrophages [238]. (7) IL-10 helps to stimulate B cell development and proliferation helped by the chemokine CXCL13 [239]. There are IL-10-related cytokines (i.e. IL-19, 20, 22, 24, 26) that activate Stats, often through shared receptors like IL-20R and IL-22R [240].

It has been clarified that IL-10 activates via Stat3 the transcriptional repressor ETV3 and the corepressor SBNO2 [241], and they repress NF- κ B. Meantime, it

turns out that IL-27 is immunosuppressive [242], and that IL-27(or IL-6) induce Stat3-mediated production of IL-10. In fact, IL-10 explains how IL-27 will inhibit the production of Th-17 cells. Cytokines related to IL-10 (type II cytokine) are IL-19, IL-20, IL-22, IL-24, IL-26, IL-27, IL-28 and IL-29.

12.16 Those Basic Signal Cascades

In Fig. 4.1, attention was drawn to the growth factor $\rightarrow$ MKKK $\rightarrow$ MKK $\rightarrow$ MAPK cassette. The reader may need appreciation of how other upstream kinases (Tpl-2, Tak1, Ask, p21-activated kinase [PAK], etc.) fit the schemes. Table 12.12 puts each in its place. One will recall that Raf is an MKKK, MEK is an MKK, and that the essential MEKs are ERK1/2. Upstream of MKK4/7 are MEK kinase I (MEKKI), Ask-1, mixed lineage kinase (MLK3), TAK1 and Tpl-2 (Cot). Their actions are now being clarified.

TAK1 is a MAPKKK (MAP kinase kinase kinase) that has a pivotal role in the activation of the IKK complex and JNK in response to proinflammatory signals (Fig. 12.1), like IL-1, TNF α , LPS and TGF β . Using RNA interference (RNAi) to knockdown Tak1 in Jurkat T cells revealed suppression of I κ B phosphorylation in response to TCR stimulation, blocked IL-2 formation and T cell signalling and so proliferation.

As in Table 12.12, Tpl-2 is an MAPKKK. It is of note that inhibition of Tpl-2 in human monocytes and synoviocytes will block formation of TNF α and so inflammation. In T cells, under chronic stimulation Tpl-2 is a tumor suppressor gene that is antiproliferative.

Figure 12.11 recapitulates the growth factor cascade and superimposed are the points at which pharmaceutical inhibitors can act.

Having digested all this complexity, one might like to contemplate the *inflamed synovium of the patient with RA*, in which one has to account for formation of cytokines like IL-1 β , TNF α , oncostatin, GM-CSF and IL-6 by macrophages and DCs and by fibroblast-like synoviocytes. There are CD4 and CD8 T cells producing IL-2 and IFN γ . CD4 T cells activate fibroblasts, chondrocytes and osteoclasts [243, 244]. Ectopic germinal centres are formed by T and B lymphocytes with some plasma cells. Interestingly, IFN γ does not seem to play a major role in the RA joints, but there is recruitment of CCR6-expressing Th17 lymphocytes [245].

Rheumatoid pathophysiology is so complicated that it cannot be tackled in detail here. TLRs 2, 3, 4 and 7 occur in RA joints. TLR2 and TLR4 signalling can account for production of cytokines [246]. TLRs could provide the impetus for the chronic inflammation. Indeed, activation of TLR4 is important for the survival of Th17 cells. A point mutation of Zap70 of T lymphocytes predisposes to autoimmune arthritis [247, 247a, 247b]. It would help to be able to focus on a causative antigen for erosive arthritis. The mineral oil pristane induces erosive arthritis in DA1F rats, and there is then overexpression of the heterogenous nuclear ribonucleoprotein RA33 [248]. There is T cell upregulation in the synovial membranes, and they activate macrophages, B cells and fibroblasts. There are Th17 cells in rheumatoid synovium (Fig. 12.12) [244, 249], and they will be the source of IL-17, which can amplify

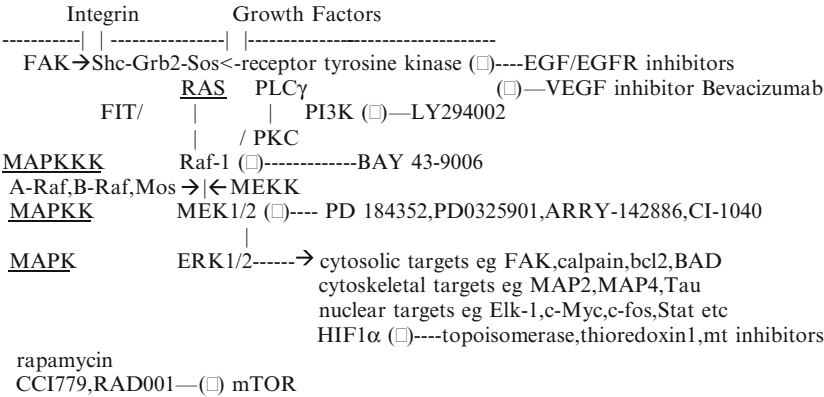


Fig. 12.11 Inhibitors of the growth factors → mitogen-activated protein kinase (MAPK) cascade. There are negative controls like MAPK phosphatases (dual-specificity phosphatases, *DUSPs*) and Sprouty proteins

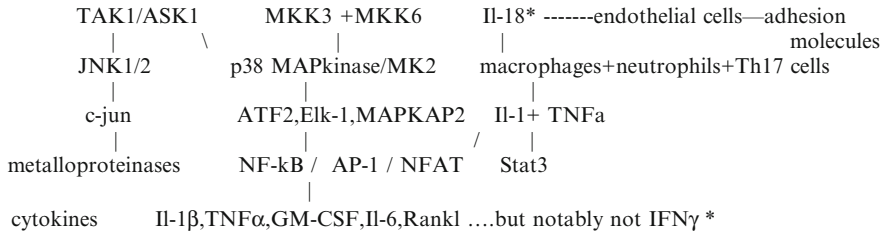


Fig. 12.12 Formation of cytokines in the rheumatoid synovium. Lack of interferon-γ (*IFNγ*) in rheumatoid arthritis (RA) synovium suggests that Th1 lymphocytes are not the protagonists

joint destruction, in part by upregulation of FcγR receptors on cartilage [250] and by activation of osteoclasts. IL-15 is overexpressed in RA and will activate monocytes and so reactive CD4 T cells. The B cells form plasma cells that produce autoantibodies to IgG (rheumatoid factor), to citrullinated peptides like fibrinogen and vimentin and to the 33-kDa antigen RA33. The TNFα family member Tweak induces proinflammatory cytokines, and it causes cartilage and bone erosion [251]. Neutralizing Tweak will have to be considered. Yet, much evidence suggests that TNFα initiates the local destructive process [252, 253], and that is driven by IL-18 [254]. Mice that lack the PI3Kγ gene have much less synovial inflammation and destruction. Hence, PI3Kγ inhibitors arrest disease progression in collagen-induced arthritis [255]. CTLA4 Ig (abatacept) will be useful as a T cell blocker in RA therapy [256]. It is a soluble fusion protein containing the extracellular domain of CTLA4 linked to an Ig Fc region, and it works by stopping co-stimulation through CD28 on T cells as a result of binding to CD80/CD86 on APCs. SOCS1/SOCS3 molecules are expressed by macrophages and the endothelial cells in RA, but most T cells are negative. So, that is another situation to be explained [257].

Fortunately, insight into mechanisms of autoimmune arthritis has been gained by studies of the treatment of murine collagen-induced arthritis with the tyrosine kinase inhibitor imatinib mesylate [258]. The synovial fibroblasts are subjected to platelet-derived growth factor receptor (PDGFR) signalling, so there is proliferation. In fact, fibroblast-like synoviocytes almost have a tumour-like phenotype under the direction of NF- κ B, which enhances their proliferation and matrix-degrading activity [259]. Indeed, there do seem to be novel cytoskeletal rearrangements within the synovial fibroblasts [260]. Hence, in Fig. 12.11 we contemplated new pharmaceutical means of thwarting the growth factor cascades. In the meantime, BAFF synthesis by synoviocytes is under consideration [261] for BAFF will provide the impetus for the maintenance of local B lymphocytes. One can control the B cells by combining standard methotrexate therapy with injections of rituximab (anti-B cell CD20) [262].

IL-2 is a T cell growth factor and a B cell growth factor, and it supports the activities of NK cells and monocyte-macrophages. IL-2 helps proliferation of T cells and development of the Th1/Th2 cells. It also supports regulatory T cells. It enhances Fas-mediated activation induced cell death (AICD) and it inhibits any Th-17 cells [263a].

Calcium ions bind to cytosolic calmodulin that activates calcineurin, whose phosphatase action dephosphorylates NFAT, so that it enters nuclei to bind to the IL-2 promoter [263b]. That promoter is also supported by CaMkinase IV [263c, 263d] but is repressed by Cabin-1 and histone deacetylase II, and by CaMkinase II [b]. Fine control over IL-2 transcription is also helped by cAMP mediated inhibition via CREM, and by the action of Soc-3. c-Fos of the AP-1 transactivator is stimulated by the CD28 costimulator pathway via p300/CBP, CREB-binding protein action. A CD28 signal also inactivates GSK-3 (glycogen synthase kinase) so that NFAT is retained within nuclei.

References

1. Delves PJ, Roitt IM. The immune system. *N Engl J Med* 2000;343:3–49, 108–117
2. Banchereau J, Briere F, Caux C et al., Immunobiology of dendritic cells. *Annu Rev Immunol* 2000;18:767–811
3. Beutler B, Hoebe K, Du X, Ulevitch RJ. How we detect microbes and respond to them: the Toll like receptors and transducers. *J Leukoc Biol* 2003;74:479–485
4. Saboe I, Read RC, Whyte MKB et al., Toll like receptors in health and disease. *J Immunol* 2003;171:163–165
5. Shimaoka T, Nakayama T, Kume N et al., SR-PSOX mediate bacterial phagocytosis by antigen presenting cells. *J Immunol* 2003;171:1647–1651
6. Foti M, Granucci F, Ricciardi-Castagnoli P. A central role for tissue-resident dendritic cells in innate responses. *Trends Immunol* 2004;25:650–654
7. Bae EM, Kim WJ, Suk K, Kang YM et al., Reverse signaling initiated from GITRL induces NF- κ B in the inflammatory activation of macrophages. *Mol Immunol* 2008;45:523–533
- 8a. Mantovani A, Sica A, Locati M. New vistas on macrophage differentiation and activation. *Eur J Immunol* 2007;37:14–16
- 8b. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 2008;8:958–969
- 8c. Benoit M, Desnues B, Mege J-L. Macrophage polarization in bacterial infections. *J Immunol* 2008;181:3733–3739

9. Ogawa K, Funaba M, Chen Y, Tsujimoto M. Activin A as Th2 cytokine in the promotion of the alternative activation of macrophages. *J Immunol* 2006;177:6787–6794
10. Edwards JP, Zhang X, Frauwirth KA, Mosser DM. Three activated macrophage populations. *J Leukoc Biol* 2006;80:1298–1307
11. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* 2007;117:175–184
12. Visintin A, Iliev DB, Monks BG. MD2: review. *Immunobiology* 2006;211:437–447
13. Bowie A, O'Neill LAJ. The IL-1 receptor/Toll like receptor superfamily: signal generators for proinflammatory interleukins and microbial products. *J Leukoc Biol* 2000;67:508–514
14. Hennek P, Golenbock DT. TIRAP: how Toll receptors fraternize. *Nat Immunol* 2001;2:828–830
15. Suzuki N, Saito T. IRAK-4: a shared NFkB activator in innate and acquired immunity. *Trends Immunol* 2006;27:566–572
16. Doyle SE, O'Connell RM, Miranda GA et al., Toll-like receptors induce a phagocytic gene program through p38. *J Exp Med* 2004;199:81–90
17. Kawai T, Akira S. Pathogen recognition with Toll-like receptors. *Curr Opin Immunol* 2005;17:338–344
18. Hoebe K, Jansson E, Beutler E. The interface between innate and adaptive immunity. *Nat Immun* 2004;5:971–974
19. Takeda K, Akira S. TLR signaling pathways. *Semin Immun* 2004;16:3–9
20. Blander JM. Coupling TLR signaling with phagocytosis. *Trends Immunol* 2007;28:21–24
21. Kawai T, Adachi O, Ogawa T et al., Unresponsiveness of MyD88 deficient mice to endotoxin. *Immunity*. 1999;11:115–122
22. Godowski PJ. A smooth operator for LPS responses. *Nat Immun* 2005;6:544–546
23. (a) Palsson-McDermott EM, O'Neill LAJ. Signal transduction by the lipopolysaccharide receptor TLR4. *Immunology* 2004;113:153–162; (b) Gray NJ, Gangloff M. Structure and function of Toll receptors and their ligands. *Annu Rev Biochem* 2007;76:141–165
24. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune response. *Nat Immun* 2004;5:987–995
25. Sugimoto K, Ohata M, Miyoshi J et al., A serine-threonine Cot/Tp12 modulates bacterial DNA induced IL-12 production and T helper cell differentiation. *J Clin Invest* 2004;114:857–866
26. Parker LC, Whyte MKB, Vogel SN et al., TLR2 and TLR4 agonists regulate chemokine receptor expression in human monocytes. *J Immunol* 2004;172:4977–4986
27. Vivarelli MS, McDonald D, Miller M, Kelliher RS, Geha RS. RIP links TLR4 to Akt and is essential for cell survival in response to lipopolysaccharide. *J Exp Med* 2004;200:399–404
28. Malissen B, Ewbank JJ. Tailoring the response of dendritic cells to pathogens. *Nat Immun* 2005;6:749–750
29. Woodgett JR, Ohashi PS GSK3: an in Toll-erant protein kinase. *Nat Immun* 2005;6:751–752
30. Liew FY, Xu D, Brint EK, O'Neill LAJ. Negative regulation of Toll-like receptor mediated immune responses. *Nat Rev Immunol* 2005;5:446–458
31. Han J, Ulevitch RJ. Limiting inflammatory responses during activation of innate immunity. *Nat Immunol* 2005;6:1198–1205
32. Mansell A, Smith R, Doyle SL et al., SOCS1 negatively regulates TLR signalling by mediating Mal degradation. *Nat Immunol* 2006;7:148–155
33. Wertz IE et al., De-ubiquitination and ubiquitin ligase domains of A20 down-regulate NF-kB signaling. *Nature* 2004;430:694–699
34. Parmeswaren N, Pao CS, Leonhard KS et al., Arrestin2 and GPCR5 interact with NFkB1 and negatively regulate LPS stimulated ERK1/2 activation in macrophages. *J Biol Chem* 2006;281:34159–34170
35. Takeshita F, Ishii KJ, Kobiyama K et al., Traf4 acts as a silencer in TLR-mediated signaling through association with Traf6 and Trif. *Eur J Immunol* 2005;35:2477–2485
36. Hawlisch H, Belkaid Y, Baelder R et al., C5a negatively regulates Toll-like receptor 4 induced immune responses. *Immunity* 2005;22:415–426
37. Divanovic S, Trompette A, Atabani SF et al., Negative regulation of TLR4 signaling by the Toll-like receptor homolog RP105. *Nat Immun* 2005;6:571–578

38. Negishi H, Ohba Y, Yanai H et al., Negative regulation of Toll-like receptor signaling by IRF4. *Proc Natl Acad Sci U S A* 2005;102:15989–15994
- 39a. Lemke G, Rothlin C. TAM receptors. *Nat Rev Immunol* 2008;8:327–336
- 39b. O'Neil, Luke A J. Positive and negative regulation of Toll-like Receptor Signal Transduction. *Immunity* 2008;29:12–20
40. Nau GJ, Schlesinger A, Richmond J, Young RA. Cumulative Toll-like receptor activation in human macrophages treated with whole bacteria. *J Immunol* 2003;170:5203–5209
41. De Gregorio R, Rappuoli R. Inside sensors detecting outside pathogens. *Nat Immun* 2004;5:1095–1100
42. Creagh EM, O'Neill L. TLRs, NLRs and RLRs: a trinity of pathogen sensors that cooperate in innate immunity. *Trends Immunol* 2006;27:352–357
43. Franchi L, McDonald C, Kanneganti T-D et al., Nucleotide-binding oligomerization domain-like receptors: intracellular pattern recognition molecules. *J Immunol* 2006;177:3507–3513
44. Girardin SE, Boneca IG, Viala J et al., NOD2 is a sensor of peptidoglycan through MDP detection. *J Biol Chem* 2003;278:8869–8872
45. Totemeyer S, Sheppard M, Lloyd A et al., IFN γ enhances production of NO from macrophages via nucleotide oligomerization domain-2. *J Immunol* 2006;176:4804–4810
46. Martinon F, Tschopp J. NLRs join TLRs as innate sensors of pathogens. *Trends Immunol* 2005;26:449–454
- 47a. Skehlik C, Reed JC. The pyrin connection: novel players in immunity and inflammation. *J Exp Med* 2004;200:551–558
- 47b. Skehlik C, Dorfleutner A. COPs and POPS: modulators of inflammasome activity. *J Immunol* 2007;179:7993–7998
48. Park J-H, Kim Y-G, McDonald C et al., Rick/RIP2 mediates innate immune responses through NOD1/2 but not TLRs. *J Immunol* 2007;178:2380–2386
49. (a) Netea MG, Azam T, Ferwerda G et al. TREM1 amplifies signals induced by NLR pattern recognition receptors. *J Leukoc Biol* 2006;80:1454–1461; (b) Tessarz AS, Weiler S, Zanzinger K et al. NTAL (non-T cell activation linker) negatively regulates Trem1/Dap12 cytokine production. *J Immunol* 2007;178:1991–1999
50. Martinon F. Orchestration of pathogen recognition by inflammasome diversity. *Eur J Immunol* 2007;37:3003–3006
51. Andrei C, Marqicco P, Poggi A et al., Phospholipases C and A2 control lysosome mediated IL-1 β secretion. *Proc Natl Acad Sci U S A* 2004;101:9745–9750
52. Yoo NJ, Park WS, Kim SY et al. NOD1 enhances prointerleukin 1- β processing through interaction with procaspase-1. *Biochem Biophys Res Commun* 2002;299:652–658
53. Bruey JM, Bruey-Sedano M, Newman R et al., PAN.1/NALP2 an inducible inflammatory mediator regulates NF-kappa B and caspase-1 activation in macrophages. *J Biol Chem* 2004;279:51897–51907
54. Kahlenberg JM, Dubyak GR. Differing caspase-1 states in monocyte versus macrophage models of IL-1 beta processing and release. *J Leukoc Biol* 2004;65:676–684
55. Sekiyama A, Ueda H, Kashiwamura S-I et al., A stress induced superoxide mediated caspase-1 activation pathway causes IL-18 upregulation. *Immunity* 2005;22:669–677
56. Roy CR, Zamboni DS. Cytosolic detection of flagellin. *Nat Immun* 2006;7:549–551
57. Drenth JPH, van der Meer JWM. The Inflammasome-a linebacker of innate defense. *N Engl J Med* 2006;355:730–732
58. Wang J, Wang X, Hussain S et al., Distinct roles of NFkB subunits in regulating inflammatory and T cell stimulatory gene expression in dendritic cells. *J Immunol* 2007;178:6777–6788
59. Sun H-J, Xu X, Wang X-L et al., Formation of interleukin 12. *Chin J Biochem Biophys* 2006;38:194–200
60. Lu J, Sun H, Wang X et al., IL-12 p40 promoter activity is regulated by the reversible acetylation mediated by HDAC1 and p300. *Cytokine* 2005;31:46–51
61. Gilchrist M, Thorsson V, Li B et al., Systems biology approaches identify ATF3 as a negative regulator of TLR4. *Nature* 2006;441:173–178

62. Hacker H, Mischah H. Cell type specific activation of MAPkinases by CPG-DNA controls Il-12 release from APCs. *EMBO J* 1999;18:6973–6982
63. Cooper AM, Khader SA. Il-12p40. *Trends Immunol* 2007;28:33–38
64. Grenningloh R, Kang BY, Ho I-C. Ets-1, a functional cofactor of T-bet, is essential for Th.1 inflammatory responses. *J Exp Med* 2005;201:615–626
65. Yao Y, Li W, Kaplan MH, Chang C-H. Interleukin 4 plays a key role in Th.1 differentiation by instructing dendritic cells to produce less interleukin 10. *J Exp Med* 2005;201: 1899–1903
66. Agrawal S, Agrawal A, Doughty B et al., Different Toll-like receptor agonists instruct dendritic cells to induce distinct T helper responses via differential modulation of ERK-mitogen activated protein kinase and c-fos. *J Immunol* 2003;171:4984–4989
67. Schade AE, Levine AD. Cutting edge: extracellular signal-related kinases 1:2 function as integrators of T cell receptor signal strength. *J Immunol* 2004;172:5828–5832
68. Bhalla US, Ram PT, Iyenger R. The strength of a signal can induce negative feedback circuits. *Science* 2002;297:1018–1021
69. Duckworth BC, Cantley LC. Conditional inhibition of MAP kinase cascade by wortmannin: dependence on signal strength. *J Biol Chem* 1997;272:27665–27700
- 70a. Damiano JS, Newman RM, Reed JC. Multiple roles of CLAN in the mammalian innate immune response. *J Immunol* 2004;173:6338–6345
- 70b. Hu X, Chakravarty SD, Ivashkiv LB. Regulation of interferon and Toll-like receptor signaling during macrophage activation. *Immunol Revs* 2008;226:41–56
71. Murray HW. Interferon-gamma, the activated macrophage and host defence against microbial challenge. *Ann Int Med* 1988;108:585–608
72. Xu W, Schlagwein N, Roos A et al., Human peritoneal macrophages show characteristics of M-CSF driven anti-inflammatory type 2 macrophages. *Eur J Immunol* 2007;37:1594–1599
73. Janssen MJ, Hendriks T, Huyben CM et al., Increased cytolytic activity and production of reactive oxygen and nitrogen intermediates by peritoneal macrophages during development of macrophage dysfunction in sepsis. *Scand J Immunol* 1995;44:361–368
74. Bae EM, Kim W-J, Suk K et al., Reverse signaling from GITRL induces NF- κ B activation of macrophages. *Mol Immunol* 2008;45:523–533
75. Zasloff M. Fighting infections with vitamin D. *Nat Med* 2006;12:388–390
76. Martineau AR, Wilkinson K, Newton S, Floto R et al., Vitamin D inducible suppression of Mycobacteria: the role of cathelicidin LL37. *J Immunol* 2007;178:7190–7198
77. Tobias PS, Ulevitch RJ. Lipopolysaccharide binding protein and CD14 in LPS dependent macrophage activation. *Immunobiology* 1993;187:227–232
78. Lien E, Means TK, Heine H, Yoshimura S et al., Toll like receptor 4 imparts ligand specific recognition of bacterial lipopolysaccharide. *J Immunol* 2000;105:497–504
79. Melendez AJ, Mohd Ibrahim FB. Antisense knockout of sphingosine kinase-1 in human macrophages inhibits C5a receptor dependent signal transduction, calcium signals, enzyme release, cytokine production and chemotaxis. *J Immunol* 2004;173:1596–1603
80. Scott MJ, Hoth JJ, Stagner MK et al., CD40-CD154 interactions between macrophages and NK cells during sepsis are critical for macrophage activation. *Clin Exp Immunol* 2004;137: 469–477
81. Park HS, Jung HY, Park EY et al., Direct interaction of TLR4 with NADPHox.4 isozyme is essential for LPS induced production of ROS and activation of NF κ B. *J Immunol* 2004;173:3589–3593
82. Vivarelli MS, McDonald D, Miller M et al., RIP links TLR4 to Akt and is essential for cell survival in response to LPS stimulation. *J Exp Med* 2004;200:399–404
83. Nieves D, Moreno JJ. Role of 5 lipoxygenase pathway in the regulation of RAW264.7 macrophage proliferation. *J Biol Chem* 2006;281:1022–1030
84. Bone RC, Sprung CL, Sibbald WJ. Definitions for sepsis and organ failure. *Crit Care Med* 1992;20:724–726
85. Sitkovsky MV, Lukashev S, Apasov S et al., Physiological control of immune response and inflammatory tissue damage by hypoxia inducible factors and adenosine A2A receptors. *Annu Rev Immunol* 2004;22:657–682

86. Bryn T, Mahic M, Enserink JM et al., The cyclic AMP-Epac1-Rap1 pathway acquires immunoregulatory function in mature macrophages. *J Immunol* 2006;176:7361–7370
87. Lorenowicz MJ, van Gils J, de Boer M et al., Epac1-Rap1 signaling regulates monocyte adhesion and chemotaxis. *J Leukoc Biol* 2006;80:1542–1552
88. Hirasaka K, Kohno S, Goto J et al., Deficiency of cbl-b enhances infiltration and activation of macrophages and causes peripheral insulin resistance in mice. *Diabetes* 2007;56: 2511–2522
89. Rauh MJ, Sly LM, Kalesnikoff J et al., The role of SHIP1 in macrophage programming and activation. *Biochem Soc Trans* 2004;32:785–786
90. Lemke G, Lu Q. Macrophage regulation by Tyro 3 family receptors. *Curr Opin Immunol* 2003;15:31–36
91. Maruyama K, Takada Y, Ray N et al., RANKL and osteoprotegerin regulate proinflammatory cytokine production in mice. *J Immunol* 2006;177:3799–3805
92. Zhang M, Caragine T, Wang H et al., Spermine inhibits proinflammatory cytokine synthesis in human mononuclear cells: a counterregulatory mechanisms that restrains the immune response. *J Exp Med* 1997;185:1759–1768
93. Freeman MW, Moore KJ. eLIXiR for restraining inflammation. *Nat Med* 2003;9:168–169
94. Dauphinee SM, Karsan A. Lipopolysaccharide signaling in endothelial cells. *Lab Invest* 2006;86:9–22
95. O'Toole T, Peppelenbosch MP. PI-3kinase mediates CD14 dependent signaling. *Mol Immunol* 2007;44:2362–2399
96. Yeh W-C, Chen N-J. Another Toll road. *Nature* 2003;424:736–737
97. Nakayama K, Okugawa S, Yanagimoto S et al., Involvement of IRAK.M in peptidoglycan induced tolerance in macrophages. *J Biol Chem* 2004;279:6629–6634
98. Jiang Z, Ninomiya-Tsuji J, Qian Y et al., IRAK dependent Il-1 induced signal complexes phosphorylate TAK1 and TAB2 at the plasma membrane and activate TAK1 in the cytosol. *Mol Cell Biol* 2002;22:7158–7167
99. Cheung PC, Nebreda AR, Cohen P. TAB3, a new binding partner of the protein kinase TAK1. *Biochem J* 2004;378:27–34
100. Chen Y-Q, Zhou Y-Q, Wang MH. RON receptor tyrosine kinase protects murine macrophages from apoptotic death induced by LPS. *J Leukoc Biol* 2002;71:359–366
- 101a. Ralph JA, Morand EF. MAPK phosphatases as novel targets for rheumatoid arthritis. *Expert Opin Therap Targets* 2008;12(7):795–808
- 101b. Procyk KJ, Rippo MR, Testi R. Lipopolysaccharide induces JNK in macrophages by a novel cdc42/Rac independent path involving phosphatidylcholine dependent phospholipase C. *Blood* 2000;96:2592–2597
102. Cuschieri J, Billgren J, Maier RV. PC-PLC is required for LPS mediated macrophage activation through CD14. *J Leukoc Biol* 2006;80:407–414
103. Hsu Y-W, Chi K-H, Huang W-C, Lin W-W. Ceramide inhibits LPS mediated nitric oxide synthase and Cox.2 induction in macrophages. *J Immunol* 2001;166:5388–5397
104. Foey AD, Brennan FM. Conventional protein kinase C and atypical PKC differentially regulate macrophage production of TNF α and IL-10. *Immunology* 2004;112:44–53
105. Pengal RA, Ganeson LP, Wei G et al., LPS induced production of interleukin 10 is promoted by serine/threonine kinase Akt. *Mol Immunol* 2006;43:1557–1564
106. Annane D, Bellissant E, Cavaillon J-M. Septic shock. *Lancet* 2005;365:63–78
107. Lang T, Mansell A. The negative regulation of Toll-like receptor and associated pathways. *Immunol Cell Biol* 2007;85:425–434
108. Panderey S, Agrawal DK. Immunobiology of Toll-like receptors. *Immunol Cell Biol* 2006; 84:333–341
- 109a. Liang J, Jiang D, Griffith J et al., CD44 is a negative regulator of LPS-TLR signaling in mouse macrophages. *J Immunol* 2007;178:2469–2475
- 109b. An H, Hou J, Zhou J et al., Phosphatase SHP-1 promotes TLR- and RIG1 activated production of type I interferon by inhibiting the kinase IRAK1. *Nat Immunol* 2008;9:542–550
110. Wang Y, Tang Y, Teng L et al., Association of β -arrestin and TRAF6 negatively regulates TLR II-1 receptor signaling. *Nat Immunol* 2006;7:139–147

111. Fraser DA, Arora M, Bohlson SS et al., Anti-inflammatory activity of complement protein C1q in human monocytes. *J Biol Chem* 2007;282:7360–7367
112. Schroder K, Swelt MJ, Hume DA. Signal integration between IFN γ and TLR signaling pathways in macrophages. *Immunobiology* 2006;211:511–524
113. Shortman K, Liu Y-J. Mouse and human dendritic subtypes. *Nat Rev* 2002;2:151–161
114. Galgani M, De Rosa V, De Simone S et al., Cyclic AMP modulates the functional plasticity of immature dendritic cells by inhibiting Src-like kinases through protein kinase A mediated signaling. *J Biol Chem* 2004;279:32507–32514
115. Marteau F, Communi D, Boeynaems J-M, Gonzalez NS. Involvement of multiple P2Y receptors and signaling pathways in the action of adenine nucleotide diphosphates on human monocyte derived dendritic cells. *J Leukoc Biol* 2004;76:796–803
116. Santambrogio L, Potoicchio I, Fessler SP et al., Involvement of caspase cleaved and intact API complex in endosomal remodelling in dendritic cells. *Nat Immun* 2005;6:1020–1028
117. Kantenga S, Jornot L, Devenoges C, Nicord LP. Superoxide anions induce the maturation of human dendritic cells. *Am J Resp Crit Care Med* 2003;167:431–437
118. Sedlik C, Orbach D, Venon P et al., A critical role for Syk in Fc receptor mediated antigen presentation and induction of dendritic cell maturation. *J Immunol* 2003;170:846–852
119. Flohe SB, Bruggenabb J, Lendermans S et al., Human HSP60 induces maturation of dendritic cells via a Th.1 phenotype. *J Immunol* 2003;170:2340–2348
120. Luft T, Maraskowsky E, Schnorr M et al., Tuning the volume of the immune response, strength and persistence of stimulation determines migration and cytokine secretion of dendritic cells. *Blood* 2004;104:1066–1074
121. Setterblad N, Roucard C, Bocaccio C et al., Composition of MHC class II enriched lipid microdomains is modified during maturation of primary dendritic cells. *J Leukoc Biol* 2003;74:40–48
122. Serbina NV, Salazar-Mather TP, Biron CA et al., TNF α /iNOS producing dendritic cells mediate innate immune defence against bacterial infection. *Immunity* 2003;19:59–70
123. Van Gisbergen KPJM, Sanchez-Hernandez M, Geijtenbeck TBH, van Kooyk Y. Neutrophils mediate immune modulation of dendritic cells through glycosylation dependent interactions between Mac-1 and DC-SIGN. *J Exp Med* 2005;201:1281–1292
124. Reth M. Hydrogen peroxide as second messenger in lymphocyte activation. *Nat Immun* 2002;3:1129–1135
125. Matsue H, Edelbaum D, Shalhevet Det al., Generation and function of oxygen species in dendritic cells during antigen presentation. *J Immunol* 2001;171:3010–3018
126. Vulcano M, Dusi S, Lissandre D et al., Toll-like receptor mediated regulation of NADPH oxidase in human dendritic cells. *J Immunol* 2004;173:5749–5756
127. Maemura K, Zheng Q, Wada T et al., Reactive oxygen species are essential mediators in antigen presentation by Kupffer cells. *Immunol Cell Biol* 2005;83:336–343
128. Bhattacharyya S, Sen P, Wallet M et al., Immunoregulation of dendritic cells by interleukin 10 is mediated through suppression of the PI3kinase/Akt pathway and of I κ B kinase. *Blood* 2004;104:1100–1109
129. Ronger-Savle S, Valladeau J, Claudy A et al., TGF β inhibits CD1d expression on dendritic cells. *J Invest Derm* 2005;124:116–118
130. Cohen N, Mouly E, Hamdi H et al., GILZ expression in human dendritic cells redirects their maturation and prevents antigen specific T lymphocyte response. *Blood* 2006;107:2037–2044
131. Piercy J, Petyrova S, Tchilion EZ, Beverley PCL. CD45 negatively regulates TNF and IL-6 production in dendritic cells. *Immunology* 2006;118:250–256
132. Vlad G, Cortesini R, Suci-Foca N. Bidirectional interaction of antigen specific regulatory T cells and tolerogenic antigen presenting cells. *J Immunol* 2005;174:5907–5914
133. Vivier E, Dalad M. Tolerogenic dendritic cells. *Blood* 2006;107:2591–2592
134. Tracey KJ, Vlassara H, Cerami A. Cachectin/tumor necrosis factor. *Lancet* 1989;i:1122–1125
135. Andersson U, Erlandsson-Harris H, Yang H, Tracey KJ. HMGB1 as a DNA binding cytokine. *J Leukoc Biol* 2002;72:1084–1091
136. Oppenheim JJ, Yong DE. Alarmins: chemotactic activators of the immune response. *Curr Opin Immunol* 2005;17:359–365

137. Tang D, Kang R, Xiao W et al., Nuclear hsp72 as a negative regulator of oxidative stress induced HMGB1 release. *J Immunol* 2007;178:7376–7384
138. Goeddel DV. Signal transduction by TNF α . *Chest* 1999;166:suppl 69S–73S
139. Wajant H, Pfizenmaier K, Scheueriel P. TNF α signaling. *Cell Death Differ* 2003;10:45–65
140. MacEwan DJ. TNF ligands and receptors-a matter of life and death. *Br J Pharmacol* 2002;135:855–875
141. Hsu H, Shu H-B, Pan M-G, Goeddel DV. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF-R I signal pathways. *Cell* 1996;84:299–308
142. Wullaert A, Heyninck K, Beyaert R. Mechanisms of crosstalk between TNF α induced NF- κ B in hepatocytes. *Biochem Pharmacol* 2006;72:1090–1101
143. Temkin V, Karin M. From death receptor to reactive oxygen species and c-Jun N-terminal protein kinase: RIP1 odyssey. *Immunol Rev* 2007;220:8–21
144. Heller RA, Song K, Fan N, Chang DJ. The p70 TNF-R mediates cytotoxicity. *Cell* 1002;70:47–56
145. Liu J, Lin A. Wiring the cell signaling circuitry by NF κ B and JNK cross-talk and its applications in human disease. *Oncogene* 2007;26:3267–3278
146. Duckell CA. Apoptosis and NF κ B. *J Clin Invest* 2002;109:579–580
- 147a. Gupta S, Kim C, Yu L, Gollapudi SA. A role of FADD in increased apoptosis in aged humans. *J Clin Immunol* 2004;24:24–29
- 147b. Natoli G, Austenna L. A birthday gift for TRADD. *Nat Immunol* 2008;9:1015–1016.
- 147c. Pincheira R, Castro AF, Ozes O, Idumalla P, Donner DB. Type I TNF Receptor forms a complex with and uses Jak2 and c-Src to selectively engage signal pathways that regulate transcription factor activity. *J Immunol* 2008;181:1288–1298
148. Lombardo E, Alvarez-Barrientos A, Maroto B et al., TLR4 mediated survival of macrophages is MyD88 dependent and requires TNF α autocrine signaling. *J Immunol* 2007;178:3731–3739
149. Dempsey PW, Doyle SE, He JQ, Cheng Q. The signaling adaptors and pathway activated by the TNF superfamily. *Cytokine Growth Factor Rev* 2003;14:193–209
150. Papa S, Bubici C, Zazzeranni F et al., NF- κ B mediated control of the JNK cascade. *Cell Death Differ* 2006;13:712–729
151. Reddy SAG, Huang J, Kiao WSL. PI 3-kinase as a mediator of TNF α induced NF κ B activation. *J Immunol* 2000;164:1355–1365
- 152a. Gupta SA. A decision between life and death during TNF α induced NF κ B activation. *J Clin Immunol* 2002;22:185–194
- 152b. Varfolomeev E, Goncharov T, Fedorova AV et al., cIAP 1/2 are critical mediators of TNF α induced NF- κ B activation. *J Bio Chem* 2008;283:24295–24299
153. Wang J, Li C, Liu Y et al., JAB1 determines the response of RA synovial fibroblasts to TNF α . *Am J Pathol* 2006;169:889–902
154. Wang CY, Mayo MW, Korneluk RG et al., NF κ B antiapoptosis. *Science* 1998;281:1680–1683
155. Shen H-M, Pervaiz S. TNF receptor superfamily-induced cell death: redox dependent exution. *FASEB J* 2006;20:1589–1598
156. Meylan E, Tschoopp J. The RIP kinases: crucial integrators of cellular stress. *Trends Biochem Sci* 2005;30:151–159
157. Kelliher MB, Grim S, Ishida Y et al., The death domain kinase RIP mediates the TNF-induced NF- κ B signal. *Immunity* 1998;8:297–303
158. Festjens F, van den Berghe T, Cornelis S, Vandenabeele P. RIP-1, a kinase on the crossroads of a cell's decision to live or die. *Cell Death Differ* 2007;14:400–410
159. Malewicz M, Zeller N, Yilmaz ZB, Weih F. NF κ B controls the balance between Fas and TNF cell death pathways during T cell receptor induced apoptosis via the target gene A20. *J Biol Chem* 2003;278:32825–32833
160. Pettus BJ, Bielawski J, Porcelli AM et al., The sphingosine kinase: sphingosine-1-phosphate pathway mediates cyclooxygenase 2 induction and PGE2 production in response to TNF α . *FASEB J* 2003;17:1411–1421
161. Andrade RM, Wessendorp M, Portillo JAC et al., TRAF6 dependent CD40 signaling primes macrophages to acquire anti-microbial activity in response to TNF α . *J Immunol* 2005;175:6014–6021

162. Zakharova M, Ziegler HK. Paradoxical anti-inflammatory actions of TNF α : inhibition of IL-12 and IL-23 via TNFRI in macrophages and dendritic cells. *J Immunol* 2005;175:5024–5033
163. Toh M-L, Aeberli D, Lacey D et al., Regulation of IL-1 and TNF-receptors and function by endogenous MIF. *J Immunol* 2006;177:4818–4825
164. Hoi AY, Hickey MJ, Hall P et al., MIF deficiency attenuates macrophage recruitment, GN and lethality in MRL/lpr mice. *J Immunol* 2006;177:5687–5696
165. Calandra T, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat Rev Immunol* 2003;3:791–800
166. Bucala R, Donnelly SC. Macrophage migration inhibitory factor. *Immunity* 2007;26: 281–285
167. Anderson P. Post-transcriptional regulation of tumour necrosis factor α production. *Ann Rheum Dis* 2000;59(Suppl 1):i3–i5
- 168a. Cavaillon J-M. Chap 6 Molecules Mediators Cytokine Receptors in Immunology. From Cell Biology to Disease Ed. R.A Meyers. Wiley-VCH Verlag 2007 (ISBN 978-3-527-31770-7)
- 168b. Lord DJ, McIntosh BC, Greenberg PD, Nelson BH. The IL-2 receptor promotes lymphocyte proliferation and induction of c-myc, bcl-2, bcl-x genes through the transcription domain of Stat 5. *J Immunol* 2000;164:2533–2541
169. Kovanen PE, Rosenwald A, Fu J et al., Analysis of γ c family cytokine target genes: DUSP5 as a regulator in IL-2 signaling. *J Biol Chem* 2003;278:5205–5213
170. Tenbrock K, Juang Y-T, Tolnay M, Tsokos GC. The cyclic AMP response element modulator suppresses IL-2 production in stimulated T cells by a chromatin dependent mechanism. *J Immunol* 2003;170:2971–2976
171. Gong D, Malek TR. Cytokine dependent Blimp1 expression in activated T cells inhibits IL-2 production. *J Immunol* 2007;178:242–252
172. Lawrence A, Tato CM, Davidson TS et al., Interleukin 2 signaling via Stat5 constrains T helper 17 cell activation. *Immunity* 2007;26:371–381
173. Ivanov II, McKenzie BS, Zhou L et al., The orphan nuclear receptor ROR γ directs the differentiation program of IL-17 + T helper cells. *Cell* 2006;126:1121–1133
174. Skov S, Rienech K, Bovin LF et al., Histone deacetylase inhibitors: a new class of immunosuppressives targeting a novel signal pathway for CD154 expression. *Blood* 2003;101: 1430–1438
175. Stepkowski SM, Kao J, Wang M-E et al., Mannich base NC1153 promotes longterm allograft survival. *J Immunol* 2005;175:4236–4246
176. Ii M, Matsunaga N, Hazeki K et al., TAK242 selectively inhibits TLR4 mediated cytokine production through suppression of signaling. *Mol Pharm* 2006;69:1288–1295
177. Sevigny CP, Li L, Awad AS et al., Activation of adenosine 2A receptors attenuates allograft rejection and alloantigen recognition. *J Immunol* 2009;178:4240–4249
178. Ohteki T, Tada H, Ishida K et al., Essential role of dendritic cell derived IL-15 as a mediator of inflammatory responses in vivo. *J Exp Med* 2006;203:2329–2338
179. Wilks AF, Harpur AG. Cytokine signal transduction and the JAK family of protein tyrosine kinases. *Bioessays* 1994;15:313–320
180. Leaman DW, Leung S, Li X, Stark GR. Regulation of Stat dependent pathways by growth factors and cytokines. *FASEB J* 1995;10:1578–1588
181. Trinchieri G. Interleukin 12, a proinflammatory cytokine with immunoregulatory functions. *Annu Rev Immunol* 1995;13:251–275
182. Ahn H-J, Tomura M, Yu WG et al., Requirement for distinct Janus kinases and Stat proteins in T cell proliferation versus IFN γ production with IL-12 stimulation. *J Immunol* 1998;161: 5893–5900
183. Yoshimura A, Naka T, Kubo M. SOCS proteins, cytokine signalling and immune regulation. *Nat Rev Immunol* 2007;7(6):454–466
184. Yu CR, Mahdi RM, Ebong S et al., SOCS3 regulates proliferation and activation of T helper cells. *J Biol Chem* 2003;278:29752–29759
- 185a. O'Neill LAJ, Dinarello CA. The IL-1 receptor/Toll-like receptor superfamily; crucial receptors for inflammation and host defence. *Immunol Today* 2000;21:206–209
- 185b. O'Neill LAJ. The interleukin 1/Toll-like receptors superfamily: 10 years of progress. *Immunol Revs* 2008;226:10–18

186. Eder J. Tumour necrosis factor and Il-1 signalling: do MAP kinases connect it all. *Trends Pharm Sci* 1997;18:319–322
187. Martindale JL, Holbrook NJ. Cellular response to oxidative stress: signaling for suicide and survival. *J Cell Physiol* 2002;192:1–15
188. Cimino F, Esposito F, Ammendola R, Russo T. Gene regulation by reactive oxygen species. *Curr Top Cell Regul* 1997;35:123–148
189. Bonizzi G, Piette J, Merville M-P, Bours V. Cell type specific role for reactive oxygen species in NFkB activation by interleukin 1. *Biochem Pharm* 1999;59:7–11
190. Huang W-C, Chen J-J, Inoue H, Chen C-C. Tyrosine phosphorylation of I- κ B kinase α/β is involved in TNF α induced cyclooxygenase -2 expression. *J Immunol* 2003;170:4756–4775
191. de Oliveira-Marques V, Cyrne L, Marinho HS, Antunes F. A study of NF- κ B activation by H2O2: relevance in inflammation and synergy with TNF α . *J Immunol* 2007;178:3893–3902
192. Yao J, Kim TW, Qin J et al., Il-1 induced TAK1 dependent versus MEKK3 dependent NF- κ B activations bifurcate at Il-1Receptor associated kinase modification. *J Biol Chem* 2007;282:6075–6089
193. Wullaert A, Heyninck K, Janssens S, Beyaert R. Ubiquitin: tool and target for intracellular NFkB inhibitors. *Trends Immunol* 2006;27:533–540
194. Wan YY, Chi H, Xie M et al., The kinase TAK1 integrates cytokine receptor signaling for T cell development, survival and function. *Nat Immunol* 2006;7:851–859
195. Alford KA, Glennie S, Turrell BR et al., Hsp27 in inflammatory gene expression and TAK1 mediated signaling. *J Biol Chem* 2007;282:6232–6241
196. Li X. IRAK4 in TLR/Il-1R signaling: possible clinical applications. *Eur J Immunol* 2008;38:614–618
197. Aggarwal BB. Signal pathways of the TNF superfamily. *Nat Rev Immunol* 2003;3:745–746
198. Takeuchi M, Rothe M, Goeddel DV. Anatomy of TRAF2. Distinct domains for NFkB activation and association with TNF signaling proteins. *J Biol Chem* 1996;271:19935–19942
199. Unoue J-I, Ishida T, Tasukamoto N, Kobayashi N et al., TNF α receptor associated factor (TRAF) family adapter proteins that mediate cytokine signaling. *Exp Cell Res* 2000;254:14–24
200. Grech AP, Amesbury M, Chan T et al., TRAF2 differentially regulates the canonical and non-canonical NFkB activation in mature B cells. *Immunity* 2004;21:629–642
201. Chambers TJ. Regulation of the differentiation and function of osteoclasts. *J Pathol* 2000;192:4–13
202. King CG, Kobayashi T, Cejas PJ et al., TRAF6 is a T cell intrinsic negative regulator required for maintenance of immune homeostasis. *Nat Med* 2006;12:1088–1092
203. Hacker H, Redecke V, Blagoev B et al., Specificity in TLR signaling through distinct effector function of TRAF3 and TRAF6. *Nature* 2006;439:204–207
204. Banchereau J, Bazan F, Blanchard D et al., The CD40 antigen and its ligand. *Annu Rev Immunol* 1994;12:811–922
205. van den Blink B, Juffermans NP, ten Hove T et al., p38 MAPkinase inhibition increases cytokine release by macrophages. *J Immunol* 2001;166:582–587
206. Munroe ME, Bishop GA. Costimulatory function for T cell CD40. *J Immunol* 2007;178: 671–682
207. Baccam M, Woo S-Y, Vinson C, Bishop GA. CD40 mediated transcriptional regulation of the Il-6 gene in B lymphocytes. *J Immunol* 2003;176:3099–3108
208. Bishop GA, Hostager BB, Brown KD. Mechanisms of TNF-receptor associated factor (TRAF) regulation in B lymphocytes. *J Leukoc Biol* 2002;72:19–23
209. Xie P, Hostager BS, Munroe ME, Moore CR, Bishop GA. Cooperation between TRAF1 and 2 in CD40 signaling. *J Immunol* 2006;176:5388–5400
210. Qian Y, Qin J, Cui G et al., Act.1, a negative regulator in CD40 and BAFF mediated B cell survival. *Immunity* 2004;21:575–587
211. Walls TH. Tnf/Tnf.R family members in costimulation of T cell receptors. *Annu Rev Immunol* 2005;23:23–68
212. Del Prete F, DeCarli M, D’Elios M et al., CD30 mediated signaling promotes development of human T helper type 2 T cells. *J Exp Med* 1995;182:1655–1661

213. Gommermann JL, Browning JL. Lymphotoxin/ Light, lymphoid microenvironments and autoimmune disease. *Nat Rev Immunol* 2003;3:642–655
214. Takahashi C, Mittler RS, Vella AT. 4–1BB is a bonefide CD8 T cell survival signal. *J Immunol* 1999;162:5037–5040
215. Myers LM, Vella AT. Interfering T cell effector and regulatory function through CD137(4–1BB) costimulation. *Trends Immunol* 2005;26:440–447
216. Weinberg AD, Evans DE, Thalhofer C et al., Generation of T cell memory: molecular and cellular events following OX40 engagement. *J Leukoc Biol* 2004;75:962–972
217. Kim MY, Bekiaris V, McConnell FM et al., OX40 signals during priming on dendritic cells inhibit CD4 cell proliferation. *J Immunol* 2004;174:1433–1437
218. Nocentini G, Riccardi C. GITR: a multifaceted regulator of immunity belonging to the TNF receptor superfamily. *Eur J Immunol* 2005;35:1016–1022
219. Bushell A, Wood K. GITR ligation blocks allograft protection by regulatory T cells without enhancing effector T cell function. *Am J Transplant* 2007;7:759–68
220. Bae E, Kim WJ, Kang YM et al., GITR related protein mediated macrophage stimulation may induce cell adhesion and cytokine expression in RA. *Clin Exp Immunol* 2007;148:410–418
221. Schmidt AM, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *J Clin Invest* 2001;108:949–995
222. Dalton TP, Shertzer HG, Puga A. Regulation of gene expression by reactive oxygen. *Annu Rev Pharmacol Toxicol* 1999;39:67–99
223. Morbini P, Villa C, Campo Iet al., The receptor for advanced glycation end-products and its ligands: a new inflammatory pathway in lung disease. *Modern Pathol* 2006;19:1437
224. Dougan M, Dranoff G. Inciting inflammation: the RAGE about tumor promotion. *J Exp Med* 2008;205:267–270
225. Zen K, Chen CX, Chen YY et al., Receptor for AGEs mediates neutrophil migration across intestinal epithelium. *J Immunol* 2007;178:2483–2490
226. Pullerits R et al., Soluble receptor for AGEs triggers a proinflammatory cascade via β 2-integrin Mac-1. *Arthr Rheum* 2006;54:3898–3907
227. Lu C, He JC, Cai W et al., AGE Receptor-1 is a negative regulator of the inflammatory response to AGE in mesangial cells. *Proc Natl Acad Sci U S A* 2004;101:11767–11772
228. Fukami K, Ueda S, Yamagishi S-I et al., AGEs activate mesangial TGF β -Smad signaling via an angiotensin II type I receptor interaction. *Kid Int* 2004;66:2137–2147
229. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin 10 and the interleukin 10 receptor. *Annu Rev Immunol* 2001;19:683–765
230. O'Garra M, Vieira PL, Vieira P, Goldfeld AE. Il-10 producing and naturally occurring CD4 + T regs: limiting collateral damage. *J Clin Invest* 2004;114:1372–1377
231. Williams LM, Ricchetti G, Sarma V, Smallie T, Foxwell BMH. Interleukin 10 suppression of myeloid cell activation. *Immunology* 2004;113:281–292
231. (a) Mosser DM, Zhang X. Interleukin 10: new perspectives on an old cytokine. *Immunol Rev* 2008;226:205–218
232. Grutz G. New insights into the molecular mechanisms of Il-10 mediated immunosuppression. *J Leukoc Biol* 2005;77:3–15
233. Antoniu T T, Parek-Min K H, Ivashkiv L B. Kinetics of Il-10 induced gene expression in human macrophages. *J Immunol* 2005;210:87–95
234. Riley JK, Takeda K, Akina S, Schreiber RB. Interleukin 10 Receptor signaling through the Jak-Stat pathway. *J Biol Chem* 1999;274:16513–16521
235. Dean JL, Sully G, Clark AR, Saklatvala J. The involvement of AU-rich element-binding proteins in p38 MAP kinase mediated RNA stabilization. *Cell Signal* 2004;16:1113–1121
236. Murray PJ. The endogenous Il-10/Stat3 mediated anti-inflammatory response. *Curr Opin Pharmacol* 2006;6:379–386
237. Cao SA, Liu J, Chesi M et al., Differential regulation of Il-12 and Il-10 gene expression in macrophages by leucine-zipper transcription factor c-Maf. *J Immunol* 2002;169:5715–5725
238. Riemann M, Endres R, Liptay S et al., Bcl-3 negatively regulates transcription of the interleukin-10 gene in macrophages. *J Immunol* 2005;175:3560–3566

239. Perrier P, Martinez FO, Locati M et al., Distinct transcriptional programs activated by IL-10 with or without lipopolysaccharide in dendritic cells: induction of the B cell activating chemokine CXCL13. *J Immunol* 2004;172:7031–7042
240. Renauld J-C. Class II cytokine receptors and their ligands. Key antiviral and inflammatory modulators. *Nat Rev Immunol* 2003;3:667–669
241. El Kasmi KC, Smith AM, Williams L et al., A transcriptional repressor and corepressor induced by Stat3 regulated anti-inflammatory signaling pathway. *J Immunol* 2007;179:7215–7219
242. Jankovic D, Trinchieri G. IL-10 or not IL-10. *Nat Immunol* 2007;8:1281–1283
243. Vincenti F, Luggen M. T cell costimulation. *Annu Rev Med* 2007;58:347–358
244. McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. *Nat Rev Immunol* 2007;7(6):429–442
245. Hirota K, Yoshitomi H. Preferential recruitment of CCR6 expressing Th-17 cells to inflamed joints via CCL20 in RA and animal model. *J Exp Med* 2007;204:2803–2812
246. Sacre SM, Andreaskos E, Kirikidis S et al., Toll-like receptor adaptor proteins MyD88 and Mal contribute destructive processes in a human model of RA. *Am J Pathol* 2007;170:518–525
- 247a. Sakaguchi S, Sakaguchi N, Yoshitomi H et al., Spontaneous development of autoimmune arthritis due to genetic anomaly of T cell signal transduction. *Semin Immunol* 2006;18:199–106
- 247b. Thomas R, Turner M, Cope AP. High avidity auto reactive T cells with a low signalling capacity through the T cell receptor: Central to RA pathogenesis? *Arthr Res and Therapy* 2008;10(4):210 Biomed Central
248. Hoffmann MH, Tuncel J, Skriner K et al., RA33 is a major stimulator of autoimmunity in rats with pristane induced arthritis. *J Immunol* 2007;179:7568–7576
249. Kikly K, Liu L, Na S, Sedgwick JD. The IL-23/Th17 axis. *Curr Opin Immunol* 2006;18:670–675
250. van den Berg WB, Van Lant PL, Joosten L et al., Amplifying elements of arthritis and joint destruction. *Ann Rheum Dis* 2007;66(Suppl iii):iii45–48
251. Perper SJ, Browning B, Burkly LC et al., Tweak is a novel arthritogenic mediator. *J Immunol* 2006;177:2610–2620
252. Feldmann M, Maini RN. Anti-TNF α therapy of RA. *Annu Rev Immunol* 2001;19:163–196
253. Rossol M, Meusch U et al., TNF activation of monocytes. *J Immunol* 2007;179:4239–4248
254. Dai S-M, Shan Z-Z, Xu H, Nishioka K. Cellular targets of interleukin 18 in rheumatoid arthritis. *Ann Rheum Dis* 2007;66:1411–1418
255. Firestein GS. Inhibiting inflammation in rheumatoid arthritis. *N Engl J Med* 2006;354:80–82
256. Pollard LC. Inhibiting costimulatory activation of T cells. *Drugs* 2007;67(1):1–9
257. Rahman A. Regulators of cytokine signalling in RA. *Rheumatology* 2007;46:1745–1746
258. Paniagua RT, Sharpe O, Ho PP et al., Selective tyrosine kinase inhibition by imatinib mesylate for the treatment of autoimmune arthritis. *J Clin Invest* 2006;116:2633–2642
259. Li X, Makarov SS. Essential role of NF- κ B in the tumor-like phenotype of arthritic synovio-cytes. *Proc Natl Acad Sci U S A* 2006;103:17432–17437
260. Vasilopoulos Y, Gkretsi V, Armaka M, Aidinis V, Kollias G. Actin cytoskeleton dynamics linked to synovial fibroblast activation as a novel pathogenic principle in TNF-driven arthritis. *Ann Rheum Dis* 2007;66(Suppl iii):iii23–iii28
261. Alsaleh G, Messer L, Semaan N. BAFF synthesis by rheumatoid synovio-cytes. *Arthritis Rheum* 2007;56:3202–3214
262. Smolen JS, Aletaha D, Koeller M, Weisman MH, Emery P. New therapies for treatment of rheumatoid arthritis. *Lancet* 2007;370:1861–1873
- 263a. Hoyer K, Dooms H, Barron L, Abbas AK. Interleukin 2 in the development and control of inflammatory disease. *Immunol Revs* 2008;226:19–28
- 263b. Liu JO. Calmodulin dependent phosphatase, kinases and copressor involved in T cell activation. *Immunol Revs* 2009;228:184–198
- 263c. Ho N, Gullberg M, Chatila T. AP-1 dependent transcriptional activation of IL-2 gene by CaMkinase IV. *J Exp Med* 1996;184:101–112
- 263d. Racioppi L, Means AR. Calcium/calmodulin dependent kinase IV in immune and inflammatory responses. *Trend Immunol* 2008;29:600–608

Chapter 13

T Lymphocytes

Abstract All aspects of T lymphocytes and how they are motivated are outlined. Interferon- γ drives Th1 lymphocytes in defence against pathogens, and ILs 4 and 13 incite Th2 lymphocytes in allergic reactions. Asthma allergy is a pressing problem with a seemingly never-ending list of immunological aspects.

13.1 Activation of T Lymphocytes

Antigen-presenting cells (APCs) such as dendritic cells (DCs) process antigen to neopeptide that is presented bound to major histocompatibility (MHC) class II glycoprotein molecules to T helper cells [1], whose role is to help B cells become plasma cells, that secrete appropriate specific immunoglobulin (Ig) antibody to the antigen. The T lymphocytes have T cell receptors (TCRs) with a unique structure i.e. α -/ β -subunits for neopeptide/MHC antigen recognition (Fig. 13.1) and the CD3- ζ complex signal-transducing subunits. They connect with underlying phosphotyrosine kinases (PTKs) p59 Lck, p59 Fyn and Zap70, initiating an integrated signal for interleukin 2 (IL-2) formation. Associated costimulatory glycoprotein molecules are required, like the CD4 and CD8 adhesion molecules, the CD2 sheep cell receptor, integrin LFA-1 and the CD28 co-stimulator.

This chapter emphasises the cascades for signal transmission in T lymphocytes [2]. Whilst the TCR α / β has only a short cytoplasmic tail devoid of catalytic activity, the associated CD3 subunits (γ , δ , ϵ and ζ) each have immunoreceptor tyrosine activation motifs (ITAMs) that become phosphorylated, leading to T cell activation. Each ITAM consists of a short amino acid sequence that is a duplicate of YXXL/I, with Y indicating tyrosine, L leucine, I isoleucine and X any amino acid. APC engagement of the TCR, via the *immune synapse*, leads to rapid and transient phosphorylation of tyrosine molecules within the ITAMs, creating temporary binding sites for -SH2-bearing molecules like Zap70, adaptor SLP76, phosphokinase PLC γ 1 and Grb2.

TCR signalling is determined by the numbers of TCRs and their avidity, by the duration of APC-T cell contacts, and by requisite costimulatory signals. Effector

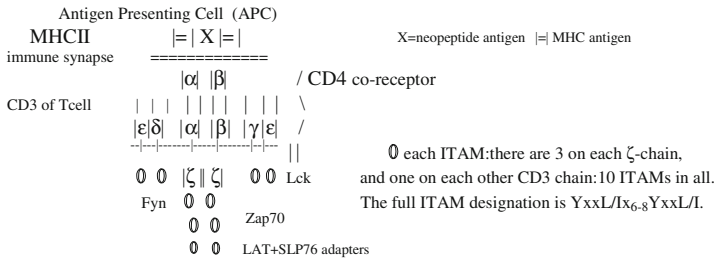


Fig. 13.1 Structure of T cell receptor (TCR) to emphasise the immunoreceptor tyrosine activation motifs (*ITAMs*). The CD3 signal-transducing complex comprises a CD3 $\epsilon\delta$ heterodimer, a CD3 $\epsilon\delta$ heterodimer and a CD3 $\zeta\zeta$ homodimer. Binding of the TCR together with CD4 coreceptor to the same peptide-major histocompatibility complex (*MHC*) molecule leads to T cell activation. A single class I MHC-peptide complex on an antigen-presenting cell (*APC*) will activate CD8 T cells. About 200 class II MHC-peptide complexes are needed to activate CD4 cells. The Tec family of tyrosine kinases, which are adjacent to Linker of Activated T cells (*LAT*) (Fig. 13.4) serve to amplify Lck and Zap70 and thus TCR signals [2]. *Zap* ζ chain-associated protein of 70 kDa. The adapter *LAT* has nine tyrosines that, when phosphorylated, serve as docking domains for signal components with Src-homology domain 2 (*SH2*) motifs like PLC γ 1 and adaptor Grb2. *LAT*, with Gads associated with SLP76, form a scaffold for PLC γ 1 to interact with the TCR. As the TCR is stimulated, there is transient inactivation of phosphatase SHP2 by the formed ROS. Accordingly signal complexes of Vav1 or ADAP associate with the SLP76 (Fig. 13.4). Vav1 connects with WASP which directs formation of actin filaments, so that there will be internalization of TCR complexes, and other endosomal sorting processes

CD4 T cells express excess signalling intermediates CD3 ζ ++ and pZap70++. Zap70 is also high in memory cells.

13.1.1 General Scheme of T Lymphocyte Activation

A signalling pathway is determined by organised phosphorylations of proteins along a cascade (or network) that leads to activation of specific transcription factors (TFs) so that there can be induction of appropriate target genes. One will recall that T cell activation is induced by (a) recognition of antigen, (b) binding of costimulatory molecules and (c) cytokines (Table 13.1).

The scheme in Fig. 13.2 indicates how in T lymphocytes there is a TCR that determines the cell activation sequence [4, 5]. This consists of a ligand-binding module that is noncovalently associated with four dimeric signal molecules: TCR α/β , CD3 $\delta\epsilon$, CD3 $\gamma\epsilon$ and $-\zeta$. The organisation is via intramembrane polar contacts [5]. Consequent on activation of the TCRs, membrane-associated Src family kinases Lck and then Zap70 are recruited to doubly phosphorylate the ITAMs on the CD3 chains (Fig. 13.1). The phosphorylated domains on the receptors are then sites for the temporary coalescence of kinases, adaptor molecules *LAT* and SLP-76 and

Table 13.1 PTPases controlling T lymphocyte activation

Name	Role	Function
CD45	+ Regulator	+ Regulates Lck, Fyn and Janus phosphotyrosine kinases (JAKs)
PEP	– Regulator	Proximal site of action on the T cell receptor (TCR)
LMPTP	–	Dephosphorylates Zap70
SHP-1	–	Inhibits Zap70 and spleen tyrosine kinase (Syk), supports immunoreceptor tyrosine inhibitory motifs (ITIMs); limits PI3 kinase signalling
SHP-2	+/–	Augments extracellular signal-related kinase (ERK) activation; works also with CTLA4 and programmed death 1 (PD-1)
HePTP (PTPN7)	–	Regulates ERK and p38
VHR (dual-specificity phosphatase 3, DUSP3)	–	Dephosphorylates ERK and Jun N-terminal kinase (JNK)
PTP (protein tyrosine phosphatase)	–	Counteracts PI3 kinase (Fig. 2.6), hence CD28 costimulation is required
PP2A(protein phosphatase 2A)	–	Binds and inhibits both CD28 and CTLA4; targets Akt
PHLPP	–	Pleckstrin homology (PH) domain leucine-rich repeat protein phosphatase targets Akt
PTP-PEST	–	Binds C-terminal Src kinase (Csk) and dephosphorylates Cas, Shc
PTPN11 (protein tyrosine phosphatase 11)	+	Normal haemopoietic development

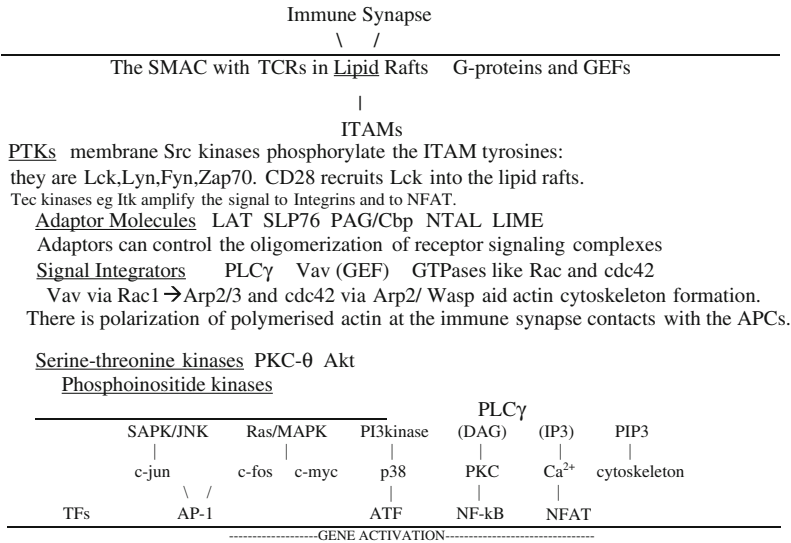


Fig. 13.2 Scheme of T cell receptor (*TCR*) signalling

controlling phosphatases. Phosphorylated LAT will trigger the phosphorylation of phospholipase $C\gamma 1$ ($PLC\gamma 1$), forming IP_3 and the sequence $Grb2:Sos-Ras \rightarrow$ mitogen-activated protein kinase (MAPK). At these distal levels, the cascades are organised either as *signalosomes* or as networks.

The PI3 kinase (PI3K)-Akt path responds to the TCR, co-stimulatory receptors and cytokines. The resulting $PI(3,4,5)P_3$ is then controlled by protein tyrosine phosphatase PTEN, SHP-1 and SHP-2 phosphatases.

Within the membranes of T lymphocytes, lipid rafts enriched in cholesterol and sphingolipids enable formation of supramolecular activation clusters (SMACs), which together form an immunological synapse (IS) between the antigen-presenting DC and the T cell signalling system. The function of the IS must be to enable a few TCRs to be triggered by a few MHC antigen–neopeptide ligands [6]. However, when T cells are tolerised [7], immune synapses are not formed because there is defective translocation of TCRs and protein kinase $C\theta$ ($PKC\theta$) into the lipid raft fractions.

Within the SMACs, there are the TCRs with their CD3 molecules, Src kinases p56Lck and p59 Fyn and $PKC\theta$ [8]. Genetic evidence from knockout mice suggests a critical role for Lck [9]. Lck interacts with the cytoplasmic tail of the CD4 and CD8 molecule coreceptors. During antigen presentation, CD4 or CD8 bind to MHC molecules on APCs, bringing Lck to the TCRs to effect ITAM phosphorylation. Interestingly, HIV-1 infection targets Lck as a key step in T cell activation, resulting in an inability to assemble the immune synapses. Activation of Lck triggers a cascade that recruits Zap70 to amplify the signal (Fig. 13.4) by assembly of molecules of palmitoylated transmembrane adaptor protein LAT [10]. The LAT adaptor molecules bear multiple tyrosine-based motifs that direct connections to intracellular signalling pathways (Figs. 13.1 and 13.3). Partitioning of PIP_2 ($PtdIns\ 3.4P_2$) into the lipid rafts must mean that PIP_2 activates $PLC\gamma 1$ and PI3K [11]. So, on TCR stimulation, Lck incites the phosphorylation of the $TCR\zeta$ molecules and the CD3 ITAMs, the Zap70 and the adaptors LAT and SLP76, and activates $PLC\gamma 1$. Hence, there is influx of Ca^{2+} ions. Furthermore, the activated Lck invites migration of co-stimulator molecules like CD28 into the contact site

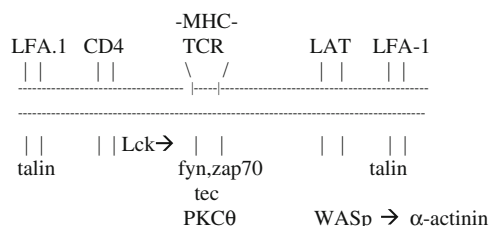


Fig. 13.3 Signalling from the supramolecular activation cluster (SMAC) to immune response genes. The central SMAC with major histocompatibility complex (MHC) T cell receptor (TCR) molecules within lipid rafts contains protein kinase $C\theta$ ($PKC\theta$). The peripheral SMAC (pSMAC) of leucocyte function-associated antigen 1 (LFA-1) molecules is characterised by talin, which connects with the actin cytoskeleton. CD4 enhances sensitivity by helping Lck accumulation at the immunological synapse. Lck induces phosphorylation of the TCR immunoreceptor tyrosine activation motifs (ITAMs); thus, there is ζ -chain phosphorylation and hence Zap70 activation. Talin helps LFA-1 upregulation

[12, 13]. Lck that is attached to ITAM-bound Zap70 forces the coreceptors CD4 or CD8 to surround the engaged SMAC (Fig. 13.3). The *peripheral SMAC* is a ring of integrin LFA-1 molecules. LFA-1-mediated adhesion of the T cell to the APC acts as an adjunctive signal to those created by the TCR/CD28 molecules [14].

Lck controls the activation threshold of naïve T cells [6]. There is positive-feedback regulation of Lck via the action of extracellular signal-related kinase (ERK) and negative regulation by phosphatase SHP-1. Fyn phosphorylates different adaptor molecules like SLAP (SLP76-associated protein), ADAP (adhesion- and degranulation-promoting adaptor protein) and PAG (cf. Fig. 13.4).

Critical signalling molecules like Itk (IL-2-inducible tyrosine kinase), PLC γ 1 and Vav depend on interaction of their pleckstrin homology (PH) domain modules with phosphatidyl-inositol (PtdIns) lipids. Itk is a Tec kinase [3]. Tec helps polarisation of surface integrins. Vav1 GEF helps (a) TCR-induced Ca²⁺ influx; (b) activation of ERK MAPK and nuclear factor kappa B (NF- κ B); (c) organisation of the actin cytoskeleton, clustering of TCRs and polarisation of T cells, and similarly (d) the inside-out activation of LFA-1 integrin. From the point of view of actin assembly at the immune synapse, note that Vav determines Rac1 induction of Arp2/3, and

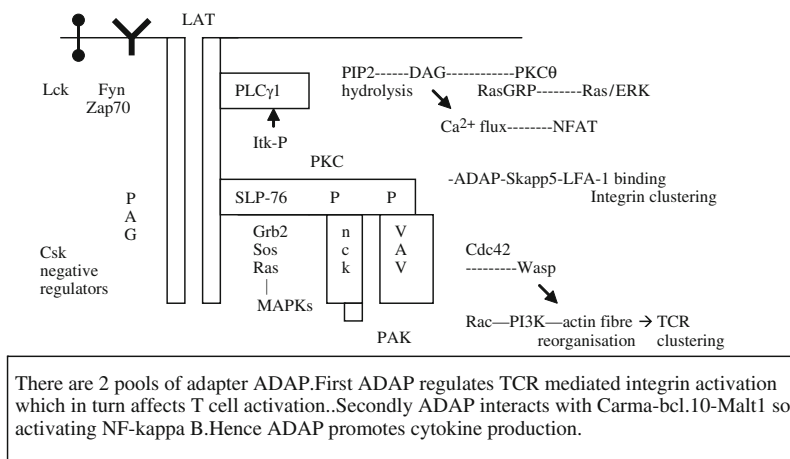


Fig. 13.4 T cell activation and adaptor proteins. There are two pools of adapter adhesion- and degranulation-promoting adaptor protein (ADAPs). First, ADAP regulates T cell receptor (TCR)-mediated integrin activation, which in turn affects T cell activation. Second, ADAP interacts with Carma-bcl-10-Malt1, activating nuclear factor kappa B (NF- κ B). Hence, ADAP promotes cytokine production. There is processive phosphorylation of the adaptor Linker of Activated T cells (LAT), first by Zap70. Recruited interleukin-2-inducible tyrosine kinase (Itk) activates phospholipase C γ 1 (PLC γ 1) by phosphorylation. Note the importance of Ras for extracellular signal-related kinase (ERK)/mitogen-activated protein (MAP) kinase activation. In the standard way, Shc/growth factor receptor-bound protein 2/Son of Sevenless (Shc/Grb2/Sos) will lead to Ras activation. In addition, Ras can be stimulated by RasGRP at the instigation of Itk Tec kinase. Adaptor SLP76, Vav, Itk and PLC γ 1 work together in a complex. Ras activation via exchange factor RasGRP occurs in response to the protein kinase C activator called PMA [4]. Protein associated with GEMS (PAG)

cdc42 also induces Arp2/3 and so F-actin polymerisation [15]. With respect to the contribution of the CD28 costimulator, CD28 connects to the adapter SLP76, which then recruits Vav. So, there can be Ca^{2+} ion influx and formation of IL-2. Vav induction of p38 MAPK allows proinflammatory cytokine production.

13.1.2 Adaptor Proteins

Adaptors like LAT and SLP76 are polypeptides with specific modules but no kinase or phosphatase activity, which organise the signalling networks.

Observe that LAT is a key scaffold molecule, around which a complex of signal proteins, the signalosome, is organised. The phosphorylated LAT serves for docking of the SH2 domains of PLC γ , PI3K, and more. After a T cell is activated by a DC, PI3K is induced for some 9 h. There is a transient peak of PI3K followed by prolonged activation that helps IL-2-induced proliferation. There is LAT within lipid rafts. LAT is essential for Syk/Zap70 recruitment of PLC γ , for Grb2-associated Sos activation of the Ras-MAPK, and for Gads-SLP76 adaptor interaction with Vav1. The kinase Itk (Fig. 13.4) connects the TCR-CD28 trigger to Ca^{2+} ion influx [16] since it activates PLC γ 1. Itk promotes Th2 cells. The adaptor molecule SLP76, working with activated PLC γ 1, recruits PKC θ to the immune system [17], and this will aid NF- κ B activation. The CD28 co-stimulator of the TCR ensures that SLP76 is connected to Vav, and it with Rac, so there is cytoskeletal reorganisation. Vav is a guanine-nucleotide exchange factor (GEF) that activates PI3K. Vav1 attached to the LAT activates the sequence Grb2-Sos-Ras $\rightarrow$ MAPK. Vav activates PLC γ 1 to generate diacylglycerol (DAG), and it recruits RasGRP1. As shown in Fig. 13.4, Vav activation will lead to actin fibre reorganization, which helps clustering of the TCRs. In fact, via the adaptors LAT and SLP76, activation of the TCRs recruits Nck and WASP (Wiskott-Aldrich syndrome protein), and initially they colocalise with Zap70 [18]. On the other hand, adaptor SLP76 via adaptor ADAP leads to LFA-1 integrin clustering, which helps to bind T lymphocytes to DCs by the peripheral SMAC. Furthermore, T cell activation is helped by β -integrins, which are upregulated when Zap70 activates LAT/SLP76 [19, 20a]. Itk couples PI3K and LCK to downstream activation of integrins [20b] and Raps is a key regulator.

Another adaptor, PAG, activates the cytoplasmic Csk (C-terminal Src kinase), which is a critical negative regulator of Src family kinases. Accordingly, it helps to set a threshold for TCR activation. Protein kinase A phosphorylates and activates PAG-bound Csk, and it stops signalling through the TCR [17].

To complete the picture of T cell activation, one will know that PLC γ 1 hydrolyses membrane PI, leading to early Ca^{2+} ion influx followed by a sustained influx of Ca^{2+} i. Calcineurin phosphatase is the downstream effector of the Ca^{2+} signal that leads to activation of NFAT, so that the promoter for IL-2 is induced co-incident with binding to that promoter of p65/p50 NF- κ B and activator protein 1 (AP-1) (c-fos/c-jun (Fig. 13.5)). So, there is RNA polymerase II-directed activity.

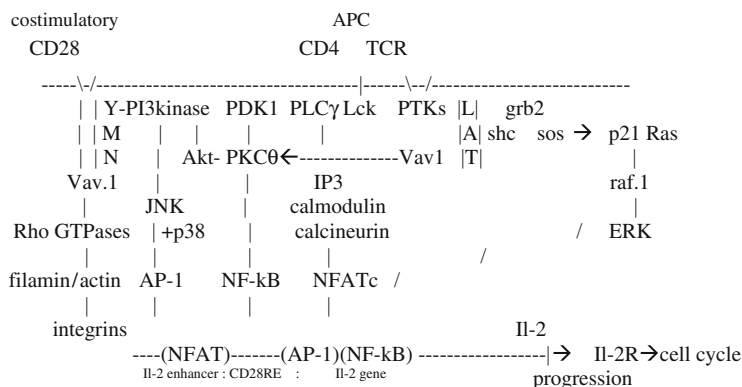


Fig. 13.5 T cell costimulation leading to T cell proliferation. CD28 costimulator is vital for the activation of naïve T cells. CD28 orchestrates Lck movement into lipid rafts and T cell receptor (TCR) activation [25]. CD28 → Vav1 supports the SLP76-Vav-interleukin-2-inducible tyrosine kinase (*Itk*) signalosome [26]. CD28 costimulation acts by stopping the nuclear export of NFATc proteins [27]. Protein kinase Cθ (*PKCθ*) is the only PKC isoform to translocate to the cSMAC. Interleukin-1 receptor-associated kinase 4 (*IRAK4*) phosphorylates the *PKCθ*. Note how nuclear factor of activated T cells (*NFAT*), nuclear factor kappa B (*NF-κB*) (p50, p65), activator protein 1 (*AP-1*) (*c-fos/c-jun*) determine production of cytokine interleukin 2 (*IL-2*). Combinations of these transcription factors (*TFs*) are generally in control at promoters for the cytokines. CD28 costimulation can occur independently of PI3 kinase via adapter SLP76 and Vav induction of NF-κB [28]. Transforming growth factor-β (*TGFβ*) blocks IL-2 transcription via inhibition of an octamer-binding enhancer. p38 mitogen-activated protein kinase (*MAPK*) features in **Fig. 13.5**. When p38 MAPK inhibitors are used, there is a dramatic increase of IL-2 and upregulation of extracellular signal-related kinase (*ERK*) 1/2. Hence, p38 restricts the output of IL-2 [29]. The output of IL-2 is intended to be brief. So, IL-2 is tightly regulated by other γ-cytokines acting through the signal transducer and activator of transcription 5 (*STAT5*) supported by Stats 6 and 4 [30]

Activation of p21 Ras leads to MAPK activation, which supports NFAT [21]. *PKCζ* phosphorylates the transactivation domain of NFAT. *PKCθ*, together with Akt, affects NF-κB signalling in TCR/CD28-stimulated cells. There are Toll-like receptors (TLRs) 3/5/9 on T cells that help their survival [22a] and RAGE is upregulated to help boost IL-2 and IFN-γ formation [22b].

13.1.3 CD28 Costimulation

CD28 regulates the threshold for naïve T cell activation but does not affect memory cells or T effector lymphocytes. A CD28 signal acting on PI3K and thus Akt ensures glycolysis that maintains ATP levels [23]. Aerobic glycolysis in mitogen-stimulated T lymphocytes increases lactate production 40-fold. Antigen stimulation of TCRs leads to cell proliferation by IL-2-dependent and -independent pathways. TCR stimulation induces the first two thirds of G1 of the cell cycle, whilst CD28 support of the IL-2 activation promotes the S-phase of DNA synthesis. CD28 helps

cell cycle progression by downregulating the inhibitor p27^{kip-1} [24a]. Costimulation also induces phosphorylation/activation of cofilin, enabling actin formation (Fig. 13.5). Costimulation arises from a unique compartment in the SMAC (Fig. 13.3) where CD28 and PKC conjugate [24b].

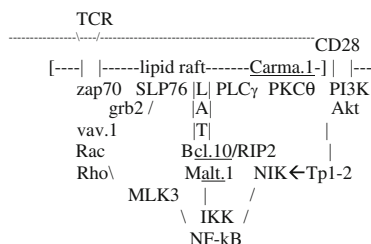
Two distinct pathways are involved in CD28 regulation of the transcription of IL-2: Vav1-actin cytoskeleton-receptor clustering path and a CD28-PI3K-Akt-GSK3 (glycogen synthase kinase 3) path. CD28 via PI3K recruits PKC θ to the cSMAC, promoting NF- κ B activation and IL-2 gene transcription [31]. Then, there is a PI3K-independent pathway that leads to IL-2 mRNA stability [31]. TCR stimulation also leads to NFATc activation of the IL-2 promoter (Fig. 13.5). Now, we also appreciate that CD28 increases the level of c-fos of the AP-1 transactivator that binds to the IL-2 gene enhancer. Activated p300/CBP (Fig. 4.8) is recruited to the Fos promoter; thus, there is histone H4 hyperacetylation [32a]. The immunosuppressive drug azathioprine converts to 6thio-guanosine triphosphate, which acts by inhibiting CD28 co-stimulation. Fatty acids EPA and DHA are known to displace IL-2R from lipid rafts so accounting for suppression by fish oils [32b].

CD28 activation of PI3K creates PtdIns products that activate Rho GTPases called Rho-A, Rac1 and cdc42, so that there is reorganization of actin [33a, 33b, 34] for creation of the immune synapse. The GEF activity of Vav1 has a key role in this [35, 36]. Now, we know that Vav is upstream of PI3K [37]. Vav is phosphorylated by Lck and Syk and LAT from the CD28. Vav then activates PLC, PI3K, ERK, NF- κ B [38] and Rac GTPase. Rac helps to activate PI3K, PAK (p21-activated kinase) and the p38 and JNK cascades. PAK may mediate interplay between CD3 and CD28 signals. The adaptor protein Nck recruits PAK and WASp to the synapse for the formation of actin [39]. Vav1 also controls the expression of integrins.

Various costimulatory molecules aid T cell activation. CD28 works on naïve T cells. LFA-1 is integral to the function of the SMAC [14]. Natural killer (NK) cell receptors (Chap. 15) like KIR (Killer Ig-like Receptor) and NKG2D are functional on T cells, and they induce JNK activation. 4-1BB works on activated T cells, both CD8 and CD4. When EphB6 molecules are cross-linked, stimulation of p38 MAPK releases many cytokines. Ephs facilitate T cell activation and proliferation [40]. ICOS (inducible costimulator) enhances CD4 and CD8 T cell activation, and it induces Th1/Th2 cytokines, especially Th2. Whenever B7H molecules on DCs contact ICOS, the ERK, JNK and p38 MAPK pathways are all induced, and the Th2 cells form IL-4 and IL-10 [41]. IL-12, acting with IL-23, leads to ICOS expression on both Th1 and Th2 cells. ICOS enhances Th2 cytokine expression. ICOS also stimulates for Treg cells. The co-stimulator OX40 (CD134) appears late after B7-CD28 interaction, and then it stimulates protein kinase B; thus, antiapoptotic proteins are formed that ensure T cell longevity [42].

To proliferate, T cells require two sequential signals. First, antigenic stimulation of the TCR/CD3 complex drives cells from the quiescent Go to G1 phase of the cell cycle (Fig. 13.6). Second, the binding of IL-2 to its high-affinity receptor causes G1 to S-phase transition and so proliferation. Strong TCR signals lead to expression of both IL-2 receptor (IL2-R) and IL-2. Weak TCR signals lead only to expression of IL-2Rs but not any IL-2 formation. Vitamin A, or its metabolite all-trans retinoic acid, stimulates IL-2-induced signalling in a JAK-dependent manner, enhancing

Fig. 13.6 T cell receptor (*TCR*)/CD28-induced acting via CBM complex (CARM1/BC/10/Matt-1) NF- κ B activation



cyclin D3 expression and hence promoting cell proliferation [43]. One will find in the context of T cell anergy that there are repressors of the IL-2 promoter. Transforming growth factor- β (TGF β)-induced Smad3/4 does this, and that action is helped by Tob [44].

13.1.4 NF- κ B Activation and T Cell Co-stimulation

TCR-induced recruitment of the I-kappa B kinase complex into lipid rafts leads to NF- κ B activation [45, 46]. NF- κ B is required for the differentiation of Th1 lymphocytes [47] and their clonal expansion. CD28 coengagement can signal to NF- κ B via two different pathways. First, PI3K-Akt-PKC θ phosphorylates Bcl-10, which activates NF- κ B. Second, via adapter Grb2 $\rightarrow$ Vav, there is activation of IKK α and so NF- κ B. When CD28 costimulation activates NF- κ B, AP-1 and the CD28RE (CD28 response element), those cells are then resistant to apoptosis. NF- κ B promotes the antiapoptotic Bcl-2, and of course, CD95 (Fas) signalling is impaired [48].

There could be at least four signal pathways to NF- κ B activation [49]. More is now known about the upstream kinases that activate NF- κ B [50]:

1. NF- κ B1. The adaptor SLP76 activates Vav1, a GEF for the GTPases Rac/Rho, activating mixed lineage kinase 3 (MLK3), which is an MAP3K (MAP kinase kinase kinase) for NF- κ B [51].
2. CARMA1, a member of the membrane-associated guanylate kinase (MAGUK) scaffold proteins is associated with lipid rafts [52]. It is stimulated by PKC θ , and it passes the impulse to Bcl-10, which through the Malt1 paracaspase activates the IKK complex and thus NF- κ B. So, there is a Carma1 “signalosome”: the CBM complex [53]. PKC θ phosphorylates three critical serines (Ser564, 649, 657) on the Carma. Oligomerisation of CARMA1/Bcl-10/MALT1 is sufficient for downstream IKK activation [54]. The CBM also activates JNK2.
3. PKC θ activates Akt, and so Cot kinase (Tpl-2) and thereby NIK (NF- κ B-inducing kinase) and NF- κ B [47]. Pim kinase activates Tpl-2 and thereby NF- κ B in a similar manner [47]. PKC θ is critical for development of Th2 cells but not for Th1 cells [55].
4. When Toll-like receptors are involved, RIP2 leads to NF- κ B activation.

There are controls. A20, known for its ability to ubiquitinate and so to stop TNF α activation of NF- κ B [56], also stops TLR activation of NF- κ B. Negative signalling function of 120 involves deconjugation of K63-linked ubiquitin chains from TRAF6 and RIP1. ZAS3 represses nuclear translocation of the p65 [57]. There can be downregulation of NF- κ B by Bcl-10 [58] and by IL-10. For therapy, either adenosine or cAMP and its analogues will, via protein kinase A stimulation, lead to inhibition of NF- κ B [59].

13.2 Control of Lymphocyte Activation by Phosphatases

There are more genes that control PTPs than there are for the PTKs [60]. In PTP knockout mice, disabling phenotypes involving the immune system are observed. The lymphoid tyrosine phosphatase PTPN22 on human chromosome 1p13 suppresses TCR signalling during thymic development. Otherwise, there can be survival of autoreactive T cells. Actually, PTPN22 is relevant to the onset of type 1 diabetes [61]. PTPs are heavily glycosylated. Generally, PTPs are sited at specific cell locations, as on protein complexes.

Some 30 phosphatases [62, 63] in differing cell compartments control T cell signalling. PTPs are activated by serine/threonine protein kinases since their regulation is by reversible tyrosine phosphorylation at several sites. Note the sensitivity of the PTP catalytic cysteine residue to reversible oxidation [64]. Consider that Akt in T lymphocytes is controlled by CTLA4 acting via the phosphatase PP2A. SHP-2 with PD1, CTLA4, BTLA-1 (B and T lymphocyte activator 1), CEACAM1 or CD31 aids negative control of T lymphocytes. In Th2 cells, SHP-1 is inhibitory for IL-4 signalling, and in Th1 cells SHP-1 controls differentiation and function, possibly by affecting the controls for IL-12.

The common leucocyte antigen CD45 is a positive regulator of antigen and Ig receptor signalling by its dephosphorylation of the C-terminal negative regulatory tyrosine residue of Src-family PTKs p56 Lck and p59 Fyn. CD45 acts as a rheostat for the threshold of T cell activation [65], being required for TCR-induced phosphorylation of cellular proteins, for the activation of PLC γ and thus for Ca²⁺ mobilisation. By removing phosphate from the Tyr505 of Lck, CD45 activates it. On account of its size, CD45 lies outside the lipid rafts and can only encounter Lck at the boundaries. It is excluded from the TCR microclusters, but nevertheless it is present to a degree in cSMACs, where it will help inactivate the receptor proximal tyrosine kinases [66]. CD45 isoforms regulate the coreceptor molecules (Fig. 13.1) CD4 and CD8 [67]. CD45 can suppress the PAK kinases [62] and thus the functioning of cytokine receptors. CD45 modulates the activity of the Csk inhibitory PTK [68]. Oligosaccharide clustering of CD45 facilitates galectin-1-induced T cell death [69].

Cytosolic PTPases like SHP-1 [70], SHP-2 [71] and SHIP [72] act to terminate signals from the TCRs. SHP-1 dephosphorylates Zap70, dampening signalling [73]. The moth-eaten mouse, which lacks SHP-1, shows inflammation in many organs [74]. Yet, PEP is a more powerful negative regulator than SHP-1 [75].

Similar SHP phosphatases downregulate vital signals when KIRs of NK cells, signal regulatory proteins (SIRPs) [76] or paired immunoglobulin-like receptors (PIRs) [77] operate on myeloid cells and DCs. Another myeloid inhibitory receptor that is linked to SHP-1 is IREM-1 [78]. The CD28 costimulator is controlled by MKP6 phosphatase [79]. The CD148 phosphatase inhibits Zap70 and controls the MAPKs [80]. PTPH1 inhibits TCR signalling by dephosphorylation of ITAM residues in CD3 ζ and by dephosphorylation of Lck [81]. TCR ζ downregulation in chronic inflammation is actually mediated by myeloid suppressor cells [82].

13.3 Downregulation of T Lymphocyte Activation

Information on these various controls is still being assembled. PECAM-1 (CD31) can inhibit TCR transmission (Table 13.2) [110], especially in the particular situation when T cells are traversing vascular endothelium. PECAM-1 has ITIMs and recruits the phosphatase SHP-2. Galectin 1 interferes with the organization of the TCR contact site, the SMAC. A galectin has carbohydrate recognition domains (CRDs) that bind to α -galactosyl-containing glycoconjugates. If galectin 1 clusters CD45 molecules or glycoforms of the T cell mucin CD43, cell death ensues [116, 117]. TCR signalling is terminated after the receptors are endocytosed [118]. That occurs when CD2AP/CIN joins with locally phosphorylated Cbl. Zap70 is then destroyed. In fact, PKC θ is involved in downregulation of engaged TCRs, and PKC α downregulates nonengaged TCRs [119].

Adaptor molecule Cbl negatively regulates tyrosine kinases of T cells [120] like Syk activity. Cbl can dismantle immune synapses. So, cbl-b regulates the threshold of signalling in T cells. Cbl-b negatively regulates CD28 costimulation [121]. Cbl and cbl-b are E3 ubiquitin protein kinases that can destroy the p85 unit of PI3K, so that there is attenuation of the activation of Vav and Akt. Also, when CTLA4 ligates the TCRs, phosphatase PP2A will inactivate Akt [121]. Clearly, cbl-b is a key regulator of potential for autoimmunity. Elimination of cbl-b allows Vav activation. If cbl is bound by protein 14-3-3, its inhibitory potential is lost.

Csk is an inhibitory PTK that works with SHP-2 to inhibit T cell activation. Csk is driven by protein kinase A responding to cAMP. Csk is pulled into lipid rafts at the immune synapse by Cbp [122], a molecule that also links to the cytoskeleton and dismantles immune synapses [122]. Yet, at the time of CD28 co-stimulation, β -arrestin/PDE4 (phosphodiesterase 4) complexes (Fig. 1.2) are recruited into T cell lipid rafts, and they then attenuate Csk activation by protein kinase A.

Following antigen stimulation of T cells, there has to be control over the expanding T cell population and likewise of the T cell memory pool. This control is by means of the surface molecule LAG3 (lymphocyte activation gene 3, CD223), on adjacent lymphocytes, which are Tregs. LAG3 binds to MHC II molecules, and it is a negative competitor of CD4 [123]. Within the thymus, there is the CYLD enzyme that, by binding to and deubiquitinating Lck, regulates proximal TCR signalling [126]. Lack of negative regulator CYLD causes cylindromas turban tumor

Table 13.2 Downregulation of T lymphocyte activation

1. Signal events at the T cell receptor (TCR) are negated by phosphatases CD45 [83a], SHP-1 [83b], SHIP [84] or negative regulatory molecules C-terminal Src kinase (Csk) and the ubiquitin ligase c-Cbl that regulates tyrosine kinases [85]. Adaptor PAG activates Csk. Adaptor molecule Grb2-associated binder 2 (Gab2) inhibits PI3 kinase [86] and blocks NFAT and NF- κ B transcription. TCR stimulation of the LAT adaptor leads to phosphorylation of Dok2, which complexes with SHIP, and levels of PI(3,4,5)PtdIns are reduced [84]. LAB (non-T cell activation linker, NTAL) adaptor also downregulates [87]
2. Coligation of Notch 1 with the TCR/CD28 inhibits T cell activation, proliferation and cytokine production [88]. Notch can block (ERK) activation through induction of the (MAPK) phosphatase (MKP) [89]. However, Notch helps Th1/Th2 cell production
3. When there is antigen persistence, chronic inflammation or interferon- γ (IFN γ) drive, TCR ζ -chain downregulation curbs the T cell response [90]. Interleukin 15 (IL-15) negatively regulates proliferation and the activation of CD4 T cells [91]. Also, lymphocyte activation gene 3 (LAG3) regulates the expansion of activated effector and memory T cells via an influence on sphingolipid metabolism
4. In the late stages of T cell priming, CTLA4 inhibits TCR signals [92] and blocks CD28 activation [93, 94]. CTLA4 engages phosphatase SHP-2 and dephosphorylates the CD3 ζ -chains. CTLA4 inhibits integrin-mediated stimulation of T cells [95]. CTLA4 is expressed constitutively in Treg cells, which keep control over the CD4/CD8 T cells
5. T cell coinhibitory receptors (CTLA4, programmed death 1 [PD-1], B and T lymphocyte activator [BTLA]) are controlled by ligands on antigen-presenting cells (APCs) [96], namely, by CD80/86-CTLA4, B7-H1 or B7-DC:PD-1, B7H3/4:PD-1 [97–99]. Other inhibitory receptors for T cells are PD-1 and BTLA [100, 101]. They act by conscripting phosphatases [102]. PD-1 determines CD8 T cell exhaustion in response to chronic viral infection. Then, semaphorin 7A is a negative regulator of T cell responses [103]
6. In DP thymocytes an MAP4K called Mink is a negative regulator [104] that will stop autoreactivity. CaM (calmodulin) kinase II, acting with SHP-2, regulates thymocyte selection [105]
7. Socs1 (suppressor of cytokine signaling 1) curtails IL-2 signalling [106]. Tim1 on Th2 cells and Tim3 on Th1 cells downregulate their molecular counterpart [107]. Tim2 acts as a negative regulator downstream of phospholipase C (PLC) activation. It stops the activation of NFAT and (AP-1) [108]. It especially acts to control Th2 cell responses in the course of airway inflammation [109a]. Galectin 9, by engaging Tim3 on Th1 cells, leads to downregulation of IFN γ formation IAP CD47 (Fig. 9.11) down regulates Th1 cells [109b]
8. Galectin 1 or PECAM1 [110] promotes peripheral tolerance of T lymphocytes by inhibition of TCR signalling. IL-2 is suppressed. Galectin 1 mediates the action of Treg cells [111]
9. Regulatory T cells exert their suppression by direct contact, during which cAMP is released, and in part by release of (TGF β) or IL-10. When IL-10 acts, IL-10 receptor inhibits CD28 phosphorylation [112] and PI3 kinase activation. Many Tregs also inhibit dendritic cells [113]
10. By killer inhibitory receptors (KIRs) on CD8 cytotoxic T cells
11. Via the action of prostaglandin E 2 (PGE2) [114] or NO from local macrophages or by adenosine (Chap 3)
12. By other negative regulators like DAF complement protector, by caterpillar 16.2, by adaptor protein LAX and by (Egr2/3), early response genes directed by c-Cbl. Short isoform I.1 of hypoxia-inducing factor 1 α (HIF1 α) negatively regulates both CD4 and CD8 T cells [115]

syndrome. Of course, ceramide can inhibit IL-2 production by T cells by stopping PKC θ -induced activation of NF- κ B [125]. When T lymphocytes are activated, TRIM (T cell Receptor Interacting adaptor Molecule) binds to CTLA4 in the trans-Golgi, it reaches immune synapses in proportion to the strength of TCR stimulation [94, 126], and it enters the lipid rafts. There, CTLA4 binds to CD80/CD86 ligands on the DCs with higher affinity than the CD28 molecule does.

CTLA4 stops proliferation of T cells and stops cytokine production by stopping nuclear accumulation of AP-1, NFAT and NF- κ B (Fig. 13.2). CTLA4 opposes production of interferon- γ (IFN γ) and thus has an outstanding effect in curbing CD8 T cell proliferation [127]. When acting on DCs, CTLA4 upgrades their indole dioxygenase and thereby causes tryptophan catabolism, so that they are weakened. In fact, 2,3-indoleamine dioxygenase (IDO)-competent DC subsets of mice, set up by CTLA4-B7 engagement, then suppress T cells and block their ability to respond to DCs [128]. CTLA4 inhibits formation of IL-2 and the cell cycle progression of T cells [129, 130]. It mainly inhibits Th2 cells [131]. CTLA4 interacts with TCR ζ -chains, and it stops Zap70 phosphorylation and so proximal TCR signalling [92, 132] (Fig. 13.1). CTLA4 attracts the phosphatase SHP-2. CTLA4 blocks CD28-induced activation [94], and it inhibits LFA-1 integrin-induced effects [133]. CTLA4 action results in inhibition of NF- κ B. Indeed, it suppresses production of inducible nitric oxide synthase (iNOS) and the induction of IFN γ . CTLA4 can cause apoptosis of T lymphocytes or anergy of T cells [134]. It decreases ERK activity but maintains JNK activity [86]. It stops upregulation of the cell cycle protein cyclin D3 and the kinase cdk4. It also stops degradation of the p27^{kip.1} cell cycle inhibitor.

The details of CTLA4 action are being worked out by the use of CTLA4 transgenic mice [121]. By virtue of its properties, CTLA4 mitigates against the emergence of autoimmune diseases like diabetes, hypothyroidism and Grave's disease [135]. It contributes to antigen-specific downregulation of cellular responsiveness in infections like filariasis [136]. Polymorphisms of CTLA4 are now being related to susceptibility to autoimmune diseases like multiple sclerosis. Several cytokine regulators like IRAK1 (Fig. 12.5) are relevant to susceptibility to organ-specific immunity [137].

13.3.1 *The SLE CD4 T Lymphocyte*

T cells are altered in special ways in systemic lupus erythematosus (SLE). Extrinsic factors leading to loss of T cell tolerance mean that presentation of antigens resembling self can induce B lymphocyte responses. Intrinsic genetic factors, like lack of Fas, Cbl-b, CTLA4, SLe1 or RasGRP1, contribute to T cell dysregulation and autoimmunity [138]. A failure by SLE cells to produce IL-2 is the basis of a breakdown in tolerance. In SLE, antilymphocyte antibody is produced that is anti-TCR/CD3. Hence, there is activation of the Ca²⁺-calmodulin kinase IV cascade, causing downregulation of IL-2 transcription [139]. Moreover, there is elevation of the

phosphatase PP2A, which is a negative regulator of IL-2 production [140]. Since lack of IL-2 means that Forkhead transcription factor p3 (Foxp3) is reduced, in SLE patients the regulatory Treg cells are decreased.

Generally, there is formation of Th2 lymphocytes, which must arise by cross talk between TCR activation of Ras and promotion of the IL-4 pathway. The Ras molecular switch is aberrant [138]. Defective Ras $\rightarrow$ Erk signalling may account for inhibition of AP-1 (Fos/Jun). Since there is decreased CD3 ζ , T cell signalling is via the common γ -chain of the Fc receptor, FcR γ . There is a rapid Ca²⁺ influx but reduced PKC θ phosphorylation and reduced p65 NF- κ B nuclear translocation. Syk instead of Zap70 is increased, and T cell lipid rafts are already clustered [141]. In mice, loss of interferon regulatory factor 4 (IRF4)-binding protein, an activator of Rho GTPase at the immune synapse, creates an SLE syndrome [142]. This reminds one that in autoimmune mice macrophages typically have reduced Rho activity that affects their signalling [143].

13.4 Characteristics of Forms of T Lymphocyte

13.4.1 *Effector T Lymphocytes*

As shown in Fig. 13.1, naïve and resting T cells signal from the TCR via the CD3 ζ -chains that connect with Zap70. The activation of naïve T cells requires three signals: TCR triggering, CD28/B7 costimulation and cytokine binding. Antigen stimulation and costimulation must occur over 20 h. In contrast, effector T cell activation can occur within 1 h since there is reduced requirement for co-stimulation and more efficient triggering of Lck and Fyn. Effector T cells exhibit a lower level of TCR expression, and in them CD3 utilises the FcR γ subunit to activate Syk kinase [144]. On effector T cells, in distinction to naïve T cells, Lck is within abundant surface lipid rafts. Furthermore, costimulatory TNFR2 (Fig. 12.6) controls the accumulation and survival of effector T cells [145a].

CD4 effector T cells (types Th1, Th2 or Th17... see later) are concerned primarily with the quick mobilization and activation of other cells. They are helper cells. They differ from CD8 T cells that specifically clear pathogens. Curiously Blimp-1, a factor for plasma cell differentiation, has been identified as a promoter of T effector cells [145b].

13.4.2 *CD8 Cytotoxic T Lymphocytes*

CD8 cytotoxic T lymphocytes (CTLs) require antigen-specific TCR stimulation, CD28 co-stimulation, and IL-15 from DCs for their development and IL-7 for maintenance. The CD8 coreceptor molecules are tuned by a negative-feedback loop. PKC θ/δ are used. Lytic potential is developed via Vav, leading to MAPK/

ERK activation [146] and connection of receptor tyrosine kinases to RhoA-p160 Rock (Rho kinase), so that there will be organisation of actin [147] and the ability to discharge granzyme vesicles by a process involving calcineurin and PKC. In CD8 CTLs, a paralogue of T-bet called eomesodermin (Eomes) is vital for IFN γ formation and production of granzyme B and perforin. T-bet with Eomes determine expression of the perforin gene [148].

Recent work has shown that during activation of CD8 T cells, ROIs are generated and are associated with increased T cell cysteine sulfenic acid levels. The ROIs that are formed are in proportion to the antigen concentration. It has to be tightly controlled, but the process is clearly related to signal transduction [149]. Lytic granules are secretory lysosomes containing cathepsins B and D, perforin and serine esterases and LAMP1/2. As preparation for granule discharge [150, 151], there must be (a) PKC $\rightarrow$ ERK determined mobilization of the microtubule organising centre (MTOC) to the point of target cell contact, as indicated by Pyk2; (b) granule polarization; and (c) fusion of lytic granules with the CTL membrane using syntaxin11 and a Rab-binding protein [151]. IL-21 seems to support this process. Lysosomal cytotoxic granules have to fuse with endosomal exocytic vesicles assembled by exocytosis regulator hMunc 13-4. CD28 costimulation elicits large amounts of cytokines by CD4 T cells, such as IL-2 40x, IL-3 14x, IFN γ and GM-CSF 5x. However, CD28 costimulation yields lower levels in CD8 T cells, such as IL-2 5x, IL-3 2x and GM-CSF 2x [152] over resting cell levels.

The use of TNFRII (p75) as a costimulator enables survival of CD8 (or CD4) T cells during clonal expansion (cf. Fig. 12.6). TNFRII works with collagen receptor VLA1 to enable CD8 cell survival in the airways in the protection against viral infections [153a]. When CD8 T cells are anergic, an increase of their membrane CD5 is found to be a negative regulator of cell activation.

Granule exocytosis for CD8 T cell cytotoxicity entails release of perforin lytic molecules and granzymes and hydrolases, as determined by protein kinase C δ transduction of TCR signals [153b]. Death receptors of the TNF. R family including Fas.L protein are expressed on such CD8 CTLs. Hence in the target cell, Fas induces cleavage of enzyme I-CAD, inhibitor of caspase activated DNase, so that CAD will dissolve the target cell DNA.

13.4.3 Memory T Cells (CD4 and CD8)

When T cells encounter antigen, there is at first a vigorous clonal proliferation, especially of CD8 T cells that will have to combat virus-infected cells. Hyperproliferation is avoided by the p21 cell cycle inhibitor [154]. Soon, there is large-scale apoptosis, until only memory cells are left. Memory cells are maintained by continual low-level proliferation helped by the action of Bcl-6 [155]. CD8 memory cells appear to be sustained by IL-15 and IL-7. They have IL-7R α receptors, and they use PKC ζ . They are well endowed with surface receptors and so will soon reactivate when there is renewed contact with antigen. In memory CD4 cells, Zap70 is enhanced, explaining their ability to produce IFN γ [156]. An important

feature of memory CD8 T cells is their extensive lipid rafts, which means that they induce phosphorylation of LAT more efficiently, and thus they activate ERK, JNK and p38 MAPKs.

CD4+ memory cells are sustained by CD28 costimulatory pathway induction of IL-2 [157]. These cells do not require IL-7 and IL-15 or, remarkably IL-2, IL-4 or IFN λ . CD4 memory cells rely on Lck and p72 Syk phosphorylation. CD45 is more intense in the lipid rafts of their immune synapses [158]. Phosphorylation of the adapter molecule SLPS76 is deficient [159]. These memory cells do have sustained CTLA4 activity [160]. It appears that BTLA regulates CD8+ T cells homeostasis and the generation of memory cells [161].

CD8+ T cells responding to infection actually divide asymmetrically into an effector cell with IFN λ receptors, genes for IFN λ and granzyme B, and a memory cell showing IL-7R α 's [162]. Under the influence of proinflammatory cytokines [163–164], CD8+ T cells must undergo proliferate expansion at the onset of infection, and later there is a program of contraction, that requires Bim (Bcl-2 interacting mediator of cell death) and AICD (activation induced cell death) by extrinsic and intrinsic pathways (Chap 8). IL-15 is crucial for the maintenance of memory CD8+ T cells and they respond to IL-7, a survival factor that induces anti-apoptotic factors Bcl-2 and Bcl-xl. At their peak they are CD44 (adhesion molecule) high [165]. A differential requirement for Tec kinase Itk is involved in their production [166].

To maintain peripheral tolerance DC-derived IDO is required to cause tryptophan catabolism in CD8 memory T cells [167]. Furthermore during chronic infections IL-10 production limits memory CD8 T cell antiviral activity.

13.4.4 Th17 Inflammatory cells

The Th17 inflammatory cells herald a new era in appreciation of autoimmunity. Th17 cells producing IL-17 are promoted in mice by TGF β along with IL-6 [164, 165]. They are cells that express IL-21/23 receptors, CCR6 and the TF ROR γ t. They arise after TNF α priming of monocytes [166]. However, their formation is opposed by retinoic acid, which favours formation of T regulatory cells [167]. Th17 cells cannot be regulated by Treg cells.

13.4.5 T Cell Apoptosis

Once T cells are activated, they are liable to apoptosis unless they are responding to specific antigen. AICD (activation-induced cell death) occurs via Fas ligand–Fas receptor interaction (Fig. 8.3). TCR activation creates reactive oxygen species (ROS), which stimulate NF- κ B and so FasL expression. Ca²⁺ ion influx induces calcineurin $\rightarrow$ NFAT and that supports expression of FasL. For AICD to occur, restimulation via the TCRs is required [168]. By contrast ACAD (activated T cell

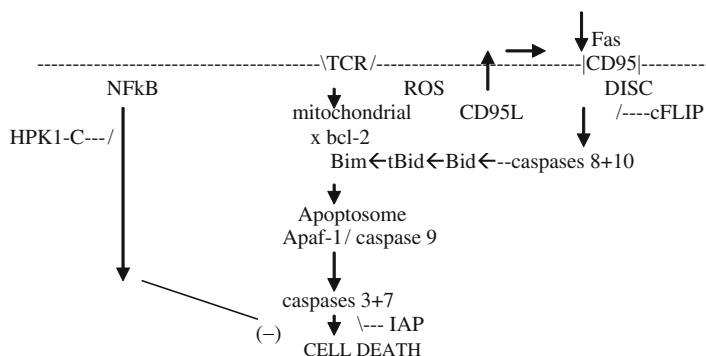


Fig. 13.7 Scheme for T cell apoptosis. T cell receptor (TCR) stimulation activates extrinsic CD95L (Fas) death pathway, and so caspases 8 and 10, and the apoptosome is formed. Apoptosis is opposed by the inhibitor of apoptosis (IAP) proteins. The disc is regulated by Fas-associated death domain-like interleukin 1 converting inhibitory protein (*c-FLIP*). NF-κB prevents apoptosis but is offset by hematopoietic progenitor kinase 1-C (*HPK1-C*) [173]

autonomous death) arises because TCR activation creates mitochondrial oxygen and thence H_2O_2 that suppresses nuclear CREB (Fig. 4.8), so that the level of Bcl-2 is lowered [169]. TCR restimulation is not required. It is the ratio of Bim versus Bcl-2 that determines T cell death [170]. CD44v7 integrin supports the survival of activated T cells by upregulating antiapoptotic genes. Thus, it offsets AICD [171]. NF-κB reduces the liability to apoptosis, but it will be suppressed by exhibition of HPK1-C (hematopoietic progenitor kinase 1) [172] (Fig. 13.7).

In the thymus, induction of a strong signal in response to self-antigen commits the thymocyte to apoptosis. Negative selection of DP thymocytes is a much-studied topic [174]. It is thought that different strengths of signal are translated to obtain Grb2-Sos activation of downstream MAPKs. The p38 and JNK MAPKs operate negative selection of thymocytes [174]. Sustained Lck activity and thus MAPK activation appear to explain the genesis of CD4 T cells, whilst transient low Lck activity and MAPK activity produces CD8 T cells [175].

Peripheral immune tolerance of T cells can be imposed by restimulation-induced cell death (RICD). In this process, Rac1 causes cytoskeletal reorganization and ERM (ezrin, radixin, moesin) protein dephosphorylation with the result that Fas molecules translocate into lipid rafts, and apoptosis can ensue.

Chronic exposure to $TNF\alpha$ attenuates TCR signalling, and it downmodulates T cell proliferative responses and cytokine secretion. TNF determines that autoreactive T cells will undergo apoptosis. $TNF\alpha$ is thus a potent means of terminating T cell responses.

13.4.6 Anergic CD4 T Cells

Anergic T cells are formed when there is stimulation via the TCR (signal 1) but no costimulation (signal 2) by CD28 or by ICOS [176]. Anergic T cells can also be induced by IL-10. These cells then suppress other T cells by a contact-dependent,

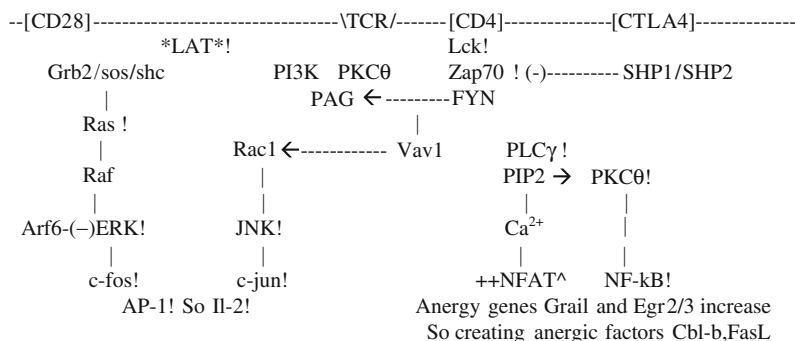


Fig. 13.8 Signal transduction defects in T cell anergy. ! signifies reduced; ^ signifies enhanced.
**A defective Linker of Activated T cells (LAT) scaffold

cytokine-independent mechanism. Essentially, there is a TCR proximal block (Fig. 13.8) that is reversible. If B7 of the APC does not bind CD28, there is poor signal transduction via Ras/MAPK; ERK is not activated, so c-fos is reduced. Hence, via B7 → CTLA4, the phosphatases SHP1/2 are recruited to the TCR and prevent phosphorylation of the CD3 ζ-chains and Zap70.

There appear to be different forms of anergic T cell [177], but actually “clonal anergy” is based on *in vitro* studies, and models of “adaptive tolerance” have been ascertained *in vivo* [178]. Clonal anergy reflects poor T cell activation due to a Ras → MAPK block. There is sustained Ca^{2+} influx, so NFAT without AP-1 induces anergy genes, and IL-2 transcription stops [179–181]. Ikaros gene will repress IL-2. Such anergy can arise from continuous antigenic stimulation of TCRs.

Alternatively, adaptive tolerance [177] is consequent on a low density of peptide-MHC complexes on the DCs [180]. There is defective coupling of TCRs to PLCγ1 and poor Ca^{2+} ion mobilisation [181] and poor IL-2 production, resulting in blocked cell proliferation. Ubiquitin E3 ligases cause degradation of PLCγ1 and PKCθ [182a]. The action of E3 ubiquitin ligase Cbl-b, which inhibits T cell activation, is determined by early growth response (Egr) genes Egr-2 and Egr-3 [183a] and TRAF6 [183b]! C-Cbl might also ubiquitinate and degrade TCR/CD3 and CD3ε chains [183c].

Importantly, all these features might result from impaired palmitoylation and function of the scaffold LAT [184]. Yet, downstream there is defective DAG-dependent recruitment of RasGRP1 to the immune synapse, and this is due to activity of DAG kinase resulting in conversion of DAG to phosphatidic acid [185]. Recall that there should be enough available DAG to activate PKCθ and protein kinase D (Fig. 7.1). So, clonal anergy results from defective Ras imposed by inhibitors of DAG that are expressed following T cell activation [178].

The small GTPase ARF6 (ADP ribosylation factor 6) is a marker of anergic T cells [186]. When expressed in Jurkat T cells, ARF6 prevents TCR activation of ERK1/2 and actin reorganization. Hence, it reduces IL-2 transcription. One must point out that cAMP-responsive CREB/CREM (cAMP response element modulator) operates also at the IL-2 promoter.

The E3 ubiquitin ligases are now recognised to regulate T cell signalling [182a]. Thus, GRAIL (gene related to anergy in lymphocytes) characterises anergic CD4 T cells. Grail together with cbl-b and Itch E3 ligase [182b] causes ubiquitination of PLC γ 1, PKC θ and Rho GDI (GDP-dissociation inhibitor) so that there is RhoA GTPase inhibition [178, 187]. Hence, there is aberrant recycling of TCRs and PKC θ to endosomes and so their lysosomal degradation [186, 187].

In Sect. 11.9 (Fig. 11.6), mTOR (mammalian target of rapamycin) was discussed. It is now appreciated that mTOR integrates signals and determines the outcome of TCR engagement: whether there should be cell activation or T cell anergy [188]. In fact, mTOR integrates environmental signals. The presence or absence of costimulation, the action of growth factors and the actions of inhibitory ligands and inhibitory cytokines could all determine the outcome of antigen recognition. Amongst the possible inhibitory agents is extracellular adenosine, which exists in the microenvironment under conditions of stress or inflammation. We have previously considered adenosine as an agent that will raise intracellular cAMP and thereby cause the suppression of immune cells.

Cells can be monitored by their ability to phosphorylate S6 kinase at Thr421 or Ser424. Also, the presence of CD71, the transferrin receptor, on cells indicates their ability to proliferate, helped by mTOR. Rapamycin creates anergic T cells, and it is known to inhibit mTOR. Should T cells be presented with signal 1 but not the costimulatory signal 2 from the APCs, tolerance (anergy) occurs, and then mTOR is inactive [188]. If anergy is reversed, by application of IL-7, IL-2 or other growth factor, then mTOR is activated.

An aspect that has been elucidated recently is that the adaptor PAG is associated with Fyn in unstimulated T cells. Fyn phosphorylates several negative regulators of T cell signalling such as PAG. CD8 T cells that are committed to proliferation and cytokine production use the Src family kinase Lck, but that is restrained by Fyn [189]. Furthermore, as indicated in Fig. 13.4, PAG-associated Fyn regulates calcium signalling and promotes anergy in T lymphocytes [190].

13.4.6.1 T Cell Tolerance

As just explained, T cells can become unresponsive after encountering antigen [191–192] in the absence of signals from CD28 and when IL-2 is lacking. The preceding section dealt with several mechanisms for T cell anergy. Self-antigens obviously do account for peripheral clonal deletion of T cells. Sometimes, a peripheral T cell is tolerant since it never receives signal 1 (the antigen), and this represents immunological ignorance.

In tolerant T cells, there might be (a) defective activation of Lck and Zap70, (b) activation of Src kinase p59^{fyn}, (c) reduced activation of Ras and ERK, or (d) decreased binding of AP-1 to the IL-2 promoter. Recent work by Li et al. [193] revealed that tolerated T cells have impaired cdk2 and cdc2 kinase activity (see Chap. 17) with consequent failure to phosphorylate Smad3; accordingly, there is increased cell cycle inhibitor p15 expression. In fact, Smad3 knockdown prevents induction of T cell tolerance.

13.4.7 *Quiescence of T Cells*

Quiescence of T cells is an actively maintained unique metabolic situation. Naïve T cells, maintained by IL-7 and IL-4, and memory T cells, using IL-7 and IL-15, are quiescent. Binding of IL-7 or IL-4 increases cell surface transporters like Glut1, and fatty acids and amino acids are used to generate ATP in mitochondria.

IL-7 induces TFs like lung Kruppel-like factor (KLF) [194], Tob1 and the Forkheads Foxo3A and Foxj1. KLF action can arise from ERK1/2 inhibition by D4 dopamine receptors [195]. KLF promotes cell cycle inhibitors p21 and p27, and it inhibits cyclin D and c-Myc [194]. KLF overexpression reduces c-Myc levels by 90%, and thus cdc25a phosphatase for activation of cdk2 is suppressed, and there is expression of GADD45 (growth arrest and DNA damage-inducible gene). Tob [196], an antiproliferative APRO gene, contributes. It accounts for reduced formation of cytokines like IL-2. Tob is associated with Smads 2/4 and enhanced Smad DNA binding. Socs3 also confers T cell quiescence [197].

KLF2 is also implicated in maintaining quiescence of endothelial cells. KLF induces Smad7 and therefore thwarts the action of TGF β in its induction of PAI-1 and thrombospondin 1 [198].

13.4.8 *Suppression of T Cells*

Study of lymphocyte quiescence is new. TGF β will play a role since it inhibits the proliferation of CD4 T cells, and it suppresses expression of Fc γ R receptors. Reduced expression of TGF β has been demonstrated to exacerbate experimental asthma [199]. Actually, at sites of chronic inflammation, T cells that express receptors for TGF β are reduced [200]. Conversely, the arrival of IL-10 promotes cells that are expressing TGF β receptors [200].

There is another means by which adjusted T cells stop an excessive inflammatory response. This is the downregulation of the TCR ζ -chains [201]. This occurs in infectious diseases like HIV and leprosy, in autoimmune conditions like SLE or rheumatoid arthritis, and in response to various cancers. So, the ζ -chain seems to be a sensor of sustained exposure to a chronic immune response. The ζ -chain mRNA levels are not affected adversely. Rather, there is a posttranslational mechanism that targets the ζ -chains for lysosomal degradation [201]. By now, various means of suppressing T cells are recognised. It will take time to establish which of these are of physiological relevance. By focussing on specific surface antigens like neuropilin, one can disorganise lipid rafts and thus inhibit T cell activation [202]. Of course, there are standard immunosuppressive agents (Sect. 12.8) that suppress cytokines. There has been much work on peptide ligands for TCR clefts. Low-affinity peptide ligands create Th1 lymphocytes, whereas high-affinity ligands induce Th2 cells. Altered peptide ligands could be used to thwart autoimmunity [203]. Recombinant TCR ligand can activate the NFAT of T cells, uncoupled from the NF- κ B, and CD4 T cells are then inhibited [204]. When IDO is induced in DCs, lowering their tryptophan content, those DCs

will suppress T cells [129, 205]. It is known that CTLA4-expressing T cells, particularly CTLA4⁺ Treg cells, induce IDO in DCs [205, 206].

13.4.9 T Regulatory Lymphocytes (*nTreg*, *iTreg*, *Tr-1*)

The regulatory T cells (Tr) are a network of diverse cells. There are natural killer T cells (NKTs) arising from the thymus. The principal CD4⁺ CD25⁺ regulatory lymphocytes also arise in the thymus, and they give rise to dedicated peripheral CD4 or CD8 Treg cells. Generally, thymus-derived nTreg cells work by means of cell–cell contact and not via cytokines. Then, there are peripheral inducible (iTreg) cells that are formed in the presence of TGFβ and IL-2 and have a high level of IL-2R expression; they express Foxp3 and high folate receptor 4. They act by release of TGFβ [207, 208] or by release of prostaglandin E2 (PGE2) or by transcellular transfer of cAMP, which suppress effector T cells [209], and via induction of IDO in APCs. DCs exposed to glucocorticoids that exhibit Gilz induce Treg cells [210]. Peroxisome proliferator-activated receptor-γ (PPARγ) agonists like ciglitazone induce Tregs [211]. Tr-1 cells produce IL-10. Tregs use granzyme B and perforin to kill other cells [212]. They use galectin 1 [213], and they use adenosine and cAMP to suppress Th1 lymphocytes [214, 215]. IL-21 subverts their action [216], and cytokine IL-27 thwarts their development [217], as does OX40 [218].

Estrogens help to expand Treg cells [219]. IL-2 is required for their survival [220]. Formation of Treg also requires IFNγ [221]. Yet, TNFα, acting through TNFRII receptors, will downgrade unstimulated Tregs [222]. The need for IL-2 is because the Treg themselves have defects in calcium mobilization and in activation of PLCγ and in the downstream pathways required to support synthesis of IL-2, which can actually be overcome by support of DAG metabolism [223] or by activating them with PMA and ionomycin. Treg have undetectable SLP76; thus, they cannot activate Vav and arrange actin. The pathway from Akt to mTOR is also unresponsive. Clearly, Treg are unresponsive to stimuli that induce IL-2; thus, they have a hypoproliferative anergic phenotype in vitro [224, 225]. All this is explained by the nuclear protein Foxp3, which blocks the pathways to NFAT, AP-1 and NF-κB activation on the IL-2 promoter (Fig. 13.5) [226a], so Treg do not produce IL-2. ICOS⁺ Foxp3⁺ Tregs use IL-10 to suppress dendritic cells, whilst ICOS[−] Foxp3⁺ Tregs use TGFβ [226b] cells designated Tr1 do not express FOXP3.

There are Treg that express programmed death ligand (PD-L1) so that they can control DCs or T cells that have PD receptors. Indirect suppression is when a Treg inhibits NF-κB-mediated expression of CD86 by the DCs [227]. Those DCs display B7-H3 inhibitory molecule [228]. Treg can also cause DCs to secrete nitric oxide (NO); accordingly, T lymphocytes are suppressed [229]. CTLA4 of Treg will also downregulate B7 costimulatory molecules on DCs [230]. They cause death of neutrophils [231].

The thymic-derived nTreg cells, which work by cell–cell contact, express Foxp3 [232] and high levels of CD25, GITR (glucocorticoid-induced TNF receptor), CTLA4, CD103 and even LAG3 [233]. There are even Treg that are CD25[−] [234],

or are they precursor cells? Thrombospondin has been shown to convert CD25⁻ cells to Treg [235]. Mast cells aid formation of Tregs [236]. Trs inhibit IL-2 production in target cells [237], and they inhibit PKC θ recruitment to the synapse of naïve T cells [238].

Treg utilise the surface phosphatase CTLA4 [95], and this suppresses their ERK kinases, albeit JNK is upregulated. TGF β -mediated suppression of other cells by Treg is facilitated by this CTLA4 signalling [239]. As mentioned, CTLA4 induces tryptophan catabolism in DCs [240]. GITR are not specific for Treg, but they help Treg proliferation [241], and stimulation of the GITR produces higher amounts of IL-10 [242]. Treg induction of DCs releases autocrine IL-10, which downregulates DC molecules B7-H4 [243].

Foxp3 is uniquely restricted to thymic $\alpha\beta$ T cells with suppressor activity and peripheral Tr cells. Foxp3 is not an initiator of development but works with NFAT to maintain Treg cells [244]. It is a repressor [245] that binds to the ARE2 (adenine- and uridine-rich element s) site in the promoter for IL-2, and there it modifies histone acetylation. Accordingly, Foxp3 represses the transcriptional activity of NFATc-AP-1 complexes at the IL-2 promoter [246]. Those Tregs that secrete IL-10 do not express Foxp3 [247] thus they are Tr1 cells. However, CD4⁺ CD25⁻ T cells are converted to Tregs when there is TGF β induction of Foxp3 [248a]. Inhibitory cytokine IL-35 is required for full Treg suppressive activity [248b].

13.4.9.1 CD8 Suppressor T Cells

CD8 suppresor T cells have the profile CD8⁺ CD57⁺ CD28⁻, but the function of CD57 (HNK-1, Lev7) (found also on NK cells) is not known. They secrete a Th0 broad set of cytokines, including TGF β , but not IL-10. Their action is not on lymphocytes but directed at APCs. Suppressor cells upregulate ILT3/4 on DCs, rendering them tolerogenic [248c].

13.5 Th1 and Th2 Lymphocytes

T cell precursors (Tps) can become uncommitted Th0 helper cells that release a broad spectrum of cytokines. Alternatively, under DC direction, they might become Th1 cells that produce IL-12 and IFN γ and thus be cells for cell-mediated immunity, or they become Th2 cells producing ILs -4, -5, -6 -13, as in allergic reactions. One should refer to immunology texts for details [249–251]. As shown in Fig. 13.9, the specific cytokines, surface receptors and TFs [250, 251] determine whether development occurs as Th1 or Th2 cells. In a normal response, both types can appear along with Th0 cells. CD4 helper T cells can be uncommitted Th0 cells that form a wide range of cytokines from IFN γ and IL-2 to IL-4 and IL-10. Yet, in certain pathologies the response is skewed towards Th1 or Th2 lymphocytes.

To form Th1 cells, IL-23/-27 act on the TCCR receptors [252, 253]. Thereafter, IL-12 acts via Stat4 to induce formation of IFN γ [250]. The TF T-bet is essential for

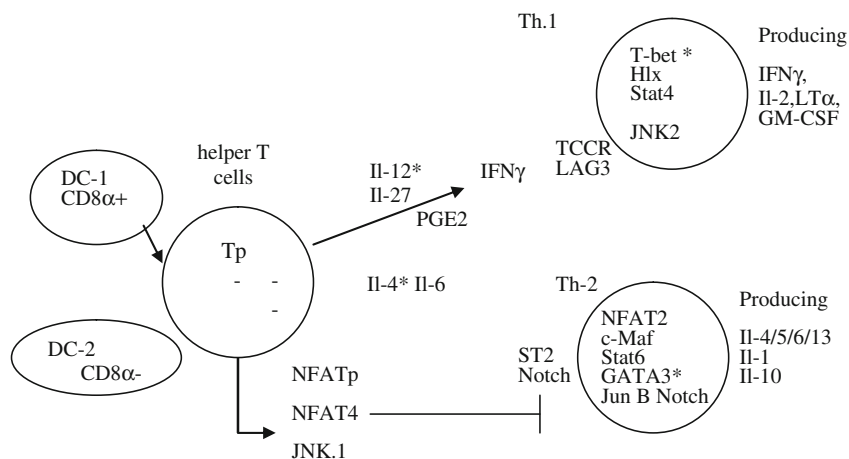


Fig. 13.9 Transcription factors for Th1/Th2 cell development. *DC* dendritic antigen-presenting cells. $CD8\alpha^{+/+}$ refers to DC types found in the spleens of mice. ST2 is the IL-1R like receptor IL-33 on Th2 cells. Note that (a) IL-12 induces signal transducer and activator of transcription 4 (*Stat4*). Interferon- γ (*IFN* γ) induces Stat1 and T-bet [272]. IL-4 induces Stat6. (b) IL-27 induces Th1 cells via p38 mitogen-activating protein kinase (*MAPK*)/T-bet and (*ICAM-1/LFA-1/ERK1/2*) pathways [273]. (c) T-bet is required for selectin ligand expression in Th1 cells [274]. T-bet is encoded by coactivators of the Mastermind-like family (*MAML*), that is, by the complex Notch ICD-MAML-RBPJK/CSL [274], and has Sp1 transcription factor-binding sites. In antigen-stimulated Th1 cells, T-bet, *NFAT* and (*AP-1*) cooperate to activate expression of *IFN* γ [274] and inducible IL-12R β 2 receptors. Notch increases nuclear factor kappa B (*NF- κ B*) and secretion of *IFN* γ [275]. (d) Epigenetic influences determine the IL-4/5/13 loci (Fig. 4.13) [276–278]. Notch may also regulate IL-4 expression via RBPJK/CSL binding sites in a downstream conserved noncoding region 2 (*CNS-2*) enhancer. (e) Oxidative stress veers T cell differentiation toward Th2 cells [279]. (f) In murine cells, nitric oxide (*NO*) promotes Th1 cell development. (g) Prostaglandin E2 (*PGE2*) from dendritic cells acting on EP1 receptors develops Th1 cells [280]. CRTH2, chemoattractant receptor for PGD2 is for Th2 cell development.

the development of Th1 cells [254]. The continuous presence of IL-12 acting via Stat4 is needed for Th1 development [255–257]. T-bet induces not only the *IFN* γ gene but also the IL-2/IL-15R β -chain and CXCR3 for NK cells. The presence of T cell *IFN* γ receptors in the immune synapses is decisive in the Th1/Th2 choice [258a]. Th1 cells have “bulls eye” immune synapses, whereas Th2 cells show as distinct multifocal IS’s [258b]. A JNK2 signal pathway helps Th1 cell differentiation, and JNK1 inhibits Th2 cell differentiation [259, 260]. Furthermore, study of mice transgenic for gene *Hlx* (Fig. 13.10) has shown an enhanced Th1 response since there is downregulation of IL-4R α [260]. When IL-4 is present, co-polarisation of *IFN* γ receptors with the TCRs is prevented. IL-4 (determined by Stat6) induces Th2 cells, and since it inhibits the β 2-unit of the IL-12 receptor, it stops development of Th1 cells. The IL-4 gene promoter has sites where NFAT, AP-1, c-Maf and jun B bind. c-Maf induces the IL-4 promoter, and TF GATA3 (Fig. 4.13) promotes formation of IL-4/5/13. Another aspect is that Itk causes negative regulation of T-bet [261], so it promotes differentiation of Th2 lymphocytes.

By now, we know of differential biochemical features that signify Th1 or Th2 cells. Whilst Th1 cells use Stat4 for their development [262–263] and use NF- κ B/Rel signals [264], this is not so for Th2 cells, which are nevertheless resistant to apoptosis during their primary activation [265]. This must arise from their high content of glutathione [266]. Th2 cells use Stat6, and they have GATA3 [267], a zinc finger TF, and TF c-Maf of the AP-1 family [268]. On Th2 cells, there are no IL-12R β 2 as they are inhibited by IL-4. Epigenetic influences affect Th2 cell developments (Fig. 4.13). The TFs that are crucial for Th1 cell development (T-bet, Rac2, Ying-Yang, Hlx) all stimulate the IFN γ promoter (Fig. 13.9). Recall also that Tim3 on Th1 cells and Tim1 on Th2 cells help the reciprocal regulation [107]. These are type I membrane glycoproteins with a mucin domain. Anti-CD3 stimulation reveals that only in Th1 cells are Fyn and Zap70 recruited to produce a sustained increase of intracellular calcium ions. This process is inhibited by cholera toxin or by herbimycin.

On activation, Th1 cells exhibit multiple Ca²⁺ transients, whereas Th2 cells show an initial spike followed by a very low level of Ca²⁺ influx [269]. cAMP is elevated in Th2 cells. Calcium is extruded rapidly from Th2 cells. In Th2 cells, there is no formation of InsPtd(4,5)P2, and there is no activation of PLC γ 1. PKC plays a major role in Th2 cells, but its values are low in Th1 cells [4]. Upregulation of PI3K in Th2 cells means that they are resistant to Fas-induced apoptosis [270]. The T cell marker LAG is expressed by Th0 and Th1 cells. LAG3 is induced by IFN γ , and it is inhibited by IL-4. Th1 cells are CD45RB^{lo}, whilst Th2 cells are CD45RB^{hi}. Selectin (CD62L) is expressed on memory Th2 cells but is lost from Th1. In vitro, IL-4 induces CD30 of the TNF receptor family on Th2 cells, but IFN γ inhibits it. How reliable CD30 is in vivo as a marker is controversial, but certainly CRTH2 for PGD2 and ST2 that binds IL-33 mark the Th2 cells [271].

The T helpers that cause autoimmune diseases are Th17, rather than Th1, and they are formed by IL-23 in conjunction with IL-6 and TGF β . Their development is stopped by IFN γ . IL-23 driven Th17 cells are currently under scrutiny.

Th1/2 cells respond differentially to chemokines. Th1 cells bear CCR5 receptors, so that they respond to Rantes and macrophage inflammatory proteins (MIP α/β) and CXCR3 for IP10 and Mig. Th1 also bear CCR1 and CCR7. Conversely, Th2 cells carry CCR3 and -4 and CCR8 and CXCR4. CCR3 means that those cells will respond to eotaxin, Rantes and the monocyte chemotactic peptides (MCPs), and CXCR4 means that they are attracted by stromal cell-derived factor 1 (SDF1). Thus, chemokines determine which types of lymphocyte go to which particular tissues [281, 282]. Interestingly, CXCR4 physically associates with the TCR on T lymphocytes [283].

13.5.1 Interleukin 12 Signalling and Th1 Lymphocytes

IL-12 has a p40 chain and a p35 chain. IRF1 is implicated in the transcription of the p35 [284]. IL-12 p70 is powerful on account of its induction of Th1 cells [250]. Accordingly, in DCs there is feedback of its production via the action of PI3K or

JNK [285]. IL-12 starts off Th1 lymphocyte- and macrophage-mediated delayed hypersensitivity reactions, which are sustained by release of IFN γ . The IL-12 receptor has a β 1-subunit with associated Jaks, and this is for the binding of IL-12 p40. The β 2-subunit, with its cytoplasmic tyrosine kinases, is the signal initiator [286], and it binds the light-chain p35 of IL-12. There is nice control for when IL-12 stimulates Th1 cells to form IFN γ , macrophages synthesise IL-10, which, via Tyk2, dampens the process [287a]. Understandably microorganisms have decisive actions on IL-12 family members [287b].

Glucocorticoids like dexamethasone inhibit IL-12 production by T cells, and dexamethasone decreases IL-12-induced Stat4 phosphorylation and IFN γ production. IFN β releases IL-10, so it also suppresses IL-12 formation. Dexamethasone enhances IFN β induction of IL-10 formation [288].

13.5.2 Regulation of IFN γ Production

There are distinct pathways (a) for induction of IFN γ and (b) for cell proliferation. Jak3 activation leads to phosphorylation of Stat4 (Fig. 12.7) and so formation of IFN γ , whilst Jak2 activation leads to Stat5 phosphorylation and so cell proliferation [289]. Both CD4 and CD8 T cells produce IFN γ [290]. The CD4 cells use the process TCR-Stat4-IFN γ . The antigen-dependent pathway is promoted via TCR engagement in the presence of IL-12. IL-12 and IL-18 together (Fig. 13.10) induce IFN γ independently of TCR stimulation [291]. Even a weak TCR signal leads to formation of IFN γ when there is support by IL-12 and IL-18 [292]. Co-stimulator TL1A of DCs or monocytes promotes IFN γ in Th1 cells [293]. A basal level of NF- κ B that

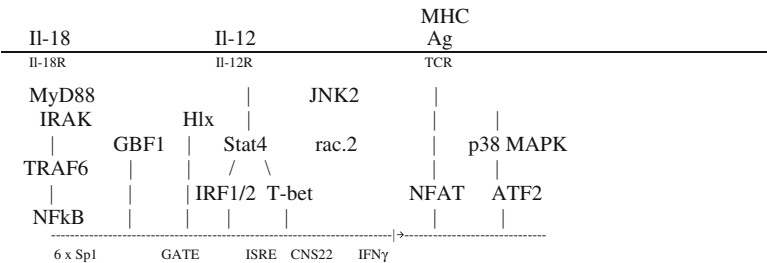


Fig. 13.10 Stimulation of interferon- γ (IFN γ) production, IRF1, acting on the interferon-stimulated regulatory element (ISRE), is involved in IFN γ action and likewise in its production. Conserved noncoding region 22 (CNS-22) is a site for histone modification of accessible chromatin [296a,296b] that is selective for T-bet that transactivates the IFN γ gene. It is then backed up by the secondary transcription factor Runx3 [297a], which augments Th1 cell activation and helps to silence the interleukin 4 (IL-4) gene. CNS1 and CNS2 sites were previously identified [297b]. H1x is a homeobox protein that raises IFN γ in CD4 T cells but reduces it in natural killer (NK) cells [298]. Phosphorylated (CREB) binds to the IFN γ proximal promoter [299]. Transforming growth factor- β (TGF β) and the Smad proteins inhibit T-bet and IFN γ expression and signalling [300]

is maintained by constitutive IL-1 α is needed for the activities of IFN [294]. The CD8 cells can produce IFN γ independent of IL-12. Furthermore, stimulation of TLRs on DCs will promote T cell production of IFN γ [295]. Clearly, there are spatially overlapping cis elements in the promoter of IFN γ . As seen in Fig. 13.10, there is an ISRE [296a] (IFN-stimulated regulatory element) responding to IRF1/2 in the promoter of the IFN γ .

IFN γ is secreted by CD4 $^{+}$ and CD8 $^{+}$ T cells and by NK and NKT cells. Immediately upstream of the IFN γ gene is its promoter, which is strongly inducible on T cell stimulation [301]. The promoter for IFN γ is like that of IL-2 since it has NFAT and NF- κ B sites and an AP-1 site and a proximal Octamer/AP-1/ATF (activating transcription factor) site next to a TATA box. Stat4, acting on behalf of the IL-12 receptor, stimulates IFN γ . In Th1 cells, there is a TF T β x that stimulates the IFN γ promoter [302]. Rac2 drives IFN γ transcription [303]. When T cells are adherent by their integrins to collagen, formation of IFN γ is much greater [304]. The differentiation of Th1 lymphocytes is preceded by demethylation of CpG DNA sites in the IFN γ promoter [305] but not so for Th2 cells. The ability to produce IFN γ is reduced in children who become atopic [306]. Rapid demethylation of the IFN γ promoter has also been demonstrated in memory CD8 T cells [307a]. Interestingly, there is a point at which IFN γ limits its own production by regulatory feedback [307a]. Yet an IFN γ + loops is required to sustain IL-12 production.

IFN γ via STAT1 [307b] activates macrophages, enhances their expression of Fc receptors, and promotes formation of ROS and RNIs by iNOS. It enhances B7 levels on APCs, and it upregulates MHC I and MHC II antigens via the TF IRF1. To combat *Mycobacterium tuberculosis*, there will be signalling in macrophages and T cells that depends on the combined effects of IL-12 and IFN γ . IFN γ is recognised as pro-osteoclastic [308]; thus, bones thin when there is chronic inflammation.

Not surprisingly, there will be curbs on IFN γ production. IFN γ itself creates negative feedback [307a]. TGF β inhibits T-bet and IFN γ formation [309]. IL-21 inhibits IFN γ production in developing Th1 cells, and this occurs because IL-21 represses the TF Eomes [310a]. Yet IL-21 arguments T cell proliferation. The stat 3/stat 1 balance is so relevant to the control of inflammation [310b]. Stat 3 counteracts inflammation whilst stat 1 promotes immunity. State 3 promotes cell proliferation whereas stat 1 inhibits it.

13.5.3 *Th17 Inflammatory Cells*

The Th17 inflammatory cells herald a new era in autoimmunity. Th17 cells producing interleukin Il-17 are induced by TGF β along with Il-6, and some Il-1[310c], through the engagement of Il-21 and Il-23 pathways [310d], as well as STAT3 and the nuclear orphan receptor ROR γ . IRF4 helps to induce the Th17 cells. Th17 cells express CCR6. Th17 cells can arise from priming of monocytes [310e] and from Th1 or Th2 lymphocytes. A synergy between TNF α and Il-17 results from an ability of IL-17 to stabilize short-lived mRNAs that are induced only transiently by TNF α . Th17 cells

have much potential because they produce the cytokines IL-17 (ie IL-17A), IL-17F, IL-6, TNF α and IL-22 and IL-26 of the IL-10 family [310f]. Some Th17 become regulatory T cells. In Chap 10 one noted that IL-17 drives neutrophil induced inflammation. Th-17 cells are implicated in asthma, uveitis, rheumatoid arthritis, Crohn's disease and psoriasis. Their formation is opposed by retinoic acid, which favours formation of Treg cells [310g]. Th17 cells cannot be regulated by standard Treg cells.

Many IL-17 target genes contain both NF- κ B and c/EBP binding sites. IL-17 activates NF- κ B and MAP kinases via the adaptor protein CIKS (connection to I κ B kinase and stress activated protein kinases, otherwise known as Act-1). The recruitment of CIKS to the IL-17R occurs via domain interactions [310h], similar to the way that Toll-like Receptors recruit the adaptor MyD88 via TIR domain interactions. CIKS is known to interact with Nemo/IKK γ (Chap 11), the regulatory subunit of the IKK complex. CIKS/Act1 can also bind to TRAF3 and it activates TAK1. Furthermore IL-17 induces I κ B ζ which is able to promote NF- κ B activity.

13.6 Interleukin 4 and Th2 Lymphocytes

In T lymphocytes, the CD28 co-stimulator activates PDK-1 (Fig. 2.8), which activates protein kinase A, which helps NFATc action to form IL-4 and thus Th2 cells [311]. Th2 cells appear in gastrointestinal mucosa in response to helminths and in the bronchial mucosa in asthma and the nose in rhinitis. Th2-dominant cells stimulate IgE antibody responses, activate mast cells and elicit tissue eosinophilia. Essentially, IL-4, acting through Stat 6 (Fig. 4.13), determines Th2 cell maturation. IL-1 costimulation helps DC2 DCs (akin to mouse CD8 α cells) (Fig. 13.9) promote development of Th2 cells, as will B lymphocytes acting as APCs for small soluble antigens. The CD30 marker is typical of cells that have been stimulated by IL-4.

13.6.1 Interleukin 13

IL-13 binds to a receptor complex made of the IL-4 receptor- α and IL-13 receptor- α 1. IL-13 can also bind to IL-13R α 2, forming a stable complex with a long half-life and thus is a decoy receptor that inhibits IL-13 responses [312]. Binding of IL-13 to its receptor and activation of JAK1/TYK2 activates Stat6. Stat 6 leads to production of chemokines, metalloproteinases, adenosine, vascular endothelial growth factor (VEGF) and TGF β 1. IL-13 induces airway leucocyte activation, mucus secretion, bronchospasm and airway fibrosis [313]. Polymorphisms of IL-13 are associated with asthma. IL-13 action can even produce bronchiolitis obliterans. Yet, there is more. In IL-10 null mice, increased expression of the IL-13R α 2 reduces airway hyperreactivity and mucus production. When IL-10 and IL-13R α 2 act together, they promote a Th2 cell response [314a]. IL-13R α 2 can stimulate TGF and so fibrosis [314b].

13.7 Respiratory Tract Allergy

The features of asthma [315, 316] include eosinophilic airway inflammation and airway muscle hyper-responsiveness, both orchestrated by Th2 cytokines from Th2 cells and mast cells. Thymic stromal lymphopoietin (TSLP), an IL-7 like cytokine, is a key initiator of allergic disease. TSLP of the thymus or originating from bronchial epithelial cells is now recognised to activate DCs in crucial ways. When TSLP is put into airway epithelium of mice, Th2 cells appear bearing CCR4, which helps their homing to the lungs [317]. TSLP also activates mast cells. In fact, inflammatory Th2 cells are induced by TSLP, and they form IL-4/5/13 along with TNF α , not IL-4/5/13 with IL-10 as in conventional Th2 cells [318, 319]. DCs that are transduced with SOCS3 also direct the requisite Th2 cell differentiation since IL-12/IL-23 signalling is blocked [320]. As well as the mast cells, eosinophils act as APCs to induce a Th2 reaction [321]. CRTH2 receptors boosted by IL-5 control eosinophils for prostaglandin D2 (PGD2) acting on CRTH2 receptors induces eosinophilic airway inflammation [322]. Likewise, 5-oxo-HETE, derived from arachidonic acid via 5LO [323a], plays an important role since it activates neutrophils, eosinophils and monocytes. iNKT cells [323b] also participate in producing IL-4, a lot of IL-5 and IL-13. Cytokine IL-13 is particularly pertinent to the asthma process, and IL-4 promotes production of Th2 lymphocytes. Indeed, IL-4 receptor polymorphisms in mice relate to airways reactivity [324]. PI3K p110 δ plays an essential role in the allergic reaction [325], and PKC ζ determines IL-4 production of Th2 cells. Now, it has been found that hepatocyte growth factor, through its ability to suppress DCs, will suppress airway hyperreactivity and eosinophilia [326]. With luck, it could also stop fibrosis beneath the airway epithelium (Fig. 13.11).

The leucocytes in the airways create ROS, and the local oxidative stress and neutrophil elastase induce mucin production by airway epithelium via transactivation of EGF receptors [327, 328]. The IL-4Rs on Clara cells induce mucus production. Interestingly, pollen grains have intrinsic NADPH oxidases, which enhance ROS production on the respiratory epithelia. An oxidising microenvironment actually favours Th2 cells and IL-4 production [329].

The chemokines TARC (thymus and activation-regulated chemokine) and MDC (macrophage-derived chemokine) are important for migration of Th2 cells into the lungs and associated lymphoid tissues [330]. Airway smooth muscle cells will produce several chemokines and cytokines. Furthermore, ADAM33 [331] metalloproteinase is expressed in bronchial smooth muscle and is thought to heighten its hypertrophic response. It is possible that IL-6 actually precipitates the asthma profile since IL-6 induces Th2 lymphocytes, and IL-6 is known to stop the development of Treg cells [332].

Interleukin 13 acts via metalloproteinases (MMPs) 2, 9, 12, and 14 to cause airway inflammation, remodelling and fibrosis [333]. IL-13, when acting on pulmonary fibroblasts, stimulates both Stat6 and Stat1. The Stat6 promotes, and the Stat1 dampens, upregulation of the TF Egr-1 and formation of (PDGF) ligands for fibroblasts [334]. IL-13 is a potent stimulator of bronchial cell CCR5 expression

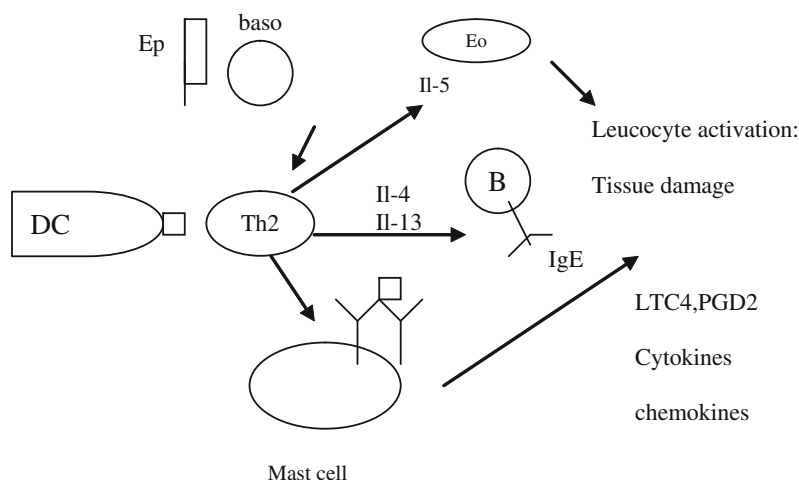


Fig. 13.11 Mucosal allergic reaction. DC dendritic cell, γ immunoglobulin E (IgE) antibody. In response to allergen release, interleukin 4 ($IL-4$) instigates a Th2 cytokine response. $IL-5$ is essential for the eosinophil response. $IL-13$ has key actions on epithelial and smooth muscle cells. The release of mediators from mast cells, basophils and eosinophils provokes an inflammatory reaction in the bronchial mucosa that is asthma or in the nose that is allergic rhinitis. For a Th2 response to occur, levels of interferon- γ ($IFN\gamma$) must be low. Differentiation of eosinophils and accumulation in the lungs is regulated by the Th2 cytokines $IL-3$, $IL-5$ and ($GM-CSF$) [361]. It follows that inhibition of allergic inflammation can be based on (a) inhibition of Th2 cells, (b) use of anti- $IL-4/5/13$, (c) eosinophil VLA4 integrin inhibition, (d) CCR3 antagonists to thwart eotaxin and Rantes, (e) use of the corticosteroids and p38 mitogen-activated protein (MAP) kinase inhibitors

[335], and that binds the mediators MIP-1 α (CCL3), MIP-1 β (CCL4) and Rantes (CCL5). $IL-13$, using Stat6-dependent pathways, induces arginase I, curtailing synthesis of NO and contributing to airway hyperresponsiveness AHR [336]. This is proven by the effect of iRNA, which stops $IL-13$ -mediated AHR. In the airways $CD8^+$ T cells are producing Th2 cytokines like $IL-13$. For their induction, these cells are dependent on the $IL-4$ that comes from $CD4^+$ T cells [337].

Can there be any more relevant cytokines? $IL-25$ has been shown to augment the expansion of TSLP-activated Th2 memory cells [338]. Also, activin A, whose serum levels are increased in asthma, provides a link to TGF β 1-induced airway remodelling [339].

Injured airway epithelial cells release GM-CSF, which is a factor that aids maturation of DCs as well as attraction of white cells. Engagement of ICAM1 on epithelial cell surfaces leads to phosphorylation of Syk, thereby activating the P38 MAPK; thus, cytokines like $IL-8$ are produced [340]. This is anticipated whenever airway-targeting viruses like rhinovirus cause exacerbation of asthma. An antisense that blocks p38 MAPK suppresses murine respiratory inflammation [341] as will blockade of PI3K δ [342]. By way of protection, the DCs in the respiratory tract produce $IL-10$, which drives Treg cell differentiation [343]. We know that $IL-10$

inhibits the expression of FcεRI receptors by mast cells [344]. During specific immunotherapy for inhaled pollens, autocrine release of IL-10 blocks allergic activation. In asthmatic mice infected with a gastrointestinal nematode *Heligmosomoides polygyrus*, asthma features were suppressed in an IL-10-dependent manner. Inhalation of PGD agonist inhibits DCs but creates Treg cells that utilise IL-10 to suppress asthma. Also, IL-10 will upregulate Smad7 and so prevent TGFβ action that favours airway remodelling [345]. By now, an anti-IgE recombinant humanised monoclonal antibody *omalizumab* is being used. It binds the IgE, which would bind to FcεRI receptors. Also one notes that the PD-1 receptor and B and T lymphocyte attenuators will all help to terminate allergic airway inflammation [346].

To combat allergic disorders, in addition to inhaled or systemic corticosteroid usage, one will have to consider thwarting the following cell signal pathways:

1. IgE-IgE receptor signal cascade (cf. Fig. 6.1)
2. Jak-Stat receptor signalling (e.g. via Socs proteins) [347] (Fig. 12.7)
3. TLR 2/4/7/8 signalling activating NF-κB
4. Leukotriene and PGD2 receptor signalling
5. Signalling to neurotrophils from epithelial cells and T cells
6. TSLP signalling via OX40 ligand inducing Th2 inflammatory Th2 cells

With regard to the last item, TSLP is expressed by airways of epithelial cells of subjects with asthma, and it causes myeloid DCs to recruit eosinophils and Th2 lymphocytes. TSLP also stimulates mast cells to produce IL-5 and IL-13 [348].

There is a surprise for, although we regard IL-12 as important for the suppression of Th2 reactions, in fact IL-12 contributes to allergic airway disease with bronchial responsiveness and eosinophilia in mice [349]. Also, BAFF (B cell-activating factor) is a product of airway epithelial cells, aiding airways inflammation [350]. Another surprise comes with the knowledge that IL-17 is a negative regulator of established allergic asthma since it inhibits the actions of DCs and chemokine synthesis [351]. Yet, IL-17 evokes migration of neutrophils [352]. The cytokine scene in asthma is indeed very complex [353]. Naturally, PBMC T-bet, the proponent of Th1 cells, turns out to be suppressed in asthma [354]. Of course, immunotherapy would expand IL-10-producing cells and elevate the IL-10/IFNγ ratio [355].

With asthma, there must be persistent activation of NF-κB. Understandably, blockade of NF-κB in airway immune cells inhibits experimental asthma [356]. There is an important modulating effect of NO in respiratory epithelium, which may vary between individuals [357]. *Pitrakinra* is a therapeutic variant IL-4Rα that has been shown to ameliorate the late phase of asthma in clinical trial [358].

Another way to thwart NF-κB activation is by use of a glycogen synthase kinase 3 beta inhibitor (TDZD-8) [359]. In ovalbumin-challenged mice, TDZD8 stops lung tissue eosinophilia and airway mucus production, and BAL levels of IL-5, IL-13 and eotaxin are reduced.

It is now clarified how eosinophils can promote either Th1 or Th2 cell responses. Eosinophils forming TNFα and IL-4 induce Th2 cells, whilst eosinophils with TNFα and IFNγ produce Th1 cells [360].

13.8 Chronic Bronchitis as Obstructive Airway Disease

Smokers will develop chronic bronchitis and airway obstruction as a result of mucosal inflammation, with neutrophil infiltration and bronchial muscle reactivity. Consequent on the activation of NF- κ B [362], there is release of IL-8 and TNF α and of MMPs, so that there is recruitment of neutrophils. Within the lungs, the elastase from neutrophils digests the air spaces, so that emphysema develops. Formation of ceramide by neutrophils and in alveolar epithelium leads to apoptosis of those cells. Whereas in asthma corticosteroid therapy stimulates glucocorticoid receptors that translocate into nuclei to bind HDAC (histone deacetylase), and hence cause muscle relaxation, in chronic obstructive pulmonary disease (COPD) the oxidant-modified HDAC cannot interact with the glucocorticoid receptors [363], and there is no airway relaxation in response to corticosteroid. Not surprisingly, airway smooth muscle cells express TLRs 2/3/4, and their activation leads to the release of chemokines. NOD proteins (Fig. 12.3) sense bacteria that invade the epithelium [364]. IL-18 (IFN γ -inducing factor) levels are raised in persons with COPD. Studies in mice show that IL-18 provoked by cigarette smoke enhances cathepsins in pulmonary macrophages, and consequently emphysema develops [365]. The failure of innate lung defence allows infection to smoulder in the lower airways [366].

References

1. Brodsky FM, Guagliardi LE. The cell biology of antigen processing and presentation. *Annu Rev Immunol* 1991;9:707–724
2. Janeway CA. The T cell receptor as a multicomponent signaling machine. *Annu Rev Immunol* 1992;10:835–873
3. August A, Fischer A, Hao S, Mueller C, Ragin M. The Tec family of tyrosine kinases in T cells: amplifiers of T cell receptor signals. *Int J Biol Cell Biol* 2002;34:1184–1189
4. Cantrell D. T cell antigen receptor pathways. *Annu Rev Immunol* 1996;14:259–274
5. Call ME, Wucherpfennig KW. Common themes in the assembly and architecture of activating immune receptors. *Nat Rev Immunol* 2007;7:841–850
6. Bromley SK, Burack WR, Johnson KG et al. The immunological synapse. *Annu Rev Immunol* 2001;19:375–396
7. Lovatt M, Filby A, Parravicini V et al. Lck regulates the threshold of activation in primary T cells. *Mol Cell Biol* 2006;26:8655–8665
8. Altman A, Villalba M. Protein kinase C theta. *J Biochem* 2002;132:841–846
9. Razzaq TM, Ozegebe P, Jury EC et al. Regulation of T cell receptor signalling by membrane microdomains. *Immunology* 2004;113:413–426
10. Jordan MA, Singer AL, Koretzky GA. Adaptors as central mediators of signal transduction in immune cells. *Nat Immun* 2003;4:110–116
11. Okkenhaug K, Vanhaesebroeck B. PI3-kinase in lymphocyte development, differentiation and activation. *Nat Rev Immunol* 2003;3:317–330
12. Bromley SK, Burack WR, Johnson KG et al. A molecular framework for two step T cell signaling. *J Immunol* 2001;166:754–764
13. Viola A. The amplification of T cell receptor signaling by dynamic membrane microdomains. *Trends Immunol* 2001;22:322–326

14. Simizu Y. LFA-1 signal as adjunct to T Cell Receptor/CD28. *Nat Immun* 2003;4:1052–1054
15. Billadeau DD, Nolz JC, Gomez TS. Regulation of T cell activation by the cytoskeleton. *Nat Rev Immunol* 2007;7:131–143
16. Kanner SB, Perez-Villar JJ. Alternating T cell activation by targeting the multidomain tyrosine kinase Itk. *Trends Immunol* 2003;24:249–253
17. Horejsi V, Zhang W, Schraven B. Transmembrane adaptor proteins:organisers of immunoreceptor signalling. *Nat Rev Immunol* 2004;4:603–614
18. Barda-Saad M, Braiman A, Titerence R et al. Dynamic molecular interactions linking the T cell antigen receptor to the cytoskeleton. *Nat Immun* 2005;6:80–89
19. Goda S, Quale AC, Woods ML et al. Control of T cell receptor activation of $\beta 1$ -integrins. *J Immunol* 2004;172:5379–5387
- 20a. Pibibila JT, Quale AC, Mueller KL, Shimizu Y. Integrins and T cell mediated immunity. *Annu Rev Immunol* 2004;22:157–180
- 20b. Burbach BJ, Medeiros RB, Mueller KL, Shimizu Y. T-cell receptor signaling to integrins *Immunol Revs* 2007; 218: 65–81
21. Masuda ES, Imamura R, Amasaki Y et al. Signaling into the T cell nucleus: NFAT regulation. *Cell Signal* 1998;10:599–611
- 22a. Gelman AE, Zhang J, Choi Y, Turka LA. Toll like receptor ligands directly promote activated CD4⁺ T cell survival. *J Immunol* 2004;172:6065–6073
- 22b. Moser B, Desai DD, Downie MP et al. RAGE expression on T cells contributes to antigen-specific celalular expansion in vivo. *J Immunol* 2007;179:8051–8058
23. Frauwirth KA, Riley JL, Harris MH et al. The CD28 signalling pathway regulates glucose metabolism. *Immunity* 2002;16:769–778
- 24a. Appleman LJ, Berezovskaya A, Grass I, Boussiotis VA. CD28 costimulation mediates T cell expansion via Il-2 independent and Il-2 dependent regulation of cell cycle progression. *J Immun* 2000;164:144–151
- 24b. Tadashi Y, Wakana K et al. Spatio-temporal Regulation of T Cell Costimulation by TCR-CD28 Microclusters and Protein Kinase C Translocation. *Immunity* 2008;29:589–601
25. Tavano R, Gri G, Molon B et al. CD28 and lipid rafts coordinate recruitment of Lck to the immune synapse of T lymphocytes. *J Immunol* 2004;173:5392–5397
26. Dennehy KM, Elias F, Na S-Y et al. Mitogenic CD28 signals require the exchange factor Vav1 to enhance TCR signaling. *J Immunol* 2007;178:1363–1371
27. Pan M, Winslow MM, Chen L et al. Enhanced NFATc1 nuclear occupancy causes T cell activation independent of CD28 costimulation. *J Immunol* 2007;178:4315–4321
28. Schmitz ML, Krappmann D. Controlling NF-kB activation in T cells by costimulatory receptors. *Cell Death Differ* 2006;13:834–842
29. Kogkopolou O, Tzakos E, Mavrothalassitis G. Conditional upregulation of Il-2 production by p38 MAPK inactivation is mediated by increased Erk1/2 activity. *J Leukoc Biol* 2006;79:1052–1060
30. Villarino AV, Tato CM, Stumhofer JS et al. Helper T cell Il-2 production is limited by negative feedback and STAT dependent cytokine signals. *J Exp Med* 2007;204:65–71
31. Sanchez-Lockhart M, Marin E, Graf B et al. CD28 mediated transcriptional and post-transcriptional regulation of Il-2 expression are controlled. *J Immunol* 2004;173:7120–7124
- 32a. Nandiwada SL, Li W, Zhang R, Mueller DL. p300/CREB mediates transcriptional coactivation by the CD28 T cell costimulator receptor. *J Immunol* 2006;177:401–413
- 32b. Zeyda M, Staffler G, Horejsi V et al. LAT displacement from lipid rafts as a molecular mechanism for the inhibition of T cell signaling by polyunsaturated fatty acids. *J Biol Chem* 2002;277:28418–28423
- 33a. Acuto O, Cantrell D. T cell activation and cytoskeleton. *Annu Rev Immunol* 2000;18:165–184
- 33b. Scheele JS, Marks RE, Boss GR. Signaling by small GTPases in the immune system. *Immunol Revs* 2007;218:92–101
34. Salazar-Fontana LI, Barr V, Samelson LE, Biere BE. CD28 engagement promotes actin polymerization through activation of small Rho GTPase cdc42 in human T cells. *J Immunol* 2003;171:2225–2232

35. Bauch A, Alt FW, Crabtree GR, Snapper SB. The cytoskeleton in lymphocyte signaling. *Adv Immunol* 2000;75:89–114
36. Michel F, Mangino G, Attal-Bonnefoy G et al. CD28 utilizes Vav.1 to enhance T cell receptor proximal signaling and NFAT activation. *J Immunol* 2000;165:3820–3829
37. Reynolds LF, Smyth LA, Norton T et al. Vav1 transduces T cell receptor signals to the activation of phospholipase C via PI3kinase dependent and independent pathways. *J Exp Med* 2002;195:1103–1114
38. Piccolella E, Spodaro F, Ranani C et al. Vav1 and IKK α subunit of I κ B kinase functionally associate to induce NF κ B activation in response to CD28 engagement. *J Immunol* 2003;170:2895–2903
39. Germain AN. The T cell receptor for antigen signaling and ligand discrimination. *J Biol Chem* 2001;276:35223–35246
40. Kuo H, Yu G, Wu Y, Wu J. Eph B6 crosslinking results in costimulation of T cells. *J Clin Invest* 2002;110:1141–1150
41. Feito MJ, Vaschetto K, Croado G et al. Mechanisms of H4/ICOS costimulation. *Eur J Immunol* 2003;33:204–214
42. Song J, Salek-Ardakani S, Rogers PR et al. The costimulation regulated duration of PKB activation controls T cell longevity. *Nat Immunol* 2004;5:150–158
43. Engedal N, Gjevik T, Blomhoff R, Blomhoff HK. All-trans retinoic acid stimulates IL-2 mediated proliferation of human T lymphocytes. *J Immunol* 2006;177:2851–2861
44. Tzachanis D, Freeman GJ, Hirano N et al. Tob is a negative regulator of activation expressed in anergic and quiescent T cells. *Nat Immunol* 2001;2:1174–1178
45. Sebald A, Mattioli I, Schmitz ML. T cell receptor complex induced lipid raft recruitment of the I κ B kinase complex is necessary for NF κ B activation occurring in the cytosol. *Eur J Immunol* 2005;35:318–325
46. Weil R, Israel A. Deciphering the pathway from the T cell receptor to NF- κ B. *Cell Death Differ* 2006;13:826–833
47. Kane LP, Mollenauer MN, Xu Z et al. Akt dependent phosphorylation regulates cot induction of NF κ B dependent transcription. *Mol Cell Biol* 2002;22:5962–5974
48. Corn RA, Aronica MA, Zhang F et al. T cell intrinsic requirement for NF κ B induction in post-differentiation IFN γ production and clonal expansion in a Th.1 response. *J Immunol* 2003;171:1816–1824
49. Strauss G, Knappe I, Metzner I, Debatin KM. Impaired death inducing signaling formation in CD95 resistant and long term activated antigen specific T cells. *J Immunol* 2003;171:1172–1182
50. Schmitz ML, Bacher S, Dienz O. NF κ B activation pathways induced by T cell costimulation. *FASEB J* 2003;17:2187–2193
51. Viatour P, Merville M-P, Bours V, Chariot A. Phosphorylation of NF- κ B and I κ B proteins: implications in cancer and inflammation. *Trend Biochem Sci* 2005;30:43–50
52. Thome M, Tschopp J. TCR-induced NF κ B activation: a crucial role for Carma 1, Bcl-10 and Malt.1. *Trends Immunol* 2002;23:1–4
53. Thome M. Carma 1, Bcl-10 and Malt.1 in lymphocyte development and activation. *Nat Rev Immunol* 2004;4:348–359
54. Rawlings DJ, Sommer K, Moreno-Garcia ME. The CARMA1 signalosome links the signalling machinery of adaptive and innate immunity in lymphocytes. *Nat Rev Immunol* 2006;6:799–812
55. Marsland BJ, Soos TJ, Spath G et al. PKC is critical for development of T helper 2 cells but not Th.1 cell responses. *J Exp Med* 2004;200:181–190
56. Heyninck K, Beyaert R. A20 inhibits NF- κ B activation by dual ubiquitin-editing functions. *Trends Biochem Sci* 2005;30:1–5
57. Hong J-W, Allen CE, Wu L-C. Inhibition of NF κ B by ZAS3, a zinc finger protein that also binds to the κ B motif. *Proc Natl Acad Sci U S A* 2003;100:12301–12306
58. Scharschmidt E, Wegener E, Heissmeyer V et al. Degradation of bcl-10 by T cell activation negatively regulates NF κ B signals. *Mol Cell Biol* 2004;24:3860–3873
59. Minquet S, Huber M, Rosenkranz L et al. Adenosine and cyclic AMP are potent inhibitors of the NF- κ B pathway downstream of immunoreceptors. *Eur J Immunol* 2005;35:31–41

60. Mustelin T, Vang T, Bottini N. Protein tyrosine phosphatases and the immune response. *Nat Rev Immunol* 2005;5:43–57
61. Bottini N, Vang T, Cucca F, Mustelin T. Role of PTPN22 in type 1 diabetes and other autoimmune diseases. *Semin Immunol* 2006;18:207–213
62. Pani G, Siminovitch KA. Protein tyrosine phosphatases in regulation of lymphocyte signaling. *Clin Immunol Immunopathol* 1997;84:1–16
63. Hermiston ML, Xu Z, Majeti R, Weiss A. Reciprocal regulation of lymphocyte activation by tyrosine kinases and phosphatases. *J Clin Invest* 2002;109:9–15
64. Chiarugi P, Cirri P. Redox regulation of protein tyrosine phosphatases during receptor tyrosine kinase signal transduction. *Trends Biochem Sci* 2003;28:509–513
65. Zamoyska R. Why is there so much CD45 on T cells? *Immunity* 2007;27:421–433
66. Kupfer A. Signaling in the immunological synapse. *Immunity* 2006;25:11–13
67. Dorman S, Sebestyen Z, Gamle J, Nagy P et al. Association of CD45 isoforms with CD4/8 regulates the actions of p56 Lck tyrosine kinase in T cell receptor signal transduction. *J Biol Chem* 2002;277:1912–1918
68. Dustin ML. Membrane domains and the immunological synapse—keeping T cells resting and ready. *J Clin Invest* 2002;109:155–160
69. Nguyen JT, Evans DP, Galvan M et al. CD45 modulates galectin-1 induced T cell death by expression of core-2 O-glycans. *J Immunol* 2001;167:5697–6007
70. Plas DR, Johnson R, Pinquel JT, Matthews RJ. Direct regulation of Zap70 by SHP.1 in T cell antigen receptor signaling. *Science* 1996;272:113–1176
71. Marengere LE, Waterhouse P, Duncan GS et al. Regulation of T cell receptor signaling by tyrosine phosphatase Syk associated with CTLA4. *Science* 1996;272:1170–1172
72. Ono M, Bollard S, Tempst P, Rav JV et al. Role of inositol phosphatase SHIP in negative regulation of immune system by Fc γ RIIB. *Nature* 1996;383:263–266
73. Brockdorff J, Williams S, Couture C, Moustelin T. Dephosphorylation of Zap70 and inhibition of T cell activation by activated SHP-1. *Eur J Immunol* 1999;29:2539–2550
74. Tsui HW, Siminovitch EA, de Souza L, Tsui FWL. Motheaten and viable motheaten mice have mutations in the haematopoietic phosphatase gene. *Nat Genet* 1993;4:124–129
75. Gjorloff-Wingren A, Saxena M, Williams S et al. Characterization of TCR-induced receptor:proximal signaling events negatively regulated by the protein tyrosine phosphatase PEP. *Eur J Immunol* 1999;29:3845–3854
76. Dietrich J, Cella M, Seiffert M et al. Signal regulatory protein β 1 is a DAP12 associated activating receptor expressed in myeloid cells. *J Immunol* 2000;164:9–12
77. Takai T. Paired immunoglobulin-like receptors and their MHC class I recognition. *Immunology* 2005;115:433–440
78. Alvarez-Errico D, Aguilar H, Kitzig F et al. IREM-1 is a novel inhibitory receptor expressed by myeloid cells. *Eur J Immunol* 2004;34:3690–3701
79. Marti F, Krause A, Post NH et al. Negative feedback regulation of CD28 costimulation by MAPK phosphatase MKP6. *J Immunol* 2001;166:197–206
80. Tangye SG, Wu J, Aversa G, de Vries JE et al. Negative regulation of human T cell activation by the receptor type protein tyrosine phosphatase CD148. *J Immunol* 1998;161:3803–3807
81. Han S, Williams S, Mustelin T. Cytoskeletal protein tyrosine phosphatase PTPH1 reduces T cell antigen receptor signaling. *Eur J Immunol* 2000;30:1318–1325
82. Ezernitch AV, Vakrin I, Cohen-Daniel L et al. TCR ζ downregulation under chronic inflammation is mediated by myeloid suppressor cells. *J Immunol* 2006;177:4763–4772
- 83a. Wong N, Lai J, Birkenhead D et al. CD45 down-regulates Lck-Mediated CD44 signaling and Modulates Actin Rearrangement in T cells. *J Immunol* 2008;181:7033–7043
- 83b. Kilgore NE, Carter JD, Lorenz U, Evavold BD. Dependence of T cell receptor antagonism on SHP.1. *J Immunol* 2003;170:4891–4895
84. Dong S, Corre B, Foulon E et al. T cell receptor for antigen induces LAT dependent activation of a negative signaling complex involving Dok2, SHIP1, Grb2. *J Exp Med* 2006;203:2509–2518

85. Krawczyk C, Bochmaier K, Sasaki T et al. Cbl-b is a negative regulator of receptor clustering and raft aggregation. *Immunity* 2000;13:463–468
86. Pratt CJ, Igras VE, Maeda H et al. Gab2 mediates an inhibitory PI 3-kinase pathway in T cell antigen receptor signaling. *J Immunol* 2000;165:4158–4163
87. Zhu M, Koonpaew S, Liu Y et al. Negative regulation of T cell activation and autoimmunity by the transmembrane adaptor protein LAB. *Immunity* 2006;25:757–768
88. Eager TN, Tang Q, Wolfe M et al. Notch.1 signaling regulates peripheral T cell activation. *Immunity* 2004;20:407–415
89. Tsukumo S-I, Yasutomo K. Notch governing mature T cell differentiation. *J Immunol* 2004;173:7109–7113
90. Bronstein-Sitton N, Cohen, Daniel L, Vaknin I et al. Sustained exposure to bacterial antigen induces IFN γ dependent T cell receptor α downregulation and impaired T cell function. *Nat Immun* 2003;4:957–964
91. Lee J-M, Chung C-Y, Vhiang W-E et al. IL-15 Receptor α is a negative regulator of T Cell Receptor activated proliferation in CD4+ T cells. *J Immunol* 2004;173:3155–3164
92. Chikuma S, Imboden JB, Bluestone JA. Negative regulation of T cell receptor: lipid raft interaction by CTLA4. *J Exp Med* 2000;197:129–135
93. Walunas TL, Bakker CY, Bluestone JA. CTLA4 ligation blocks CD28 dependent T cell activation. *J Exp Med* 1996;183:2541–2547
94. Teft WA, Kirchhof MG, Madrenas J. A molecular perspective of CTLA4 function. *Annu Rev Immunol* 2006;24:65–97
95. Gotta L, Calviello G, DiNuciuolo F et al. CTLA4 inhibits integrin mediated stimulation. *Immunology* 2002;107:209–216
96. Chen L. Coinhibitory molecules of the B7-CD28 family in the control of T cell immunity. *Nat Rev Immunol* 2004;4:336–347
97. Selenko-Gebauer NK, Majdic O, Szekeres A et al. B7.H1 on dendritic cells is involved in induction and maintenance of T cell anergy. *J Immunol* 2003;170:3637–3644
98. Prasad DVR, Richards S, Mai XM, Dong C. B7S1 that negatively regulates T cell activation. *Immunity* 2003;18:3637–3673
99. Sica GL, Choi I-H, Zher G et al. B7-H4 negatively regulates T cell immunity. *Immunity* 2003;18:849–861
100. Watanabe N, Gavrieli M, Sedy JR et al. BTLA is a lymphocyte inhibitory receptor with similarities to CTLA4 and PD-1. *Nat Immun* 2003;4:670–679
101. (a) Croft M. The evolving crosstalk between costimulatory and coinhibitory receptors: HVEM-BTLA. *Trends Immunol* 2005;26:292–294; (b) Murphy KM, Nelson CA, Sedy JR. Balancing costimulation with BTLA and HVEM. *Nat Rev Immunol* 2006;6:671–681
102. Parry RV, Riley JL, Ward SG. Signalling to suit function: tailoring phosphoinositide 3-kinase during T cell activation. *Trends Immunol* 2007;28:161–169
103. Czopik AK, Bynoe MS, Palm N et al. Semaphorin 7A is a negative regulator of T cell responses. *Immunity* 2006;24:591–600
104. Palmer E. Signaling negative selection: MINK the missing link? *Nat Immun* 2005;6:9–10
105. McGargill MA, Sharp LL, Bui JD et al. Active calcium-calmodulin dependent protein kinase II γ impairs positive selection of T cells by modulating TCR signaling. *J Immunol* 2005;175:656–664
106. Sporri B, Kovanen PE, Sasaki A et al. Jab/Socs.1 is an IL-2 induced inhibitor of IL-2 signaling. *Blood* 2001;97:221–226
107. Meyers JH, Sabatos CA, Chakravarti S, Kuchroo VK. The TIM gene family regulates autoimmune and allergic diseases. *Trends Mol Med* 2005;11:362–369
108. Nicklbein JE, de Souza AJ, Tosti R et al. Inhibition of T cell activation by Tim2. *J Immunol* 2006;177:4966–4970
- 109a. Rennert PD, Ichimura T, Sizing ID et al. T cell, Ig domain, mucin domain-2 gene deficient mice reveal regulation of Th2 immune responses. *J Immunol* 2006;177:4311–4321
- 109b. Bouguermouh S, Van VQ, Martel J et al. CD47 expression on T cell is a self-control negative regulator of type 1 immune response. *J Immunol* 2008;180:8073–8082

110. Newton-Nash D, Newman PJA. A new role for PECAM-1: inhibition of T cell receptor signal transduction. *J Immunol* 1999;163:682–688
111. Garin MI, Chu C-C, Galshaya D et al. Galectin-1, a key effector of regulation mediated by CD4⁺ CD25⁺ T cells. *Blood* 2007;109:2058–2065
112. Akdia CA, Blaser K. Mechanisms of Il-10 mediated immune suppression. *Immunology* 2001;103:131–136
113. Mirra N, Bayry J, Lacroix-Desmazes S et al. Human CD4⁺ CD25⁺ T cells restrain the maturation and antigen presenting function of dendritic cells. *J Immunol* 2004;172:4676–4680
114. Okano M, Sugata Y, Fujiwara T et al. E prostanoic acid 2 mediated suppression of antigen specific T cell responses by PGE2. *Immunity* 2006;118:343–352
115. Kukashev D, Klebanov B, Kojima H. HIF-1 α and its activation-inducible short isoform I.1 negatively regulates functions of CD4 and CD8 T lymphocytes. *J Immunol* 2006;177:4962–4965
116. Chung CD, Patch VP, Moran M et al. Galectin-1 induces partial T cell receptor phosphorylation and antagonizes TCR signal transduction. *J Immunol* 2000;165:3722–3729
117. Hernandez JD, Nguyen JT, He J et al. Galectin1 binds different CD43 glycoforms to cluster CD43 and regulate T cell death. *J Immunol* 2006;177:5328–5336
118. Malissen B. Switching off T cell receptor signaling. *Science* 2003;302:1162–1165
119. von Essen M, Nielsen MW, Bonefeld CM et al. Protein kinases PKC α and PKC θ are the major PKC isotypes in T cell receptor downregulation. *J Immunol* 2006;176:7502–7510
120. Clements JL, Boerth NJ, Lee JR, Koretsky GA. Integration of T cell receptor dependent signaling pathways by adapter proteins. *Annu Rev Immunol* 1999;17:89–108
121. Li D, Gal I, Vernes C, Alegre M-L et al. Cbl-b: one of the key molecules tuning CD28- and CTLA4-mediated T cell costimulation. *J Immunol* 2004;173:7135–7139
122. Itoh K, Sakakihara M, Yamasaki S et al. Negative regulation of immune synapse formation by anchoring lipid rafts to cytoskeleton through Cbp-EBP50-ERM assembly. *J Immunol* 2002;168:541–546
123. Workman CJ, Cauley LS, Kim I-J et al. Lymphocyte activation gene 3 (CD223) regulates the size of the expanding T cell population following antigen activation in vivo. *J Immunol* 2004;172:5450–5455
- 124a. Lineberry N, Fathman CG. CYLD; deubiquitination-induced TCR signaling. *Nat Immunol* 2006;7:369–370
- 124b. Reiley WW, Jin W, Lee AJ et al. CYLD negatively regulates the kinase Tak1 and prevents abnormal T cell responses. *J Exp Med* 2007;204:1475–1485
- 124c. Sun S-C. Deubiquitylation and regulation of the immune response. *Nature Revs Immunol* 2008;8:501–511
125. Abboushi N, El-Hed A, El-Assaad W et al. Ceramide inhibits Il-2 production by preventing protein kinase C dependent NF κ B activation: possible role in PKC θ regulation. *J Immunol* 2004;173:3193–3200
126. Egen JG, Allison JP. CTLA4 accumulation in the immunological synapse is regulated by T cell receptor signal strength. *Immunity* 2002;16:23–25
127. Pandiyan P, Hegel J, Krueger M et al. High IFN γ production of CD8 T lymphocytes is controlled by CD152(CTLA4). *J Immunol* 2007;172:2132–2140
128. Valk E, Leung R, Kang H et al. T cell receptor interacting molecule acts as a chaperone for surface expression of CTLA4. *Immunity* 2006;25:807–821
129. Mellor AL, Chandler P, Baban B, Hansen AM et al. Specific subsets of murine dendritic cells acquire potent T cell regulatory functions following CTLA4 mediated induction of indoleamine 2,3 dioxygenase. *Int Immunol* 2004;16:1391–1401
130. Krummel MF, Allison JP. CTLA4 engagement inhibits Il-2 accumulation and cell cycle progression upon activation of resting T cells. *J Exp Med* 1996;183:2533–2538
131. Jackman RP, Balamuth F, Bottomly K. CTLA4 differentially regulates the immunological synapse in CD4 T cell subsets. *J Immunol* 2007;178:5543–5551
132. Guntermann C, Alexander DR. CTLA4 suppresses proximal TCR signaling in resting human CD4 T cells by inhibiting Zap70 phosphorylation. *J Immunol* 2002; 168:4420–4429

133. Gotta L, Calviello G, DiNuciuolo F et al. CTLA4 inhibits integrin mediated stimulation. *Immunology* 2002;107:209–216
134. Greenwald RJ, Boussiotis VA, Lorschach RB et al. CTLA4 regulates induction of anergy in vivo. *Immunity* 2001;14:145–148
135. Ueda H, Howson J, Esposito L et al. Association of T cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 2003;423:506–511
136. Steel C, Nutman TB. CTLA4 in filarial infections: a role in diminished T cell reactivity. *J Immunol* 2003;170:1930–1938
137. Deng C, Radu C, Diab A, Tsen MF et al. IL-1 Receptor Antagonist Kinase I regulates susceptibility to organ specific autoimmunity. *J Immunol* 2003;170:2833–2842
138. Mor A, Philipis MR, Pillinger MH. The role of Ras signaling in lupus T lymphocytes; biology and pathogenesis. *Clin Immunol* 2007;125:215–223
139. Kammer GM. Altered IL-2 production in SLE. *J Clin Invest* 2005;115:836–840
140. Katsiari CG, Kyttaris VC, Juang Y-T, Tsokos GC. Protein phosphatase 2A is a negative regulator of IL-2 production in patients with SLE. *J Clin Invest* 2005;115:3193–3204
141. Crispin JC, Kyttaris V, Juang Y-T, Tsokos GC. How signaling and gene transcription aberrations dictate the SLE T cell phenotype. *Trends Immunol* 2008;29:110–115
142. Fanzo JC, Yang W, Jang SY et al. Loss of IRF4 binding protein leads to development of systemic autoimmunity. *J Clin Invest* 2006;116:703–714
143. Fan H, Patel VA, Longacre A, Levine JS. Abnormal regulation of the cytoskeletal modulator Rho typifies macrophages of the major murine models of spontaneous autoimmunity. *J Leukoc Biol* 2006;79:155–165
144. Krishnan D, Warke VG, Nambier MP et al. Altered T cell receptor signaling complex in effector T cells. *J Immunol* 2003;170:4189–4195
- 145a. Kim EY, Priatel JJ, Teh S-J, Teh H-S. TNFR2 (p75) functions as a costimulator for antigen-driven T cell responses in vivo. *J Immunol* 2006;176:1026–1035
- 145b. Martins GA, Cimmino L, Shapiro-Shelef M et al. Transcriptional repressor Blimp-1 regulates T cell homeostasis and function *Mol Immunol* 2006;7:457–465
146. Wei S, Gamero AM, Liu JH et al. Control of lytic function by MAPK/ERK in a human NK cell line. *J Exp Med* 1998;187:1753–1757
147. Lou Z, Billadeau DD, Savoy DN et al. A role for RhoA/Rock/LIM kinase pathway in the regulation of cytotoxic lymphocytes. *J Immunol* 2001;167:5749–5757
148. Glimcher LH, Townsend MJ, Sullivan BM, Lord GM. Recent developments in transcriptional regulation of cytolytic effector cells. *Nat Rev Immunol* 2004;4:900–911
149. Michalek RD, Nelson KJ, Holbrook BC et al. The requirement of reversible cysteine sulfenic acid formation for T cell activation and function. *J Immunol* 2007;179:6456–6467
150. Radoja S, Frey AB, Vukmanovic S. T cell receptor signal events triggering granule exocytosis. *Crit Rev Immunol* 2006;26:265–290
151. Holt OJ, Gallo F, Griffiths GM. Regulating secretory lysosomes. *J Biochem* 2006;140:7–12
152. Rochford R, Riggs JE, Clavo A et al. Differential effects of CD28 costimulation upon cytokine production by CD4/CD8 T cells. *Immunobiology* 2004;209:513–527
- 153a. Richter MV, Topham DJ. $\alpha 1\beta 1$ Integrin and TNF receptor II protects airway CD8+ effector T cells from apoptosis during influenza infection. *J Immunol* 2007;179:5054–5063
- 153b. Ma JSY, Hayder TF, Radoja S. Protein kinase C δ localizes to secretory lysosomes in CD8+ CTLs and directly mediates TCR signals leading to granule exocytosis-mediated cytotoxicity. *J Immunol* 2008;181:4716–4722
154. Arias CF, Ballesteros-Tato A, Garcia MI et al. p21 controls proliferation of activated memory T cells and affects homeostasis of T cell responses. *J Immunol* 2007;178:2296–2306
155. Ichii H, Sakamoto A, Kuroda Y, Tokihisa T. Bcl-6 acts as an amplifier for the generation and proliferative capacity of central memory CD8 T cells. *J Immunol* 2004;173:883–891

156. Chandok MR, Okoye FI, Ndejemi M, Farber DL. A biochemical signature for rapid recall of memory CD4 T cells. *J Immunol* 2007;179:3689–3698
157. Ndejemi MP, Teijaro JR, Patke DS et al. Control of memory CD4 T cell recall by the CD28/B7 costimulatory pathway. *J Immunol* 2006;177:7698–7706
158. Watson ARO, Lee WT. Differences in signaling molecule organization between naive and memory CD4 T lymphocytes. *J Immunol* 2004;173:33–41
159. Hussain SF, Anderson CF, Farber DL. Differential SLP76 expression and T cell receptor signaling in effector and memory CD4 cells. *J Immunol* 2002;168:1557–1565
160. Jago CB, Yates J, Saraiva Camara NO, Lechler RI, Lombardi G. Differential expression of CTLA4 among T cell subsets. *Clin Exp Immunol* 2004;136:463–471
161. Krieg C, Boyman O, Fu Y-X, Kaye J. BTLA regulates CD8 T cell homeostasis and memory cell generation. *Nature Immunol* 2007;8:162–171
162. Chang JT, Palanivel VR, Kiniyo I et al. Asymmetric T lymphocyte division in adaptive immune responses. *Science* 2007;315:1687–1691
163. Bevan MJ. Helping the CD8+ T cell response. *Nat Revs Immunol* 2004;4:595–602
164. Harty JT, Badovinac VP. Shaping and reshaping CD8+ T cell memory. *Nat Revs Immunol* 2008;8:107–119
165. Raberger J, Schebesta A, Sataguchi S et al. The transcriptional regulator PLZF induces the development of CD44 high memory phenotype T cells. *Proc Natl. Acad Sci. USA* 2008;105(46):17919–17924
166. Hu J, August A. Naive and innate memory phenotype CD4+ T cells have different requirements for active Itk for their development. *J Immunol* 2008;180:6544–6552
167. Liu Z, Dai H, Wan N, Wang T et al. Suppression of memory CD8 T cell generation and function by tryptophan catabolism. *J Immunol* 2007;178:4260–4266
168. Hildeman DA, Mirchell T, Kappler J, Marrack P. T cell apoptosis and reactive oxygen species. *J Clin Invest* 2003;111:305–334
169. Arnold R, Brenner D, Becker M, Frey CR, Krammer PH. How T lymphocytes switch between life and death. *Eur J Immunol* 2006;36:1654–1658
170. Jorgensen TN, McKee A, Wang M et al. Bim and Bcl2 mutually affect the expression of the other in T cells. *J Immunol* 2007;179:3417–3424
171. Marhaba R, Bourouba M, Zoller M. CD44v7 interferes with AICD by upregulation of antiapoptotic gene expression. *J Leukoc Biol* 2003;74:135–148
172. Ramaswamy M, Dumont C, Cruz AC et al. Rac GTPase sensitize activated T cells to die via Fas. *J Immunol* 2007;179:6384–6388
173. Brenner D, Golks A, Kiefer F et al. Activation or suppression of NFkB by HPK1 determines sensitivity to AICD. *EMBO J* 2005;24:4279–4290
174. Sohn SJ, Rajpal A, Winoto A. Apoptosis during lymphoid development. *Curr Opin Immunol* 2003;15:209–216
175. Yasumoto K, Doyle C, Miele L, Germain RN. The duration of antigen receptor signaling determines CD4 versus CD8 T cell lineage fate. *Nature* 2000;404:506–510
176. Powell JD. The induction of T cell anergy. *Clin Immunol* 2006;120:239–246
177. Schwartz RH. T cell anergy. *Annu Rev Immunol* 2003;21:305–334
178. Fathman CG, Lineberry NB. CD4T cell anergy. *Nat Rev Immunol* 2007;7:599–609
179. Macian F, Garcia-Cozar M, Im S-H et al. Transcriptional mechanisms underlying lymphocyte tolerance. *Cell* 2002;109:719–731
180. Mirshahidi S, Korb Ferris LC, Sadegh-Nasseri S. The magnitude of T cell receptor engagement is a predictor of T cell anergy or activation. *J Immunol* 2004;172:5346–5355
181. Wells AD, Liu G-H, Hondowicz B et al. Regulation of T cell activation and tolerance by phospholipase C γ 1 dependent integrin avidity modulation. *J Immunol* 2003;170:4127–4133
- 182a. Mueller D. E3 ubiquitin ligases T cell anergy factors. *Nat Immun* 2004;5:883–890
- 182b. Melino G, Gallagher E, Aqeilan R I et al. Itch: a HECT-type E3 ligase regulating immunity, skin and cancer. *Cell death and differentiation* 2008;15:1103–1112
- 183a. Safford M, Collins S, Lutz MA et al. Egr2 and Egr3 are negative regulators of T cell activation. *Nat Immun* 2005;6:472–480

- 183b. King CG, Buckler JL, Kobayashi T et al. TRAF6 and T cell anergy. *J Immunol* 2008;180:34–38
- 183c. Gu H. Anergic Signals: the action is upstream. *Immunity* 2008;28:598–600
184. Hundt M, Tabata H, Jeon MS et al. Impaired activation and localization of LAT in anergic T cells due to selective palmitoylation defect. *Immunity* 2006;24:513–522
185. Zha Y, Marks R, Ho AW et al. T cell anergy is reversed by active Ras and is regulated by diacylglycerol kinase α . *Nat Immunol* 2006;7:1166–1173
186. Tzachanis D, Appleman LJ, van Puijenbroek A et al. Differential localization and function of ADP-ribosylation factor 6 in anergic human T cells/marker for identification. *J Immunol* 2003;171:1691–1696
187. Soares L, Seoogy C, Skrenta H, Anandasabapathy N et al. Two isoforms of otubain 1 regulate T cell anergy via Grail. *Nat Immun* 2004;5:45–54
188. Zheng Y, Collins SL, Lutz MA et al. A role for mTOR in regulating T cell activation versus anergy. *J Immunol* 2007;178:2163–2170
189. Filby A, Seddon B, Kleczkowska J et al. Fyn regulates the duration of TCR engagement needed for commitment to effector function. *J Immunol* 2007;179:4635–4644
190. Davidson D, Schraven B, Veillette A. PAG associated FynT regulates calcium signalling and promotes anergy in T lymphocytes. *Mol Cell Biol* 2007;27:1960–1973
191. Jeon MS et al. Essential role of the E3-ubiquitin ligase c-cbl in T cell anergy induction. *Immunity* 2004;21:167–177
192. James MJ, Beleramani L, Prodromidou K et al. Anergic T cells exert antigen independent inhibition of cell-cell interactions via chemokine metabolism. *Blood* 2003;102:2173–2179
193. Li L, Iwamoto Y, Berezovskaya A, Boussiotis VA. A pathway regulated by cell cycle inhibitor p27^{kip1} and check-point inhibitor Smad 3 is involved in the induction of T cell tolerance. *Nat Immunol* 2006;7:1157–1165
194. DiSanto JP. Lung Kruppel like factor: a quintessential player in T cell quiescence. *Nat Immun* 2001;2:952–958
195. Sarkar C, Das S, Chakroborty D. Stimulation of dopamine D4 receptors induce T cell quiescence by upregulating KLF2. *J Immunol* 2006;177:7525–7529
196. Yusuf I, Fruman DA. Regulation of quiescence in lymphocytes. *Trends Immunol* 2003;24:380–386
197. Yu C-R, Mahdi RM, Ebang S et al. Socs 3 regulates proliferation and activation of T helper cells. *J Biol Chem* 2003;278:29752–29759
198. Boon RA, Fledderus JO, Volger OL et al. KLF2 suppresses TGF β signaling in endothelium. *Arterioscler Thromb Vasc Biol* 2006;27:532–539
199. Scharf W, Burdorch S, Hansen G. Reduced expression of TGF β 1 exacerbates pathology in an experimental asthma model. *Eur J Immunol* 2005;35:198–206
200. Cottrez F, Groux G. Regulation of TGF β response during T cell activation is modulated by IL-10. *J Immunol* 2001;167:773–778
201. Baniyash M. TCR ζ -chain downregulation: curtailing an excessive inflammatory immune response. *Nat Rev Immunol* 2004;4:675–687
202. Staffler G, Szekeres A, Schutz G I et al. Selective inhibition of T cell activation via CD147 through novel modulation of lipid rafts. *J Immunol* 2003;171:1707–1714
203. Enk AH, Knop J. T cell receptor mimic peptides and their potential application in T cell mediated diseases. *Int Arch Allergy Appl Immunol* 2000;123:275–281
204. Wang C, Mooney JL, Meza-Romero R et al. Recombinant TCR ligand induces early T cell receptor signaling and a unique pattern of downstream activation. *J Immunol* 2003;171:1939–1940
205. Mellor AL, Babon R, Chandler P et al. Indoleamine 2,3 dioxygenase expression in dendritic cell subsets suppresses T cell clonal expansion. *J Immunol* 2003;171:1232–1239
206. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol* 2004;4:762–774
207. Shevach EM. CD4⁺ CD25⁺ suppressor T cells. *Nat Rev Immunol* 2002;2:389–400
208. Rouse BT. Regulatory T cells in health and disease. *J Int Med* 2007;262:78–95
209. Mahic M, Yaqub S, Johansson CC et al. Foxp3 T reg cells express Cox-2 and suppress effector T cells by a PGE2 dependent mechanism. *J Immunol* 2006;127:246–254

210. Hamdi H, Godot V, Maillot MC et al. Induction of antigen specific regulatory T lymphocytes by human dendritic cells expressing the GILZ. *Blood* 2007;110:211–219
211. Wohlfort EA, Nichols FC, Nevius E, Clark RB. PPAR γ and immune regulation: enhancement of regulatory T cells. *J Immunol* 2007;178:4129–4135
212. Grossman WJ, Verbsky JW, Barchet W, Colonna M et al. Human T regulatory cells can use the perforin pathway to cause autologous target cell death. *Immunity* 2004;21:589–601
213. Garin MI, Chu C-C, Galshaya D et al. Galectin-1, a key effector of regulation mediated by CD4⁺ CD25⁺ T cells. *Blood* 2007;109:2058–2065
214. Kobie JJ, Shah PR, Yang L et al. T regulatory and primed CD4 T cells express CD73 which suppresses effector CD4 T cells by adenosine. *J Immunol* 2006;177:6780–6786
215. Bopp T, Becker C, Klein M et al. Cyclic AMP is a key component of regulatory T cell mediated suppression. *J Exp Med* 2007;204:1303–1310
216. Peluso I, Fantini MC, Fina D et al. IL-21 counteracts the regulatory T cell mediated suppression of human CD4 T lymphocytes. *J Immunol* 2007;178:732–737
217. Neufert C, Becker C, Wirtz S et al. IL-27 controls the development of inducible regulatory T cells and Th17 cells via differential effects on Stat1. *Eur J Immunol* 2007;37:1809–1816
218. So T, Croft M. OX40 inhibits TGF β and antigen driven conversion of naïve CD4 T cells into CD25⁺ Foxp3 T cells. *J Immunol* 2007;179:1427–1430
219. Polanczyk MJ, Carson BD, Subramanian S et al. Estrogen drives expansion of the CD4⁺ CD25⁺ regulatory T cell compartment. *J Immunol* 2004;173:2227–2230
220. Maloy KJ, Powrie F. IL-2 keeps CD4⁺ Treg cells fit. *Nat Immunol* 2005;6:1071–1072
221. Ho L, Crabtree G. A Foxy tango with NFAT. *Nat Immunol* 2006;7:906–908
222. Valencia X, Stephens G, Goldbach-Mansky R et al. TNF α downmodulates the function of Treg cells signaling through TNFR.II. *Blood* 2006;108:253–261
223. Hickman SP, Yang J, Thomas RM et al. Defective activation of PKC and Ras-ERK pathways limits IL-2 production and proliferation by CD4⁺ CD25⁺ regulatory T cells. *J Immunol* 2006;177:2186–2194
224. Toda A, Piccirillo CA. Development and function of CD4⁺ CD25⁺ regulatory T cells. *J Leukoc Biol* 2006;80:458–470
225. Tsang TY-S, Camara NO, Eren E et al. Altered proximal T cell receptor signaling in human CD4⁺ CD25⁺ regulatory T cells. *J Leukoc Biol* 2006;80:145–151
- 226a. Campbell DJ, Ziegler SF. Foxp3 modifies the phenotypic and functional properties of regulatory T cells. *Nat Rev Immunol* 2007;7:305–310
- 226b. Ito T, Hanabuchi S, Wang YH et al. Two functional subsets of FOXP3⁺ regulatory T Cells in human thymus and periphery. *Immunity* 2008;28:870–880
227. Mills KHG. Regulatory T cells: friend or foe in immunity to infection? *Nat Rev Immunol* 2004;4:841–855
228. Mahnke K, Ring S, Johnson TS et al. Induction of immunosuppressive functions of dendritic cells in vivo by T regulatory cells. *Eur J Immunol* 2007;37:2117–2126
229. Chen C, Lee W-H, Zhang L, Liu CP. Regulatory T cells can mediate their function through the stimulation of APCs to produce immunosuppressive nitric oxide. *J Immunol* 2006;176:3449–3460
230. Oderup C, Cederbom A, Makowska A et al. CTLA4 dependent downgrade of costimulatory molecules on DCs in T reg suppression. *Immunity* 2006;118:240–249
231. Lewkowicz P, Lewkowicz N, Sasiak A, Tchorzewski H. Lipopolysaccharide activated CD4 CD25⁺ T reg cells inhibit neutrophil function and promote their apoptosis and death. *J Immunol* 2006;177:7155–7163
232. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003;299:1057–1061
233. Huang C-T, Workman CJ, Flies D et al. Role of LAG3 in regulatory T cells. *Immunity* 2004;21:503–515
234. Uraushihara K, Kanai T, Ko K et al. Regulation of murine inflammatory bowel disease by CD25⁺ and CD25⁺CD4⁺ GITR gene regulatory T cells. *J Immunol* 2003;171:708–716

235. Grimbert P, Bouguermouh S, Baba N et al. Thrombospondin/CD47 interaction: a pathway to generate T cells from human CD4⁺ CD25⁻ T cells in response to inflammation. *J Immunol* 2006;177:3534–3551
236. Lu L-F, Lind EF, Gondek DC et al. Mast cells are intermediaries in regulating T cell tolerance. *Nature* 2006;442:997–1002
237. Duthoit CT, Mekala DJ, Alli RS, Geiger TL. Uncoupling of IL-2 signaling from cell cycle progression in naïve CD4⁺ T cells by regulatory CD4⁺ CD25⁺ T lymphocytes. *J Immunol* 2005;174:155–163
238. Sumoza-Toledo A, Eaton AD, Sarukhan A. Regulatory T cells inhibit PKC θ recruitment to the immune synapse of naïve T cells with the same antigen specificity. *J Immunol* 2006;176:5779–5787
239. Oida T, Xu L, Weiner HL et al. TGF β mediated suppression by CD4⁺ CD25⁺ T cells is facilitated by CTLA4 signaling. *J Immunol* 2006;177:2331–2339
240. Fallarino F, Grohmann U, Hwang KW et al. Modulation of tryptophan catabolism by regulatory T cells. *Nat Immunol* 2003;4:1206–1212
241. Rochetti S, Zollo O, Bruscoli S et al. GITR is costimulatory to mouse T lymphocyte subpopulations. *Eur J Immunol* 2004;34:613–622
242. Kanamura F, Youngnak P, Hashiguchi M et al. Costimulation via GITR in both conventional and CD25⁺ regulatory CD4 T cells. *J Immunol* 2004;172:7306–7314
243. Kryczek I, Wei S, Zou L et al. Induction of B7-H4 on APCs through IL-10: novel suppressive mode for regulatory T cells. *J Immunol* 2006;177:40–44
244. Fontenot JD, Rasmussen JP, Williams LM et al. Regulatory T cell lineage specification by the forkhead transcription factor Foxp3. *Immunity* 2005;22:329–341
245. Ziegler SF. FoxP3: of mice and men. *Annu Rev Immunol* 2006;24:209–226
246. Chen W, Jin W, Hardegen N et al. Conversion of peripheral naïve T cells to regulatory T cells by TGF β induction of transcription factor Foxp3. *J Exp Med* 2003;198:1875–1886
247. Vieir PL, Christensen JR, Minase S et al. IL-10 secreting regulatory T do not express Foxp3 but have comparable regulatory function to naturally occurring CD4⁺ CD25⁺ regulatory T cells. *J Immunol* 2004;172:5986–5993
- 248a. Wang Z, Hong J, Sun W et al. Role of IFN γ in induction of Foxp3 and conversion of CD4⁺ CD25⁺ T cells to CD4⁺ Tregs. *J Clin Invest* 2006;116:2434–2441
- 248b. Vignali DA, Collison LW, Workman CJ. How regulatory T cells work *Nat Rev Immunol*. 2008;8:523–532
- 248c. Chang CC, Ciubotariu R, Manavalan JS et al. Tolerization of dendritic cells by TS cells. *Nat Immunol*. 2002;3:237–243
249. Romagnani S. Lymphokine production by human T cells in disease. *Annu Rev Immunol* 1994;12:227–257
250. Scott P. Interleukin 12: initiation cytokine for cell mediated immunity. *Science* 1993;260:496–497
251. Morel PA, Oriss TB. Crossregulation between Th.1 and Th.2 cells. *Crit Rev Immunol* 1998;18:275–303
252. Kuo T, Leiden JM. Transcriptional regulation of T lymphocyte development and function. *Annu Rev Immunol* 1999;17:149–188
253. Takeda A, Hamono S, Yamaka A et al. Role of IL-27 signaling for induction of T-bet through activation of Stat.1 during initial Th.1 commitment. *J Immunol* 2003;170:4886–4890
254. Robinson DS, O'Garra A. Checkpoints in Th.1 development. *Immunity* 2002;16:755–758
255. Rengarajan J, Szabo SJ, Glimcher LH. Transcriptional regulation of Th-1/Th-2 polarization. *Immunol Today* 2000;21:479–483
256. Murphy KM, Ouyang W, Faffar JD et al. Signaling and transcription in T helper development *Annu Rev Immunol* 2000;18:451–494
257. Athie-Morales V, Smits HH, Cantrell DA, Hilken CM. Sustained IL-12 signaling is required for Th1 development. *J Immunol* 2004;172:61–69
258. Maldonado RA, Irvine DJ, Schreiber R, Glimcher LH. A role for the immunological synapse in lineage commitment of CD4 lymphocytes. *Nature* 2004;431:527–530

259. Thauland TJ, Koguchi Y, Wetzel SA et al. Th1 and Th2 cells form morphologically distinct immunological synapses. *J. Immunol* 2008;181:393–399
260. O'Garra A, Arai N. The molecular basis of T helper-1 and T helper-2 differentiation. *Trends Cell Biol* 2000;10:542–549
261. Miller AT, Wilcox HM, Lai Z, Berg LJ. Signaling through Itk promotes T helper-2 differentiation via negative regulation of T-bet. *Immunity* 2004;21:67–80
262. Mikhalkovich N, Becknell B, Caligiuri MA et al. Responsiveness of naïve CD4 T cells to polarizing cytokine determines the ratio of Th1 and Th2 cell differentiation. *J Immunol* 2006;176:1553–1560
263. Gollob J, Murphy FA, Mahjan S et al. Altered Il-12 responsiveness in Th.1 and Th.2 cells is associated with differential activation of Stat5 and Stat1. *Blood* 1998;91:1341–1354
264. Aronica MA, Mora AL, Mitchell DB et al. Preferential role for NFkB/Rel signaling in type 1 but not type 2 dependent immune response. *J Immunol* 1999;163:5116–5124
265. Carbonari M, Tedesco T, Del Porto P et al. Human T cells with type 2 cytokine profile are resistant to apoptosis induced by primary activation. *Clin Exp Immunol* 2000;120:454–462
266. Roozendaal R, Vellenga E, de Jong MA et al. Resistance of activated human Th.2 cells to nitric oxide induced apoptosis is mediated by gamma-glutamyltransferase. *Int Immunol* 2001;13:519–528
267. Zheng W, Flavell RA. GATA3 is necessary and sufficient for Th-2 cytokine gene expression. *Cell* 1997;89:587–596
268. Chen Ho BI, Lo D, Glimcher LH. C-maf promotes Th.2 and attenuates Th.1 differentiation by both Il-4 dependent and independent mechanisms. *J Exp Med* 1998;188:1859–1866
269. Sloan Lancaster J, Steinberg TH, Allen PM. Selective loss of the calcium ion signaling pathway in T cells maturing toward a T helper-2 phenotype. *J Immunol* 1997;159:1160–1168
270. Vaodhachory AS, Peto ME, Perdow SN et al. Selective upregulation of PI 3-kinase in Th2 cells: resistance to Fas mediated apoptosis. *J Immunol* 1999;163:4772–4779
271. Nagat K, Tanaka K, Ogawa K et al. Selective expression of a novel surface molecule by human Th2 cells in vivo. *J Immunol* 1999;162:1278–1286
272. Takeda A, Hamono S, Yamaka A et al. Role of Il-27 signaling for induction of T-bet through activation of Stat.1 during initial Th.1 commitment. *J Immunol* 2003;170:4886–4890
273. Owaki T, Asakawa M, Fukai F et al. Il-27 induces Th1 differentiation via p38 MAPK/T-bet and ICAM-1/ERK dependent pathways. *J Immunol* 2006;177:7579–7587
274. Rengarajan J, Szabo SJ, Glimcher LH. Transcriptional regulation of Th-1/Th-2 polarization. *Immunol Today* 2000;21:479–483
275. Osborne BA, Minter LM. Notch signalling during peripheral T cell activation and differentiation. *Immunol Rev* 2007;7:64–75
276. Ansel KM, Djuretic I, Tanasc B, Rao A. Regulation of Th2 differentiation and Il-4 locus accessibility. *Annu Rev Immunol* 2006;24:607–656
277. Ansel KM, Lee DU, Rao A. An epigenetic view of helper T cell differentiation. *Nat Immunol* 2003;4:616–623
278. Tanaka S. Conserved non-coding sequence 2 downstream of the Il-4 locus is an enhancer in NKT and CD4 Th2 cells. *Immunity* 2006;24:689–701
279. King MR, Ismail AS, Davis LS, Kemp DR. Oxidative stress promotes polarization of human T cell differentiation toward a T helper-2 phenotype. *J Immunol* 2006;176:2765–2771
280. Nagamachi M, Sakata D, Kabashima K et al. Facilitation of Th1 mediated immune response by prostaglandin E receptor EP1. *J Exp Med* 2007;204:2865–2874
281. Kim CH, Broxmeyer HE. Chemokines: signal lamps for trafficking of T and B cells for development and effector function. *J Leukoc Biol* 1999;65:6–15
282. Sallusto F, Lanzavecchia A, Mackay CR. Chemokines and chemokine receptors in T cell priming and Th-1/2 responses. *Immunol Today* 1998;10:568–574
283. Kumar A, Humphreys TD, Kremer KN et al. CXCR4 physically associates with the T cell receptor to signal in T cells. *Immunity* 2006;25:213–224

284. Liu J, Cao S, Herman LM, Ma X. Differential regulation of Il-12p35 and p40 gene expression and IFN γ production by IFN regulatory factor-1. *J Exp Med* 2003;198:1265–1276
285. Utsugi M, Dobashi K, Ishizuka T et al. C-jun N-terminal kinase negatively regulates LPS induced Il-12 production in human macrophages; glutathione redox regulation of Il-12 production. *J Immunol* 2003;171:628–635
286. Presky DH, Yang H, Minetto LJ et al. A functional Il-12 receptor complex is composed of two β -type cytokine receptor units. *Proc Natl Acad Sci U S A* 1996;93:14002–14007
- 287a. Shaw MH, Freeman GJ, Scott MF et al. Tyk2 negatively regulates adaptive Th1 immunity by mediating Il-10 signaling and promoting IFN γ dependent Il-10 reactivation. *J Immunol* 2006;176:7263–7271
- 287b. Goriely S, Neurath MF, Goldman M. How microorganisms tip the balance between interleukin-12 family members. *Nat Revs Immunol* 2008;8:81–86
288. Fahey AJ, Robino RA, Kindle KB, Heeny DM. Effects of glucocorticoid on Stat4 activation in human T cells are stimulus dependent. *J Leukoc Biol* 2006;80:133–144
289. Ahn H-J, Tomura M, Yu WG et al. Requirement for distinct Janus kinases and Stat proteins in T cell proliferation versus IFN γ production with Il-12 stimulation. *J Immunol* 1998;161:5893–5900
290. Carter LL, Murphy KM. Requirement for Stat4 in interferon γ production from CD4 and CD8 T cells. *J Exp Med* 1999;189:1355–1360
291. Yang J, Murphy TL, Ouyang W, Murphy KM. Induction of interferon γ production in Th1 CD4 T cells: two distinct pathways for promoter activation. *Eur J Immunol* 1999;29:548–555
292. Nembrini C, Abel B, Kopf M, Marsland BJ. Strong TCR signaling, TLR ligands, and cytokine redundancies ensure robust development of type 1 effector T cells. *J Immunol* 2006;176:7180–7188
293. Prehn JL, Thomas LS, Landers CJ et al. The T cell costimulator TL1A is induced by Fc γ R signaling in human monocytes and dendritic cells. *J Immunol* 2007;178:4033–4038
294. Hurgin V, Novick D, Werman A et al. Antiviral and immunoregulatory activities of IFN γ depend on constitutively expressed Il-1 α . *Proc Natl Acad Sci U S A* 2007;104:5044–5049
295. Zhang F, Wang DZ, Boothby M et al. Regulation of the activity of the IFN-gamma promoter elements during T helper cell differentiation. *J Immunol* 1998;161:6105–6110
- 296a. Hatton RD, Harrington LE, Luther RJ et al. A distal conserved sequence element controls IFN γ gene expression by T cells and NK cells. *Immunity* 2006;25:717–730
- 296b. Murphy KM. Jak3 and STAT5 signaling molecules give the green light for T Helper 1 cell differentiation. *Immunity* 2008;28:725–727
- 297a. Singh H. Shaping a helper T cell identity. *Nat Immunol* 2007;8(2):119–120
- 297b. Shnyreva M, Weaver WM, Blanchette M et al. Evolutionarily conserved sequence elements that positively regulate IFN- γ expression in T cells. *Proc Natl Acad Sci (USA)* 2004;101:12622–12627
298. Becknell B, Hughes TL, Freud AG et al. Hlx homeobox transcription factor negatively regulates IFN γ production in NK cells. *Blood* 2007;109:2481–2487
299. Samten B, Howard ST, Weis SE et al. cAMP Response element binding protein positively regulates production of IFN-gamma by T cells in response to a microbial pathogen. *J Immunol* 2005;174:6357–6363
300. Park I-K, Letterio JJ, Gorhan JD. TGF β 1 inhibition of IFN γ induced signalling and Th1 gene expression. *Mol Immunol* 2007;44:3283–3290
301. Young HA. Unravelling the pros and cons of IFN γ gene regulation. *Immunity* 2006;24:506–507
302. Takeba Y, Nagafuchi H, Takeno M et al. Tbx acts as a Th1 transcription factor and regulates IFN γ gene transcription. *J Immunol* 2002;168:2365–2370
303. Li B, Yu H, Zheng W. Role of guanosine triphosphatase Rac-2 in T helper-1 cell differentiation. *Science* 2000;288:2219–2222
304. Boisvert M, Gendron S, Chetoui N, Aoudjit F. α 2 β 1 integrin signaling augments TCR dependent production of interferon-gamma in human T cells. *Mol Immunol* 2007;44:3732–3740

305. White GP, Hollams EM, Yerkovich ST et al. CpG methylation patterns in the IFN γ promoter in naïve T cells: variations during Th1 and Th2 differentiation and between atopics and non-atopics. *Paediatr Allergy Immunol* 2006;17:557–564
306. Fitzpatrick DR, Murali-Krishna K, Shires J et al. Demethylation of the IFN γ gene occurs in memory naïve CD8 T cells. *J Immunol* 2006;176:4083–4093
- 307a. Minguela A, Pastor S, Mi W, Richardson JA, Ward ES. Feedback regulation of murine autoimmunity via dominant anti-inflammatory effects of IFN γ . *J Immunol* 2007;178:134–144
- 307b. Hu X, Chakravarty SD, Ivashkiv LB. Regulation of interferon and TLR signaling during macrophage activation. *Immunol Rev*. 2008;226:41–56
308. Gao Y, Grassi F, Ryan MR et al. IFN γ stimulates osteoclast formation and bone loss in vivo via antigen driven T cell activation. *J Clin Invest* 2007;117:122–132
309. Feuerer M, Eulenburg K, Loddenkemper C et al. Self limit of Th1 mediated inflammation by IFN γ . *J Immunol* 2006;176:2857–2863
- 310a. Suto A, Wurster AL, Reiner SL, Grusby MJ. IL-21 inhibits interferon production in developing Th1 cells through the repression of eomesodermin expression. *J Immunol* 2006;177:3721–3727
- 310b. Regis G, Pensa S, Boselli D et al. The STAT1: STAT3 seesaw of Interferon and gp130 receptor signaling *Semin Cell Dev Biol* 2008;19:351–359
- 310c. Kimura A, Naka Kishimoto T. IL-6 dependent and independent pathways in the development of IL-17 producing T helper cells. *Proc Natl Acad Sci (USA)* 2007;104:12099–12104
- 310d. Deenick EK, Tangye SG. IL-21: a new player in Th17 cell differentiation. *Immunol Cell Biol* 2007;85:503–505
- 310e. Iwamoto S, Iwai S, Tsuchiyama K et al. TNF α drives CD14 $^{+}$ monocytes to differentiate into CD70 $^{+}$ dendritic cells evoking Th-1 and Th17 responses. *J Immunol* 2007;179:1449–1457
- 310f. Schmidt-Weber CB, Akdis M, Akdis CA Th17 cells in the big picture of immunology. *J All Clin Immunol* 2007;120:247–254
- 310g. Mucida D, Park Y, Kim G et al. Reciprocal Th17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 2007;317:256–260
- 310h. Shen S, Gaffen SL. Structure function relationships in the IL-17 receptor: implications for signal transduction and therapy. *Cytokine* 2008;41:92–104
311. Nirula A, Ho M, Phee H et al. PDK-1 targets protein kinase A in a pathway that regulates interleukin 4. *J Exp Med* 2006;203:1733–1744
312. McKenzie ANJ, Fallon PG. Decoy receptors in the regulation of T helper cell type 2 responses. *J Exp Med* 2003;197:675–679
313. Jakubzick C, Choi ES, Joshi BH et al. Therapeutic attenuation of pulmonary fibrosis via targeting of IL-4 and IL-13 responsive cells. *J Immunol* 2003;171:2684–2693
- 314a. Wilson MS, Elnekave E, Mentink-Kane MM et al. IL-13R α 2 and IL-10 coordinately suppress airway inflammation and hyperreactivity in mice. *J Clin Invest* 2007;117:2941–2951
- 314b. Kasaian MT, Miller DK. IL-13 as a therapeutic target for respiratory disease. *Biochem Pharmacol* 2008;76:147–155.
315. Cohn L, Elias JA, Chupp GL. Asthma. *Annu Rev Immunol* 2004;22:789–815
316. Busse WW, Lemanske RF. Advances in asthma. *N Engl J Med* 2001;344:350–362
317. Zhou B, Comeo MR, De Smedt T et al. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immun* 2005;6:1047–1053
318. Liu Y-J, Soumelis V, Watanabe N et al. TSLP: an epithelial cell cytokine that regulates T cell differentiation by conditioning DC maturation. *Annu Rev Immunol* 2007;25:193–219
319. Liu Y-J. TSLP and OX40 ligand pathway in the initiation of dendritic cell mediated allergic inflammation. *J Allergy Clin Immunol* 2007;120:238–244
320. Li Y, Chu N, Rostami A, Zhang G-X. Dendritic cells transduced with SOCS3 direct Th2 cell differentiation. *J Immunol* 2006;177:1679–1688
321. Mackenzie J, Mattes J, Dent LA, Foster PS. Eosinophils promote allergic disease of lungs by regulation CD4 Th2 lymphocyte function. *J Immunol* 2001;167:3146–3155

- 322. Shiraishi Y, Asano K, Nakajima T et al. PGD2 induced eosinophilic airway inflammation is mediated by CRTH2 receptor. *J Pharmacol Exp Ther* 2005;312:954–960
- 323a. Jones CE. The OXE receptor. *Trends Mol Med* 2005;11:266–270
- 323b. Bendelac A, Savage PB, Teyton L. The biology of NKT cells. *Annu Rev Immunol* 2007;25:297–336
- 324. Webb DC, Matthaai KI, Cai Y et al. Polymorphisms in Il-4 receptor alpha correlate with airway hyperreactivity in allergic mice. *J Immunol* 2004;172:1092–1098
- 325. Ali K, Bilancio A, Thomas M et al. Essential role for p110 δ PI 3-kinase in the allergic response. *Nature* 2004;431:1007–1011
- 326. Okunishi K, Dohi M, Nakagone K et al. A novel role of hepatocyte growth factor as an immune regulator through suppressing dendritic cell function. *J Immunol* 2005;175:4745–4753
- 327. Takeyama K, Dabbagh K, Shim JJ et al. Oxidative stress causes mucin synthesis via trans-activation of EGF receptors. *J Immunol* 2000;164:1546–1552
- 328. Shao MXG, Nadel JA. Neutrophil elastase induces MUC5Ac mucin production in human airway epithelial cells. *J Immunol* 2005;175:4009–4016
- 329. Frossi B, DeCarti M, Piemonte M, Pucillo C. Oxidative microenvironment exerts an opposite effect on cytokine production by Th1 and Th2 cells. *Mol Immunol* 2008;45:58–64
- 330. Hirata H, Arima H, Cheng G et al. Production of Tarc and MDC by naïve T cells in asthmatic patients. *J Clin Invest* 2003;23:34–45
- 331. van Eerdewegh P, Little RD, Dupois J et al. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 2002;418:426–430
- 332. Doganci A, Saver K, Karwot R, Finotto S. Pathological role of Il-6 in experimental allergic bronchial asthma in mice. *Clin Rev Allergy Immunol* 2005;28:257–270
- 333. Lanone S, Zheng T, Zhu Z et al. Contributions of MMPs 9–12 in Il-13 induced inflammation and remodelling. *J Clin Invest* 2002;110:463–474
- 334. Ingram JL, Antao-Mnezes A, Magnum JB et al. Opposing actions of Stat1 and Stat6 on Il-13 induced upregulation PDGF ligands in pulmonary fibroblasts. *J Immunol* 2006;177:4141–4148
- 335. Ma B, Liu W, Homer RJ et al. Role of CCR5 in the pathogenesis of Il-13 induced inflammation and remodeling. *J Immunol* 2006;176:4968–4978
- 336. Yang M, Rangasamy D, Matthaai KI et al. Inhibition of arginase I activity by RNA interference attenuates Il-13 induced airways hyperresponsiveness. *J Immunol* 2006;177:5595–5603
- 337. Koya T, Miyahara N, Takeda K et al. CD8⁺ T cell mediated airway hyperresponsiveness and inflammation is dependent on CD4⁺ Il-4⁺ T cells. *J Immunol* 2007;179:2787–2796
- 338. Wang Y-H, Angkasekwinai, Lu N et al. Interleukin 25 augments type 2 immune responses by enhancing the expansion of TSLP-activated Th2 memory cells. *J Exp Med* 2007;204:1837–1845
- 339. Karagiannidis C, Hense G, Martin C et al. Activin A is an acute allergen responsive cytokine and provides a link to TGF β mediated airway remodeling in asthma. *J Allergy Clin Immunol* 2006;117:111–118
- 340. Wang X, Lau C, Wiehler S et al. Syk is downstream of ICAM1 and mediates rhinovirus activation of p38 MAPkinase in airway epithelial cells. *J Immunol* 2006;177:6859–6864
- 341. Barnes PJ. New therapies for asthma. *Trends Mol Med* 2006;12:515–520
- 342. Nashed BF, Zhang T, Al-Alwan M et al. Role of PI3kinase p110 δ in generation of type2 cytokine responses and allergic airway inflammation. *Eur J Immunol* 2007;37:416–422
- 343. Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing Il-10 mediate tolerance induced by respiratory exposure to antigen. *Nat Immun* 2001;2:725–729
- 344. Gillespie SR, de Martino RR, Zhu J et al. Interleukin 10 inhibits Fc ϵ R1 expression in mouse mast cells. *J Immunol* 2004;172:3181–3188
- 345. Fueki N, Sagara H, Akimoto K et al. Il-10 regulates TGF β signaling in cultured human bronchial epithelial cells. *Respiration* 2007;74:454–459
- 346. Deppong C, Juehne TI, Hurchla M et al. B and T lymphocyte attenuator and PD1 inhibitory receptors for acute allergic airway inflammation. *J Immunol* 2006;176:3909–3913

- 347. Inoue H, Fukuyama S, Matsumoto K, Kubo M, Yoshimura A. Role of endogenous inhibitors of cytokine signaling in allergic asthma. *Curr Med Chem* 2007;14(2):181–189
- 348. Wang Y-H, Liu Y-J. OX40-OX40L interactions. *Nat Immunol* 2007;117:3655–3658
- 349. Meyts I, Hellings PW, Hens G et al. Il-12 contributes to allergen induced airway inflammation in experimental asthma. *J Immunol* 2006;177:6460–6470
- 350. Kato A, Truong-Tran AQ et al. Airway epithelial cells produce BAFF by an IFN β dependent mechanism. *J Immunol* 2006;177:7164–7172
- 351. Schnyder-Condrian S, Togbe D, Couillin I et al. Il-17 is a negative regulator of allergic asthma. *J Exp Med* 2006;203:2715–2723
- 352. Liang SC, Lang AJ, Bennett F et al. An Il-17 F/A heterodimer protein of mouse Th-17 cells induces airway neutrophil recruitment. *J Immunol* 2007;179:7791–7799
- 353. Nakajima H, Takatsu K. Role of cytokines in allergic airway inflammation, *Int Arch Allergy Immunol* 2007;142:265–273
- 354. Ko F, Lun S, Wong CK et al. Decreased T-bet expression and changes in chemokine levels in adults with asthma. *Clin Exp Immunol* 2007;147:526–525
- 355. Piconi S, Trabattoni D, Saresella M et al. Effects of immunotherapy on the B7 family of costimulatory molecules in allergic inflammation. *J Immunol* 2007;178:1931–1937
- 356. Desmet C, Gopsset P, Bajak B et al. Selective blockade of NF-kappa B activity in airway immune cells inhibits the effector phase of experimental asthma. *J Immunol* 2004;173:5766–5772
- 357. Eriksson U, Egermann U, Bihl MP et al. Human bronchial epithelium controls Th2 responses by Th1 induced nitric oxide Stat5 dephosphorylation. *J Immunol* 2005;175:2715–2720
- 358. Wenzek S, Wilbraham D, Fuller R et al. Effect of an Il-4 variant in late phase asthmatic response to allergen challenge in asthma patients. *Lancet* 2007;370:1422–1431
- 359. Bao Z, Lim S, Liao W et al. Glycogen synthase kinase 3 beta inhibition attenuates asthma in mice. *Am J Resp Crit Care Med* 2007;176:431–438
- 360. Liu LY, Bates ME, Jarjour NN et al. Generation of Th1 and Th2 chemokines by human eosinophils: evidence for a critical role of TNF α . *J Immunol* 2007;179:4840–4848
- 361. Asquith KL, Ramshaw HS, Hansbro PM et al. The Il-3/Il-5/GM-CSF common β receptor in allergic airway inflammation. *J Immunol* 2008;180:1199–1206
- 362. Shapiro SD. COPD unwound. *N Engl J Med* 2005;352:2016–2019
- 363. Barnes PJ. Transcription factors in airway diseases. *Lab Invest* 2006;86:867–872
- 364. Ratner AJ, Aguilar JL, Shchepetov M et al. Nod1 mediates cytoplasmic sensing of combinations of extracellular bacteria. *Cell Microbiol.* 2007;9:1343–1351
- 365. Kang M-J, Homer RJ, Gall A et al. Il-18 is induced and Il-18R α in the pathogenesis of cigarette induced pulmonary emphysema and inflammation. *J Immunol* 2007;178:1948–1959
- 366. Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. *N Engl J Med* 2008;359:2355–2365

Chapter 14

Immunological Controls

Abstract Under immunological controls, transforming growth factor- β (TGF β), the interferons and cytokines and their control are discussed.

14.1 Transforming Growth Factor- β Signalling

Transforming growth factor- β (TGF β) stops the proliferation of T lymphocytes. It inhibits signalling in T cells. Binding of TGF β to its type II receptors causes their dimerization, and a complex is formed with dimerized type I receptors; the receptor T β R tetramer signals by means of intracellular SMAD proteins [1–3]. Their name is derived from the Sma and MAD (“mothers against decapentaplegic”) gene homologues in *Caenorhabditis elegans* (Sma) and the *Drosophila melanogaster* MAD. The Smads have histone acetylase activity and thus activate many genes (Fig. 14.1). Also, there are type III receptors for TGF β called β -glycan and a 180-kDa glycoprotein endoglin that help when ligand affinity for the receptor is low. These coreceptors can ensure high-affinity interactions. Endoglin increases Smad2 protein levels and potentiates TGF β signalling, leading to endothelial nitric oxide synthase (eNOS) expression in endothelial cells.

The TGF β receptor T β RI directly phosphorylates the receptor-activated R-Smads (2, 3 and 1, 5, 8). As in other situations, the duration and intensity of signalling confers specificity (Fig. 14.1). Smad effectiveness is then achieved by translocation from the cytosol into the nuclei, where they act as transcription factors. The intrinsic DNA affinity of Smads is low, and coactivators are required. The R-Smads 2/3 are in combination with a partner Co-Smad 4. A third class (I) anti-Smad (6, 7) prevents the interaction between R and Co-Smads, and thus they mediate negative feedback [7]. Smad 6 inhibits phosphorylation of Smad 2 but not of Smad 3. When Smad 7 appears in the nuclei, receptor activation induces its export into the cytosol. Fast-1 (forkhead actin signal transducer) is a winged helix transcription factor (TF) working with Smads as coactivator. The Smad 3 and 4 bind to DNA sequences termed SBEs (Smad-binding elements). Such SBE sequences are present in the promoters of TGF β -responsive genes for collagens, fibronectin and plasminogen activator inhibitor 1 (PAI-1). This is logical since one of the principal functions of TGF β is to promote

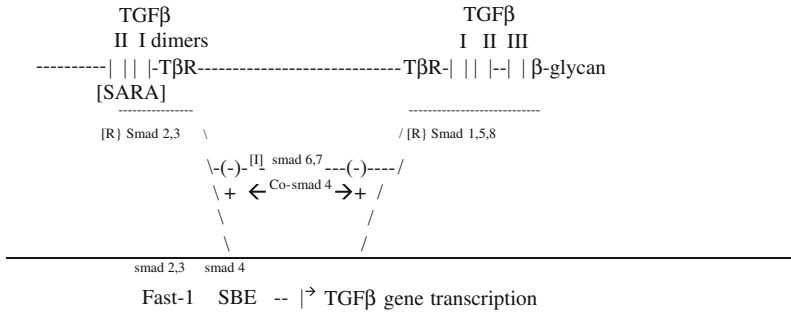


Fig. 14.1 Signaling via transforming growth factor- β ($TGF\beta$) receptors ($T\beta R$). R-Smads are recruited to $T\beta RI$ by an adaptor protein Smad anchor for receptor activation (SARA). Receptor-activated R-Smads are numbers 2, 3 and 1, 5, 8. Co-Smads are 4 α , 4 β . The phosphorylated R-Smads form signalosome complexes with Co-Smads that enter nuclei. Smad 3 is already partly within nuclei, even in the basal state. Clathrin-mediated “receptor endocytosis” enables such signalling. $TGF\beta$ can signal from endosomes via interaction with FYVE domain protein SARA, which also binds Smad 2/3 proteins. The Smads recruit chromatin-modifying proteins [4]. Smad partners include DNA binding factors like c-jun, FoxH1, and Lef-1/ternary complex factor (TCF). Inhibitory Smads (I-Smads) 6 and 7 are involved in an autoinhibitory loop [5]. I-Smads complexed with $TGF\beta$ receptors recruit Smad inhibitory regulatory factors (*Smurfs*) with E3 ligase activity and so are polyubiquitinated and then degraded [6]. Also at the nuclear membrane, MAN1 interacts with R-Smads to sequester them and to repress any signals. In nuclei, *Fast-1* is forkhead actin signal transducer. Typical target genes of $TGF\beta$ acting via Smad-binding element (SBE) are collagen $\alpha 1(I)$, fibronectin, plasminogen activator inhibitor 1 (*PAI-1*), cJun, and Jun B

the deposition of extracellular matrix proteins. Furthermore, $TGF\beta$ upregulates TIMP-1 (tissue inhibitor of metalloproteinase 1) [8], and that occurs via $TGF\beta$ induction of sphingosine kinase. So, Smads 2 and 3 with bound Smad 4 translocate into the nuclei and interact in a cell-specific manner with TFs (like Runx1, E2F), with coactivators CREB binding protein (CBP) and p300 (Fig. 4.8), and with corepressors (e.g. c-Ski, SnoN (Ski related novel protein N) [9], and $TGF\beta$ -inducing factor [TGIF]) to regulate the transcription of $TGF\beta$ -responsive genes [10]. SnoN blocks coactivators that interact with Smads 2 and 3. In fact, hepatocyte growth factor (HGF) thwarts fibrosis by promoting SnoN. The MAPK3 called TAK1 (transforming growth factor- β -associated kinase 1) Table 12.12 promotes $TGF\beta$ signalling by targeting SnoN for degradation [11]. Clearly, Smads also facilitate cross talk to other signalling pathways [12–15]. Thus, Smad 3 can directly bind c-Fos and c-Jun, which are regulated by extracellular signal-related kinase (ERK) and Jun N-terminal kinase (JNK). The Smad 3:4 heterodimer binds to the AP-1 binding site of various promoters of $TGF\beta$ target genes. Furthermore, $TGF\beta$ and interleukin 1 (IL-1) cross talk [16]. IL-1 activates SMADs, and $TGF\beta$ can activate NF- κB (Fig. 14.2).

14.1.1 Smad Activity and Other Kinase Pathways

There is complexity, but flexibility, in the $TGF\beta$ signalling pathways that is helped by the propensity of $TGF\beta$ to engage in lateral signalling with other receptors at the membrane [18]. $TGF\beta$ acting with MEK stimulates p21^{waf1/cip1}, accounting

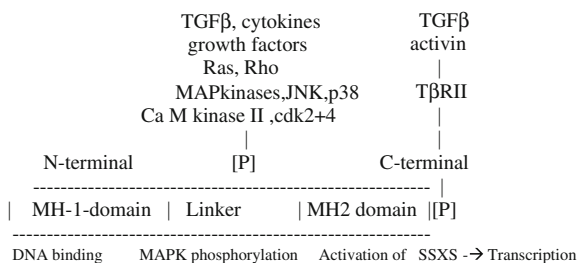


Fig. 14.2 Activation of Smads 2/3. R-Smads 2, 3 or 1, 5, 8 and Co-Smads 4 α , β have homology domains MH1 and MH2. The inhibitory Smads (*I-Smads* 6, 7) lack the MH1 domain. SSXS represents the phosphorylation site. The MH1 is for nuclear import and DNA binding. The conserved MH2 is for receptor interaction and Smad oligomerization. The proline-rich linker region is diverse. Ras/mitogen-activated protein kinase (*MAPK*) signals interact through p53 phosphorylation to connect with Smads 2/3 [17]. *TGF β does signal through non-Smad pathways.* (a) The TGF β R1 connects with Ras-*ERK*, Jun N-terminal kinase (*JNK*) or p38 MAPK. (b) It activates PI3 kinase-Akt, (c) it activates Rho-Rho kinase (*ROCK*) and (d) via cdc42/Rac and so p21-activated kinase 2 (*PAK2*), cAbl is activated

for arrested cell proliferation, and TGF β acting via JNK leads to fibronectin synthesis. In fibroblasts, TGF β activates focal adhesion kinase (FAK), causing JNK phosphorylation [19]. TGF β acting on p38 MAPK induces MSK1, a histone H3 kinase, which is involved in chromatin remodelling via its association with CBP/P300 [20]. In these ways, the actions of Smads are promoted. Conversely, there is modulation by means of JNK, which can be a repressor of TGF β 1 gene expression [21]. Added to this, there is a SMAD phosphatase PPM1A that will terminate TGF β signalling [22].

Smad 2 is activated by MEKK1 (MAP kinase kinase 1), an activator of the SAPK/JNK pathway. However, the MAPK/ERK path can often act as a negative regulator of Smad signalling. As depicted in Fig. 14.2, the ERK path targets R-Smads. It phosphorylates the MH1 domain of Smad 2 and the linker segments of Smads 1, 2 and 3. PKC likewise phosphorylates the MH1 domains of Smads 2/3, stopping DNA binding. This suggests a regulatory role.

Smad 3 is important for transcriptional regulation of cell cycle regulators. Smad 3 is involved in TGF β -induced ubiquitination and degradation of phosphatase cdc25A. It activates the p15 and p21 cell cycle inhibitors. Akt modulates TGF β by sequestering Smad 3 outside the nuclei, stopping apoptosis of cells [23, 24].

Smad signalling is regulated by ubiquitination. The effects can be positive or negative. Itch E3 ligase facilitates the formation of a complex between the TGF β receptor and Smad 2 through ubiquitination of Smad 2, leading to an increase of TGF β gene transcription. A protein Arkadia amplifies TGF β signalling [25] through degradation of the Smad 7 inhibitor. Arkadia is expressed at the gut mucosa. Axin interacts with Arkadia and promotes Smad 7 ubiquitination. Negative regulation acts at every step of the TGF β signal pathway [6, 7]. Smurf1 (Smad inhibitory regulatory factor 1) brings inhibitory Smad 7 to the plasma membrane T β RII, and so it represses TGF β signalling [26]. Smurf1 and Smurf2 are HECHT-type E3 ligases [27]. Smurf2

is E3 ligase for activated Smad 2 degradation. As stated, R-Smads are intercepted at the nuclear membrane by protein Man1, which is an R-Smad regulator.

In addition, the TGF β -Smad signal is inactivated by Ca²⁺-calmodulin (CaM)-dependent protein kinase II [28]. CaM kinase II phosphorylates Smads 2, 3 and 4 and thus inhibits their nuclear import [12]. As emphasized, Smad 7 plays an inhibitory role (Fig. 14.1). Interestingly, the DNA tumour viruses upregulate I-Smad 7. Proinflammatory cytokines IL-1 and TNF α , which stimulate NF- κ B, activate the I-Smad 7, as does interferon- γ (IFN γ) acting via Jak1/Stat1 [13].

14.1.2 TGF β and Immune Regulation

TGF β inhibits the IL-2-dependent proliferation of T lymphocytes by blocking IL-2 production, and it inhibits the development of T cells and prevents naïve T cells from acquiring effector functions [29]. It does this directly or via induction of Treg cells [30]. Smad 3 is essential for TGF β 1 to suppress IL-2 production and T cell receptor (TCR)-induced T cell proliferation [30] (Table 14.1). TGF β by Smad 3 stops TCR-induced division of CD4⁺ cells, and it stops division of CD8 T cells, albeit not via Smad 3 [31, 32]. In spite of this, TGF β enhances T cell survival. However, TGF β thwarts CD4 T cell differentiation into either Th1 or Th2 cells. Th2 cells are readily inhibited, but Th1 cells can be protected from inhibition by IFN γ . Remarkably, TGF β will convert naïve CD4⁺ CD25⁻ memory T cells in the periphery to Foxp3⁺ CD4⁺ CD25⁺ Treg cells. So, it aids tolerance [33].

There is heightened expression of TGF β in several immunoregulated diseases. The proinflammatory cytokines IL-1 β and TNF α induce TGF β expression. IL-1 β activates the p65 subunit of NF- κ B and the c-Jun subunit of AP-1 on the TGF β 1 promoter [36]. There is airway remodelling of the airways in persons with asthma that involves fibrosis and thus the action of TGF β . That can be controlled by IL-10, which upregulates the Smad 7 [37].

On surfaces of immature dendritic cells (DCs), there is TGF β bound to Latency-Associated Peptide (LAP). This arrangement means that immature DCs inhibit Th1 lymphocytes [38a] when the LAP has been removed by proteases [38b].

Table 14.1 The actions of transforming growth factor- β 1 (TGF β 1) on T lymphocytes

-
- (a) TGF β supports formation of T-bet and thus IL-2R expression and IL-15-driven development. Yet, in later development, it inhibits T-bet action
 - (b) TGF β , via Smad 3, inhibits IL-2 production and therefore T cell proliferation
 - (c) TGF β stops the division of CD4 and CD8 T cells
 - (d) TGF β inhibits formation of Th1 cells (T-bet, Stat4), and it inhibits Th2 cells (GATA3)
 - (e) TGF β prevents the formation of cytotoxic T cells (T bet and c-Myc)
 - (f) TGF β prevents activation-induced cell death (AICD)
 - (g) TGF β promotes the formation of peripheral FoxP3⁺ Treg cells, and of NKTs
 - (h) TGF β supports creation of IL-17, producing Th cells [34, 35]
-

14.1.3 TGF β Inhibition of Cell Growth

One will realize that Smad 2, Smad 3, and Smad 4 contribute to TGF β responses to a variable extent. These Smads have distinct roles in differing cell types [39]. There are some genes regulated by Smad 2, other genes regulated by Smad 3, yet others by both Smad 2 and Smad 3, and more genes that respond to TGF β in a Smad 2/3-independent manner. Consider that, TGF β is known to inhibit the growth of keratinocytes via Smad 3 [39]. Yet, in response to insulin, Akt action inhibits Smad3 activation [40]. Thus, insulin partially reverses TGF β growth inhibition [41]. Whether there is growth or apoptosis of cells is determined by opposing forces [42].

Evidently, when growth is required, inhibition by TGF β must be stopped [43]. Consider furthermore that growth factor activation of Ras (Fig. 4.3) activates ERK, which phosphorylates R-Smads, stopping their nuclear translocation. At the same time ERK activates I-Smads so that the TGF β signal is suppressed.

14.1.4 TGF β and Inhibition of Cell Proliferation

How TGF β works in relation to the cell cycle is discussed in Chap. 17 (Fig. 17.3). TGF β arrests the cycle in G1 phase by induction of the cyclin-dependent kinase inhibitor p15 and by inhibiting cell cycle regulators like cdk2 and 4 and cyclins A and E [44]. c-Myc, cdc25A and Id2 are all downregulated. Suppression of T cell proliferation (Fig. 17.8) could depend on Smad 3 induced down-regulation of cdk4 [45] or cdk2, with p57^{cip2} and p21^{cip1} contributing. The action of c-Myc is stopped. When TGF β inhibits Myc, Miz1 is released from the Myc-Max complex (Fig. 4.18), and free Miz1 then binds to the p15 promoter along with Smads 3 and 4.

There are corepressors of TGF β signalling like Ski [46] and SnoN [47], which essentially inactivate Smad complexes. They play a complicated role in cancers. Ski or SnoN are depleted (50%) or amplified (15%) in tumours.

14.1.5 TGF β and Apoptosis

TGF β will suppress release of IL-12 [48] or the IL-4-induced activation of the Jak-Stat signal pathway in activated Th2 lymphocytes. Generally, TGF β causes apoptosis of cells, but it will also protect some T cells from apoptosis [49]. So it allows expansion of memory or effector T cells [50] and TGF β maintains Treg cells [51].

TGF β acting on receptor T β R works via TAK1 to induce p38 and JNK and thereby to cause apoptosis [52] Fig. 14.3. Also, TGF β via Smad proteins induces GADD45b [53a] and so p38 on the pathway to apoptosis [53b].

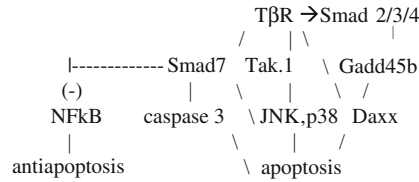


Fig. 14.3 Transforming growth factor- β ($TGF\beta$)-induced apoptosis. GADD = growth arrest and DNA damage inducible gene [53a]

14.2 Clinical Implications

14.2.1 $TGF\beta$, Connective Tissue Growth Factor, and Fibrosis

$TGF\beta$ is well known as the cytokine that causes fibrosis of damaged tissues [3]. Not only does $TGF\beta$ stimulate fibroblasts to form collagen and extracellular matrix proteins like fibronectin, but also $TGF\beta$ decreases the activity of enzymes that would degrade the matrix. $TGF\beta$, acting via connective tissue growth factor (CTGF) is responsible for kidney fibrosis that leads to end-stage renal failure and thus the need for patient dialysis. Yet, when HGF is given to mice, renal fibrosis is suppressed since the action of $TGF\beta$ is so antagonised [54]. Likewise, bone morphogenetic protein 7 (BMP7), which normally stimulates epithelial cell development, is an inhibitor of fibrosis [50, 55, 56]. Now, we know that in kidneys nephrocan of the small leucine-rich repeat protein family is an inhibitor of $TGF\beta$ [57].

$TGF\beta$ causes fibrosis by the intermediary of CTGF [55], which is an insulin-like growth factor (IGF)-binding protein. $TGF\beta$ induction of CTGF requires Smads and the PKC/Ras/MEK/ERK pathways [58]. Conversely, prostacyclin stimulation of protein kinase A blocks the ERK-Smads induction of CTGF. Likewise, prostaglandin E2 (PGE2) can stop the synthesis of collagens [59]. CTGF is required in addition to $TGF\beta$ to induce persistent fibrosis [60]. In the mesangial cells of the kidneys, $TGF\beta$ acts via $PKC\delta$ and Smad 3 to induce transcription of type I collagen [61]. An antagonist of Smad 3 would be antifibrotic. Indeed, halofuginone does just that [62]. If there are residual heparan sulphate proteoglycans in damaged tissue, they will tend to reduce fibrosis. Thus, syndecan 2 can regulate $TGF\beta$ signalling [63], and hyaluronan around tubules supports BMP7 action to offset fibrosis. *N*-Acetyl-cysteine (NAC) is now used often in therapeutics. NAC stops phosphorylation of Smads 2 and 3, and it upgrades Smad 7 [64].

14.2.2 Connective Tissue Growth Factor

CTGF upregulates extracellular matrix proteins. In kidney cells, CTGF is induced by $TGF\beta$, by mechanical strain, by high glucose as in diabetes, and by the advanced glycation end products that accumulate in diabetes. AGEs can induce CTGF-mediated

fibrosis independently of TGF β [65]. CTGF influences the signalling of TGF β and IGF-1 and the BMPs [66]. We now understand the module structure of CTGF [67] and how its units influence signalling: IGFBP/von Willebrand factor/thrombospondin 1 (TSP-1)/C-terminal module. The IGF-binding domain stimulates IGF-1. The von Willebrand factor type c domain stimulates TGF β but inhibits BMP. The TSP-1 module inhibits vascular endothelial growth factor (VEGF). The C-terminal module interacts with integrins or with heparin sulphate proteoglycans on cell membranes [68]. If CTGF is cleaved by metalloproteinases, the fragments have bioactivity [69]. It is noted that Sparc is an upstream regulator of CTGF in response to TGF β stimulation [70].

14.2.3 Scleroderma

TGF β and CTGF are together responsible for the skin fibrosis that is called *scleroderma*. This disabling condition has been an impetus to research on Smads [62, 69]. In normal fibroblasts, inhibitory I-Smad 7, induced by TGF β , binds to the activated T β R and interferes with Smad phosphorylation, thus blocking more TGF β signalling. Smad 7 also represses transcription by reducing acetylation of histones. Adenoviral gene transfer of Smad 7 curtails lung or kidney fibrosis in rodents. However, in scleroderma fibroblasts the Smad 7 negative control is lost [70]. There are other problems, like formation of autoantibodies to the PDGF receptors [71].

IFN γ is known to reduce formation of collagen, as in damaged lungs. The IFN γ -activated Stat pathway induces Smad 7, so there is negation of TGF β action [72].

Agents that inhibit fibrosis could be used in various situations like lung or kidney fibrosis [73]. TGF β can activate the nonreceptor tyrosine kinase c-Abl (Fig. 5.8), so that fibroblasts produce fibrosis. This is independent of Smad 2/3 phosphorylation [74]. The tyrosine kinase inhibitor imatinib mesylate will reduce such fibrosis [75].

14.2.4 TGF β Is Anti-inflammatory

Generally, TGF β suppresses expression of myeloid cell Fc γ receptors by affecting the common γ -subunit (Fig. 10.5) [76a]. In practice, this means that TGF β dampens Gell and Coombs type II and III immune reactions. TGF β inhibits Toll-like receptors (TLRs) 2, 4 and 5, reduces NF- κ B activation and stops formation of proinflammatory cytokines. TGF β lowers chemokine monocyte chemotactic peptide 1 (MCP-1), and thus curtails monocyte recruitment. To control TLRs, TGF β causes ubiquitination of MyD88 (myeloid differentiation marker 88) [76b].

TGF β downregulates NK, which is how cancer cells survive. When produced by keratinocytes, TGF β downregulates intraepidermal Langerhans cells. It inhibits CD1d expression on DCs; thus, they are unable to present α -galactosyl-ceramide or analogous glycolipid antigens to natural killer T cells (NKTs) or to T cells [77]. Some viruses work like this [78].

Anti-inflammatory mechanisms are manifest in the gastrointestinal (GI) mucosa. Although NF- κ B normally deals with proinflammatory responses, at the GI mucosa NF- κ B works to maintain epithelial integrity [79]. Commensal organisms like *Bacteroides* will act in the colon to negatively regulate NF- κ B by their ability to induce and activate peroxisome proliferator-activated receptor- γ (PPAR γ) [80]. In inflammatory bowel disease, there is elevation of Smad 7 in the mucosa and so decreased Smad 3, and thwarted TGF β activity enables inflammation to proceed [81a].

One will now appreciate that TGF β is anti-inflammatory, since it inhibits CD4 and CD8 T cells and it promotes Treg cells [Table 14.1]. For comparison one will recall that IL-10 (Sect. 12.15) operates as a feedback inhibitor of exuberant T cell responses to microbial antigens [81b]. Hence TGF β and IL-10 together ensure a controlled inflammatory response [81b].

14.3 TGF β and Cancer

The secretion of immunosuppressive TGF β by some cancers or display of membrane TGF β on cancer cells will mean that infiltrating immune cells are thwarted in their action against the tumour [82]. When it acts on normal epithelial cells, TGF β is a “tumour suppressor”, since it restrains cell proliferation, and apoptosis can be induced (Fig. 14.3) [83, 84]. The cell cycle kinases cdk2 and -4 phosphorylate the linker region of Smads 2 and 3 (Fig. 14.2) [85]. Smad-dependent growth inhibition of breast cancer cells has been shown to be mediated via the p38 MAPK pathway and via Rho/Rock kinase [86]. Although the majority of cancers do retain TGF β type I/II receptors, malignant cells generally are not growth inhibited by TGF β for there is activation of PI3 kinase (PI3K) and Ras signal pathways. As cancer develops, cells will lose their TGF β -mediated growth inhibition [84, 87] as a consequence of mutations of genes for one or more components of the TGF β signal pathway (Fig. 14.3) [88]. Smurf levels can decrease. In breast cancer cell lines, Dach-1 thwarts TGF β -induced apoptosis of cells [89].

The tumour suppressor activity of TGF β is mediated by Smads 2 and 3, but in most malignancies these controls are altered. Oncogenic Ras acting via MEK1/ERK kinases phosphorylates the linker regions of Smads 2 and 3, and there is inhibition of TGF β -induced Smad transcription [90].

When TGF β induces the cell cycle inhibitor p15 INK4b promoter, it uses the Smad partner C/EBP β . LIP, a variant of C/EBP β , stops TGF β -induced p15 expression. Breast cancers cells were shown to escape TGF β -induced growth arrest by virtue of their LIP expression [91]. Strains of mice with Smad mutations are predisposed to colorectal cancer. JNK has been demonstrated to induce phosphorylation of Smads 2/3 in the course of transition of human colorectal adenoma to carcinoma [92].

There comes a stage when TGF β enhances growth of cancer cells [82]. Plasma levels of TGF β often correlate with tumour progression. In due course, TGF β action imparts enhanced motility to cancer cells and elevated matrix protease activity. TGF β promotes the ability of a cancer cell to metastasize by mediating changes in the cytoskeletal architecture. TGF β can induce GTPases like RhoA, Rac, and cdc42 [12].

The relationship of TGF β to carcinogenesis is really complicated [83, 84, 93]. TGF β acts as a growth inhibitor in early-stage disease and as a prooncogenic factor in late-stage disease. Mutations and loss of T β RII receptors often occur due to epigenetic changes or genetic deletions. There can be reduction or loss of Smad 4. So, tumour cell growth is unimpeded. Furthermore, cyclin-dependent kinase (CDK) activity can cause the phosphorylation of Smad 3, so that the antiproliferative activity of TGF β is lost [94]. Eventually, TGF β via the Rho GTPases contributes to growth and invasion by cells.

TGF β is relevant to haematologic cancers [95], which, like other cancers, become TGF β resistant through decreased expression of surface T β Rs or because of repression of TGF β signalling by oncoproteins like Evi-1 or Tax of human T cell leukaemia virus. Loss of Smad 3 protein is a specific feature of pediatric T-cell acute lymphocytic leukaemia (ALL). In acute myeloid leukaemia (AML), the AML/ETO fusion protein is capable of binding to Smad 3, but instead of activating, it blocks TGF β signalling [95].

So far it has not been emphasized that tumor suppressor P53 (Figs. 8.5 and 17.7) is an essential partner of Smads and affects TGF β signalling at various points. Hence inactivation of P53 contributes to aberrant behaviour of cancer cells.

14.4 Bone Morphogenetic Proteins

BMPs are multifunctional growth factors of the TGF β superfamily that use Smads 1 and 5 for signalling. BMPs are implicated in embryonic development [46, 96] and formation of haemopoietic stem cells [97]. BMPs signal by means of type I and type II (Fig. 14.1) serine-threonine kinase receptors. Those receptors are designated as ALK3/6 (activin-like kinases 3 and 6) for the BMPs, in comparison with ALK5/5 and ALK5/1 for the TGF β receptors. Downstream of BMPs are Smads 1, 5 and 8. In BMP signalling, endofin acts as a receptor anchor for Smad 1. It has a phosphatase-binding motif to cause receptor dephosphorylation and so to negatively modulate BMP signals [98]. BMPII receptors are for vascular integrity. BMP antagonists have turned out to be negative regulators of monocyte chemotaxis [99].

14.5 The Actions of Interferons

IFN α and - β are produced by many types of cells in response to viral infection. IFN α supports formation of cytotoxic T lymphocytes and NK cells. IFN α / β elicit antiviral activity in the target cells, and they induce apoptosis of infected cells unless the viruses can inhibit IFN α / β signalling. Apoptosis is clearly a general means of limiting viral spread. Antiviral action entails formation of a low molecular weight protein synthesis inhibitor called 2-5'-A synthetase and stimulation of protein kinase R (PKR) that phosphorylates initiation factor E1F2, stopping its participation in the formation of the 40S ribosomal initiation complex [100]. Weak IFN α / β signalling will ensure that cells can respond efficiently to other cytokines.

IFN α/β genes are activated immediately by microbial agents [101, 102], explaining their vital role in innate immunity (Table 8.1). IFN α is antiviral and often antibacterial. IFN α induction of the synthesis of 2-5'A synthetase and of PKR is determined by IFN-inducible interferon regulatory factor 1 (IRF1) [103]. IRF1 is pivotal in the growth, regulatory and tumour suppressor actions of the IFNs. Yet, its action can be offset by the stronger negative regulatory IRF2 factor [104a]. IRF2 carboxyl terminus contains a repressor domain. As shown in Fig. 14.4, IRFs 3 and 7 are needed to induce the secretion of type I IFN α . IRFs 3 and 7 act on positive regulatory domains (PRDs) I and III in the promoter regions of the IFN α genes. PRDII is for NF- κ B and PRDIV is for AP-1, ATF2 and cJun heterodimer. Along with viral infection, PRDI–IV direct assembly of AP-1, IRFs, NF- κ B and HMG-1(Y) in the enhanceosome (Fig. 4.12), which recruits histone acetyltransferases like CBP/p300. IRF3 is more potent in activating the IFN β gene, whereas IRF7 activates both IFN α and IFN β .

When macrophages respond to LPS, the activation from TLR4 receptors of IRF3 that releases IFN β acts to cause release of feedback IL-10 [104b].

14.6 Signalling by IFN γ

Factors that determine IFN γ production are shown in Fig. 13.10. To effect IFN γ signalling, the IFN γ cytokine induces receptor dimerization (Fig. 14.4) and thus activation of Jak1 and Jak2. They catalyse tyrosine phosphorylation of Stat1, which forms a homodimer. Stat1 translocates into nuclei to bind there with *cis* elements of DNA termed IFN-stimulated response elements (ISREs; sequence 5'-AGTTTNTTTC-3'), residing within the promoter region of IFN-inducible genes. IFN γ -induced genes have a GAS (γ -activating sequence) promoter binding site, which is 5'-TNCNNNAA3', while secondary genes have ISRE [101].

IFN γ is an antiviral agent. It also restrains cell proliferation by various means [101, 109]. Often, it raises intracellular cAMP. IFN γ stimulates NK cells and macrophages and Th1 lymphocytes as effector cells [109–111]. IRF1 is used for this purpose [112]. IRF2 is an inhibitor of IFN-induced gene transcription, but IRF1 activator can overrule it. Generally, the control of IFN γ is achieved by Socs1 (suppressor of cytokine signaling 1) (Table 14.2).

Without doubt IFN γ is a powerful traditional proinflammatory factor that determines the development of Th1 lymphocytes. However, perhaps not so surprisingly, recent work on autoimmune collagen induced arthritis (CIA) has shown that in due course IFN γ will mitigate damaging pathology by inhibiting T cell proliferation, by causing T cell apoptosis and by inhibiting influx of neutrophils. Recall that IL-17 helps to mobilize neutrophils. Well IFN γ ultimately inhibits Th17 cell production [111b].

Interferon regulatory factor IRF4 aids development of CD4+ T cells into Th2 and Th17 cell. It has been found that IRF4 differentially regulates Th2 cytokine production [111c].

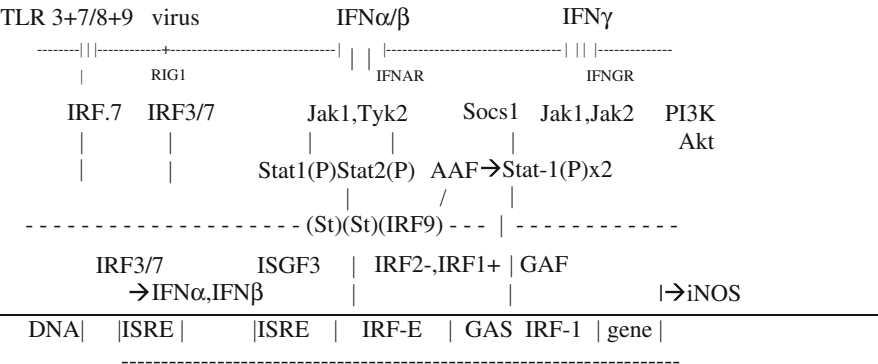


Fig. 14.4 Signalling by interferons. The signal transducer and activator of transcription (*Stat*) transcription factors form homo- or heterodimers that translocate into nuclei and there combine with DNA binding protein interferon regulatory factor 9 (*IRF9*) (p48) to form interferon-stimulated gene factor 3 (*ISGF3*). This stimulates the interferon-stimulated response element (*ISRE*) to activate interferon- α (*IFN α*)-inducible genes. Such is *IRF7* [105], the master regulator of IFN responses, which activates the *IRF1* gene by binding to γ -activating sequence (*GAS*). *IRF1*, when bound to *IRFE*, aids activation of *IFN α* -inducible genes. *IFN α* activated factor (*AAF*) induces *GAS* [101]. *IRF2* attenuates *ISRE* and *IRFE* transcriptional activation. *IRFs* 1, 3 and 7 are positive regulators of the type I IFN gene. *IRF7* can cooperate with *Smad 3*. *IRF7* is expressed poorly in cells, until it is induced by type I IFN signalling. *IRF5* regulates cytokine genes *IL-12* and *TNF α* . In the *IFN γ* system, note how *Akt* phosphorylates *Stat1* [106]. *Stat1* induces formation of suppressor of cytokine signaling 1 (*Socs1*), which then causes negative feedback of *IFN α / β* signals. During RNA virus infection (right side of Fig. 14.5), there is Toll-like receptor (*TLR*) and cytosolic *Rig-1*-dependent induction of type I IFNs in many cell types [107, 108a]. In the plasmacytoid dendritic cells (*PDCs*), single-stranded RNA encounters *TLR7/8* in endosomes. Note the position of *PI3K* $\rightarrow$ *Akt* (top right) in Fig. 14.4. Integration of signals via *PI3K* complements the *Jak-Stat* pathways transcriptional activity and controls initiation of mRNA translation [108b]. Activation of *PI3K* by IFNs α/β also activates *p70S6K* and de-activates *4E-Bp.1* a translational repressor (cf. Fig. 11.6).

Table 14.2 Induction and effects of suppressor of cytokine signaling (SOCS) proteins

Protein	Induced by	Effect/suppression of:
Cis	IL-1, -6, -12, -13, IFN γ , GM-CSF LPS	IL-2, IL-3 EPO, GH, prolactin
Socs-1	IL-2, -3, -6, -13, GM-CSF	IFN γ , IFN α , TNF α IL-2, -3, -4, -6
Socs-2	IL-1, -2, -3, -4, -6, -9, -10 G-CSF, GM-CSF, IFN γ	IL-6, LIF, IGF-1
Socs-3	IL-1, -2, -3, -4, -6, -7, -9, -10, -11, -12, -13 M-CSF, G-CSF	EPO IL-2, -3, -4, -6, -9, -10, IFN γ

G-CSF granulocyte colony-stimulating factor, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *IFN* interferon, *IGF* insulin-like growth factor, *IL* interleukin, *LPS* lipopolysaccharide, *M-CSF* macrophage colony-stimulating factor

14.7 Signalling by IFN α

During virus infections, induction of the IFN α/β genes is achieved through activation of TFs of the IFN regulatory family [108, 113, 114], namely, IRF3 and IRF7 (Fig. 14.4). The released IFN α/β stimulate receptor IFNAR [109]. Consider that a virus will act via TLR3 cell receptors and so triggers adaptor protein TRIF, which (a) via RIP (receptor-interacting protein), can stimulate caspases to cause apoptosis; (b) via TRAF6 stimulates NF- κ B, causing cytokine and chemokine release; and (c) via IKK ϵ and TRAF6 activates IRF3/7 to trigger the ISRE to cause IFN α/β release (Fig. 14.4) [115]. TRIF denotes Toll: IL-R (TIR) domain containing adaptor-inducing interferon- β [116]. We see all this on the right side of Fig. 14.5. Interestingly, the IFN α receptor 2 recruits CREB binding protein, which acetylates it on Ly399, and accordingly IRF9 is able to dock [117a, b].

Type I IFNs, by activating JAK1 and Tyk2 differentially, activate STATs 1–6 in differing cell types, which may be lymphoid or epithelial. In addition, there is activation of NF- κ B, AP-1, and PU1 [118]. Cell type-specific differences in receptor composition or in the expression of cell surface proteins modify the functions of the receptors. Different type I IFNs can activate differing MAPKs. As a result of combinations of Stats and TFs, type I IFN can promote cell survival and proliferation or apoptosis and growth inhibition, or it can enhance IFN γ so that there is promotion of T and NK cells.

IFN α is antiviral by its induction of 2-5'oligoadenyltransferase. When acting on immune cells, IFN α uses Jak1/Tyk2 and TFs Stat1 and Stat2, and they combine with p48 (IRF9) to initiate gene transcription [114, 115]. The ability of IFN α/β to synergize with IFN γ is mediated by ISGF3 (IFN-stimulated gene factor 3) proteins consisting of Stat1, Stat2 and p48. There must be separate pathways (a) for the antiviral action of IFN α (via IRF3/7) and (b) its suppression of cell proliferation [119, 120]. When IFN α acts on T cells to stop their proliferation, CD45 is recruited to the receptors. Therapeutic IFN β , marketed as Betaseron or Avonex, is used to suppress Th1 lymphocytes of patients with multiple sclerosis. It also thwarts output of IL-8 and IL-12.

Although not the case in mice, IFN α acts in humans via Stat4 to promote IFN γ -induced innate immunity [121] (Table 8.1). Pathogens acting on TLRs, via the MyD88 and TRAF6 adaptors and by means of IRF7, induce α -interferons [122] and IFN γ [123]. Hence, NK cells, DCs and CD8 T cells are activated [123], and memory T cells form. IRFs help to promote Th1/Th2 cell formation [124], but unlike IL-12, IFN α cannot alone form Th1 lymphocytes.

The contribution of IRF3 and -7 to the activation of macrophages has been examined. In adenovirus-IRF3-transduced macrophages, cell death occurred, but adenovirus-IRF7-transduced macrophages produced type I IFNs and showed enhanced expression of TRAIL (TNF-related apoptosis-inducing ligand), and so they were cytostatic for cancer cells [125].

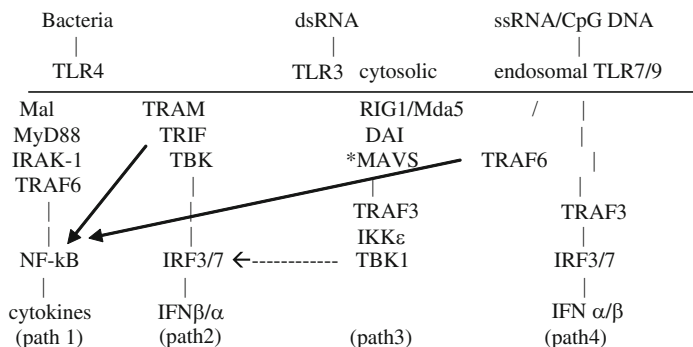


Fig. 14.5 Pathways for responses to viruses and their products. RIG1 = retinoic acid inducible gene-1 Mda5=melanoma differentiation assoc. gene DAI= DNA-dependent activator of IRFs MAVS= mitochondrial antiviral signalling protein/or IFN β promoter stimulator 1 (IPS-1), Required for IFN β production in all cells, except pDCs, but not for TLR responses. N-terminal CARD of MAVS reacts with CARD of RIG1, so activating TBK1 and IKK ϵ . Yet Pin-1 induced ubiquitination of IRF3 leads to IRF3 degradation and so termination of IFN β gene induction [139a]. adaptor* MITA (STING) is important for interaction with RNA viruses. MITA interacts with MAVS [139b]. STING = stimulator of interferon genes. SIKE is a IKK ϵ /TBK1 associated suppressor of IRF3 pathways [139c]

14.7.1 Controlling Viruses

IFN γ offers strong support for the CD8 cytotoxic T cells, the CD4 T helper cells and the NK cells, which combat microbial pathogens [126]. Both IL-12 and IL-15 help the induction of IFN γ , which boosts the killing capacity of macrophages. As indicated in Sect. 12.1.3, the TLRs [127], NLRs and RLRs (RIG-1 like receptors) aid the sensing of intracellular pathogens.

Within cells, the p47 GTPases help immunity against intracellular bacteria, viruses or protozoans [128]. The p47 GTPases are promoted by IFNs γ and α/β , which act via IRF1 and IRF9 to induce a GAS (IFN γ activation site) and ISRE [129]. Specific to protection against viruses, there are also RNA-sensing molecules, that is, cytosolic RNA helicases (ATPases), such as RIG1 [116, 130], and there is a cytosolic sensor for viral double-stranded DNA called DAI [131a]. RIG1/DAI sense dsRNA/dsDNA and activate the IRF3 and IRF7 pathways via PI3K [131b] and NF- κ B. RIG1^{null} murine fibroblasts have been used to confirm its essential role in the recognition of RNA viruses. RIG1 and MDA5 are IFN inducible and undergo amplification but are negatively regulated by another helicase, LGP2. The protein NS3-4A of hepatitis C virus is able to inhibit the adapter protein IPS1 (MAVS) that works with RIG1 to stimulate IRF3 and so production of IFN β [132]!

One might question the functions of TBK1 (Tank-binding kinase 1) and IKKi (or IKK ϵ), as shown in Fig. 14.5. They are important because they regulate IRF3 and IRF7 and different IFN α genes and IFN β [133a]. They are mediators in IFN α production in conventional DCs [133b, 134].

TLRs induce $\text{IFN}\alpha/\beta$ responses by activating distinct IRFs [127]. Viral infections incite various $\text{IFN}\alpha$ gene members with the result that there is additional release of $\text{IFN}\gamma$, so there is activation of NK cells, memory T cells are boosted, and there is even induction of isotype switching of B cells [135]. Generally, there is $\text{Fc}\gamma\text{RII}$ receptor sensitization of IFN -producing cells [136]. In DCs, the innate production of $\text{IFN}\alpha$ is cooperatively regulated by CD40 acting with $\text{IFN}\gamma$ [137]. When TLRs7/9 of endosomes promote $\text{IFN}\alpha$ production in plasmacytoid dendritic cells (pDCs), $\text{IKK-}\alpha$ is needed [138].

There are sure to be negative regulators of $\text{IFN}\alpha$ formation. The IRF3 is targeted for degradation by ubiquitination following virus infections. This ensures that the type I IFN response is not too great. The proteasome-dependent destruction of IRF3 is determined by cytoplasmic prolyl isomerase Pin-1 [139]. As shown in Fig. 14.5, TRIF is associated with TLR3 and is involved in TLR3-mediated signalling. Adaptor SARM negatively controls TRIF signalling [140], as does the phosphatase SHP-2 [141a]. $\text{IFN}\beta$ formation is negatively regulated by $\text{GSK}\beta$ glycogen synthase kinase 3- β in TLR4 stimulated cells [141b].

Now, we know of Twist, which suppresses inflammation by inhibition of $\text{NF-}\kappa\text{B}$. Twist also binds to an E-box in the $\text{TNF}\alpha$ promoter and so suppresses its release [142].

14.7.2 *Plasmacytoid Dendritic Cells*

The pDCs are the natural type I IFN -producing cells, and they account for the Th1 cell response of virus infections. Ikaros is required for pDC differentiation. They are long lived by virtue of a high bcl-2/Bax ratio in contrast to the short-lived mature DCs (mDCs). pDCs detect viruses by means of their TLR7 and TLR9 receptors and mTOR is required for type I IFN formation [143a]. In mice, Siglec H is a cell marker [143b]. Intracellular osteopontin (OPN) is an integral part of the $\text{TLR9} \rightarrow \text{IRF7}$ signal pathway [144]. When TLR9 is activated by CpGA in early endosomes, there is activation of IRF7 and so formation of $\text{IFN}\alpha$ (path2, Fig. 14.5). Conversely, in late endosomes CpGB activates TLR9, so there is induction of $\text{NF-}\kappa\text{B}$, which accounts for maturation of DCs that is part of the adaptive response [145]. One will recognise that these pathways operate opposite to the way in which there is the response to bacterial infections (Fig. 12.1). CpG DNA induction of $\text{IFN}\alpha$ in pDCs elicits important chemokines [146]. As for control, it emerges that cross-linking of $\text{IL-3R}\alpha$ (CD123) on pDCs or surface BDCA2/4 reduces levels of IRF7, and $\text{IFN}\alpha$ production is thereby reduced [147a]. The same is true for immunoglobulin-like transcript 7 (ILT7) or CD303 surface signals. Importantly exclusion of self *nucleic acids* from early endosomes of pDCs prevents immune responses to them [147b].

Myeloid DCs can be induced to be tolerogenic so that they support CD4 suppressor T cells, and IL-12 formation and Th1 cell development are downregulated. This process can be supported by means of 1,25(OH) $_2$ -D $_3$. The pDCs can prime IL-10 producing Treg cells and they can even induce energy in CD4+ T cells.

As people age they become more susceptible to viral infections. Aged pDC's show more oxidative stress and there is defective IRF-7 upregulation and diminishing IFN α production [147c].

14.8 SOCS: Negative Regulation of the Jak-Stat Pathway

Socs1 was discovered as an inhibitor of IL-6 signalling. When overexpressed in cells, Socs1 can inhibit any signal that utilizes the JAKs. Thus, Socs participate in negative feedback to regulate cytokine signalling. Proteins to which Socs bind are targeted for proteasomal degradation. The main function of Socs1 is negative regulation of IFN γ and of cytokine signalling [148, 149]. It also regulates the IFN α/β receptors, responses to LPS via TLR2 and TLR4 and responses to IL-4 and IL-15. Socs1 negatively regulates Th1 and Th2 cell differentiation (Fig. 13.9) in response to IL-12 and IL-4. Socs1 of T cells and macrophages is critical for preventing lethal inflammation [150]. In fact, nitric oxide (NO) can induce the SOCS1 [151] and so can apoptotic cells [152]. NO induces Socs1, which prevents LPS induction of IL-6. NO also induces IRAK-M, which downregulates inflammatory responses (cf. Fig. 12.5). On the other hand, Socs5 augments innate immunity during septic peritonitis. Levels of IL-12, IFN γ and TNF α are increased [153].

There are two families of negative regulatory molecule for Jak/Stats [149]. Socs molecules 1–7 are induced by cytokines (see Table 14.2 on page 303). They work in pairs like SOCS1/3, CIS1/SOCS2, SOCS4/5 and SOCS6/7. There are also the PIAS [159] (protein inhibitors of activated Stat), which block the DNA binding activity of Stats. Early on, Socs1 was shown to be a negative regulator of IFN γ activities [155–157]. Socs1 prevents the expression of major histocompatibility complex (MHC) class II molecules. Furthermore, IL-10 induces expression of Socs3 [158], which plays its role in inhibition of IFN γ tyrosine phosphorylation of Stat1. Also, IL-10 via SOCS3 stops LPS induction of CD40 [159].

There is accumulating literature on the actions of the SOCs [160]. (a) SOCs protein binds to cytokine receptors at docking sites that would be used otherwise by STATs and SHP-2. (b) SOCs function as E3 ligases that regulate degradation of JAK kinases. (c) SOCs bind directly to and inactivate tyrosine kinases such as all four JAK kinases, the insulin receptor and Tec kinases. In response to respiratory syncytial virus infection of macrophages, Socs1/3 and Cis are upregulated and the phosphorylation of IFN α -inducible Stat1/2 is suppressed. Socs1 and Socs3 regulate both DCs and macrophages. Socs1 controls presentation of antigen by DCs. Socs1 expression modulates TLR expression during DC maturation [161] and thwarts the action of GM-CSF. Socs1 regulates TLRs 2 and 4 by targeting Mal (Fig. 12.1) for destruction. IL-21 causes Socs1/3 expression and thereby stops expression of TLRs and suppresses the function of DCs [162]. By ensuring loss of sensitivity to GM-CSF, Socs ensures that TLRs do not drive the maturation of DCs but rather maturation of monocytes to macrophages [163]. In human monocytes and macrophages SOCS1 manages to suppress sustained production of TNF α , to modulate IL-6 and to down-regulate IFN β production[164].

Fig. 14.6 Regulation of cytokine signalling. The Src-homology domain 2 (SH2) domains of suppressor of cytokine signaling (SOCS) inhibit Janus phosphotyrosine kinase (*Jak*) activity, and then there is degradation of the cytokine receptors or Jaks. Socs1 and Socs3 are actually tyrosine kinase inhibitors, and they bind to JAK kinases and thereby inhibit the promotion of signal transducer and activator of transcription 1 and 3 (*Stat1/3*), respectively [177]

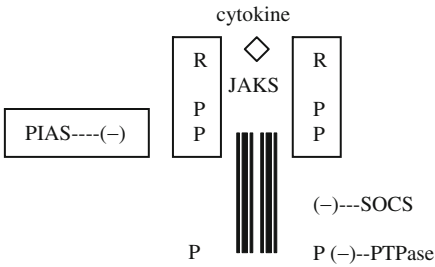


Table 14.3 Regulation of the Janus phosphotyrosine kinase-signal transducer and activator of transcription (Jak-Stat) pathways

1. Receptor-mediated endocytosis and lysosomal degradation
2. Dephosphorylation by tyrosine phosphatases SHP-1/-2 or SHIP (Src homology inositol polyphosphate 5-phosphatase)
3. Dominant-negative Stats used artificially
4. Cis/Socs (suppressor of cytokine signaling) family molecules (Table 14.2)
5. Pias (protease inhibitor of activated Stat) molecules
6. Cbl (casitas B lineage lymphoma protein), negative regulator of tyrosine kinases

There are now good examples of inhibition of cytokine transduction by Socs molecules (Fig. 14.6).

1. Cis1 is induced by Stat5, and then it binds to activated receptors for IL-2, IL-3 and EPO, and the affected receptor is degraded.
2. Socs1 regulates common γ -chain signalling, like the action of IL-15 on the homeostasis of CD8 T cells, thereby controlling autoimmune potential [165, 166].
3. Socs3 is implicated in the negative regulation of IL-6 in macrophages [167].
4. Socs3 regulates the granulocyte colony-stimulating factor (G-CSF) receptor.
5. Committed Th2 cells express Socs3, which suppresses Th1 cells; indeed, Th1 cells have shut down Socs3 expression [168]. Socs1 is relevant to Th1 cell development. IL-10 inhibition of cytokines is mediated via Socs 1 and 3 [169]. Socs2 interacts with other members of Socs and can offset their inhibitory actions, as in interferon and leptin signalling [170].

As stressed, Socs1 helps to control IFN γ [171]. Socs is implicated in the downregulation of Fc γ receptors in the kidneys [172]. Chemoattractants IL-8 and *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) regulate G-CSF signalling by inducing Socs1 [173]. Socs2 has some control over Treg cells. Socs3 helps control erythropoiesis [174]. Naïve T cells that lack SOCS3 will become Th17 cells. In view of the role of Th2 cytokines (Fig. 13.9), Socs3 is prominent in the airways of asthmatics [175]. A polymorphism of the Socs1 promoter also relates to symptoms of asthma. Socs3 will inhibit Th1 lymphocytes because it stops IL-12-mediated Stat4 signalling. When DCs are transfected with Socs3, they acquire the DC2 tolerogenic phenotype and direct the formation of Th2 lymphocytes [176].

SOCS via metabolic actions regulate insulin and leptin signalling. The multi-functional SOCS2 protein regulates the action of growth hormone and IGF-1.

Table 14.2 summarises how Cis/Socs are induced and what they do.

Consider that Socs3, by its suppression of calcineurin, inhibits the action of nuclear factor of activated T cells (NFAT), and it thus stops IL-2 production by T lymphocytes [178]. Adenovirus-carrying Socs3 has been used to ameliorate collagen-induced arthritis in mice. It controls IL-6 activity.

14.8.1 Regulation of the Jak-Stat Pathways

SOCS family proteins (Table 14.2) all have a conserved central Src-homology domain 2 (SH2) and a unique carboxyl motif at the C terminus named the SOCS box.

SHP-1 phosphatase and PIAS are present constitutively in cells. They are required to dephosphorylate receptors and Jaks or Stats. Socs are not immediately available.

PIAS proteins like PIAS1, PIAS3, PIASy or PIASx are transcriptional coregulators [179]. They work either by substrate sumoylation, as does SATB2, or through substrate interaction they determine subcellular or intranuclear localization of TFs. PIASy is a Sumo ligase for Nemo and so helps to control NF- κ B activation [180]. It has been shown how PIAS1 facilitates the recruitment of GATA3 to the promoters for IL-13 and IL-5 (cf. Fig. 4.13).

14.9 Negating Cytokines

Table 12.5 lists the cytokines and their actions. The preceding section dealt with the control of cytokines. Many researchers are taxing their minds for means of controlling cytokines therapeutically [181]. Outstanding successes have been the use of

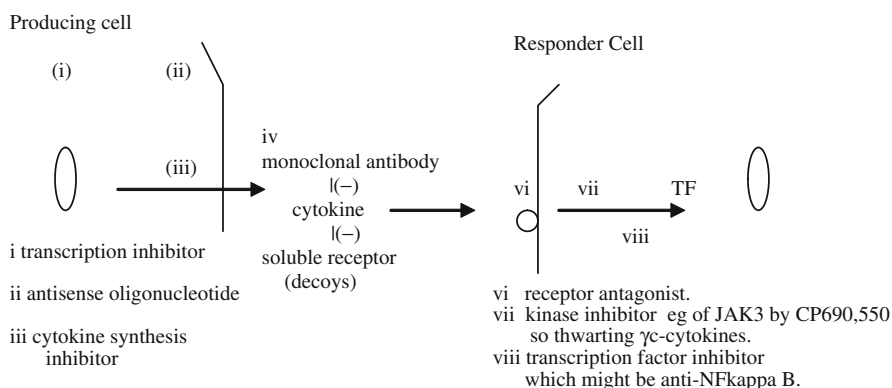


Fig. 14.7 Means of negating the output of cytokines

anti-TNF α monoclonal antibody (infliximab) or the TNFR-Fc fusion protein (etanercept) for rheumatoid arthritis. Other potential means of negating cytokines are summarized in Fig. 14.7. One might know that a soluble form of IL-13R α 2 inhibits the features of asthma in murine models. Also, a clinical trial of soluble IL-4 receptor has shown benefit in moderately severe asthma [182]. It has been emphasized repeatedly that TNF α is liable to cause necrosis of cells, but in practice that may not be so. When rheumatoid synovial fibroblasts were examined, they were found to express DR3, which is a soluble receptor that binds to FasL and protects against Fas-induced apoptosis [183] (Fig. 14.7).

Amongst the “decoys” are manufactured cytokine traps [184], created from the immunoglobulin G (IgG) constant region fused to cytokine receptor extracellular components. Drugs that inhibit NF- κ B reduce the production of cytokines [185]. Agents like phosphodiesterase 4 (PDE4) inhibitors raise cellular cAMP so that there is inhibition of NF- κ B; thus, cytokine output is curtailed [186]. Curiously, anti-inflammatory peptides like α -MSH, adrenomedullin, urocortin and vasoactive intestinal peptide (VIP) act by elevating cAMP so that protein kinase A is induced; thus, activation of NF- κ B and of JNK is thwarted. Interestingly, sphingosine-1-kinase is a negative regulator of cytokine production within CD4⁺ T cells [187].

14.10 Prostaglandin E2 and T Cell Modulation

Physiological concentrations of PGE2, produced by local macrophages or DCs, cause inhibition of proliferation of Th1 lymphocytes, support for the formation of Th2 lymphocytes and often apoptosis of B lymphocytes. Apart from the fact that PGE2 induces IL-10, by its elevation of cellular cAMP PGE2 stops expression of IL-2 receptors by Th1 cells. cAMP can inhibit the Jak-Stat1 and Stat3 pathways [188]. So, there is inhibition of Ras-dependent Raf activation [189], and at least two MAPK pathways are inhibited [190]. cAMP causes phosphorylation of CREB, the cAMP response element, so preventing NF- κ B activation of cells [191]. In macrophages, cAMP inhibits Fc γ R-mediated phagocytosis, protein kinase A suppresses formation of leukotriene B4 and TNF α , and elevation of Epac (exchange protein activated by cAMP) inhibits H₂O₂ production and bactericidal activity [192]. Likewise, adenosine A2A receptor stimulation of CD4 T lymphocytes stops production of IFN γ [193].

14.11 Interleukin 4 Signalling

IL-4 is one of the most pleiotropic cytokines. It is the major B cell growth factor. It is crucial for the differentiation and growth of Th2 cells. Its actions are antagonistic to those of IFN γ . The IL-4 receptor on Th2 lymphocytes, NK cells or basophils consists of an α -chain that binds IL-4 with high affinity and that is linked to a common γ -chain that helps transmit signals. IL-13, another cytokine that is intimately involved in

allergy, interacts with IL-13R α 1 linked to the IL-4R α . Recall that ICOS (inducible co-stimulator) receptors costimulate for the differentiation of Th2 lymphocytes. ICOS enhances IL-4 receptor signalling [194]. The stability of RNA messengers is important for the regulation of cytokines and chemokines [195]. Actually, in T cells IL-4 is regulated posttranscriptionally. mRNA for IL-4 is bound to an RNA binding protein HuR [196]. HuR is involved as a positive regulator of mRNA stability for post-transcriptional regulators of cytokine IL-13 too [197].

The IL-4R α has no intrinsic kinase activity, but it is phosphorylated when there is ligand binding. An IL-4R α has three segments: (a) an interaction site for Jak1, (b) amino acids 437–557 for activation of growth by Ras activation and for activation of PI 3-kinase and (c) a site that activates genes for Stat6 and Stat5. An upper serine-rich and acidic ID-1 region also activates Stat6 (and 5) and thus helps Th2 cell development [198]. One has to try to rationalize the several actions.

- {A} IL-4, acting via Stat6 [199], is an immunoregulatory pathway that stops the release of IL-12 and TNF α from lymphocytes, and it suppresses the IRF1 gene for IFN γ . PKC ζ , acting via Jak1 and Stat6, supports formation of Th2 cytokines [200]. Conversely, IFN γ would suppress Stat6 phosphorylation by inhibiting its recruitment to the IL-4 receptor [201]. The ID-1 region will still activate Stat5/6 when the IL-4R tyrosines are deficient [202, 203].
- {B} Via the IL-4 receptor γ c and Jak3, the PI3K path activates the Akt serine-threonine kinase, and B cell survival is promoted. Via PI3K $\rightarrow$ mTOR, G2/M transit of cells is promoted [204]. From the γ c via c-cbl [203], the adaptor protein 4PS

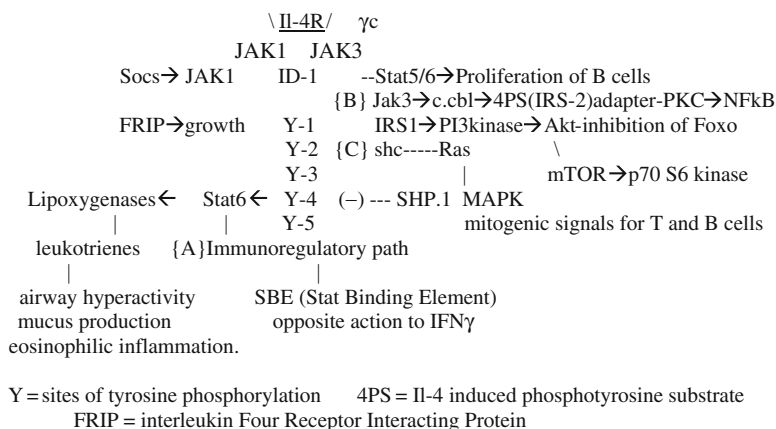


Fig. 14.8 Signaling via the interleukin 4 (IL-4) receptor. {A} The *immunoregulatory* pathway via Stat6 which binds to SBE motifs in the promoters of IL-4 inducible genes. {B} Cell *proliferation* pathway via 4PS/IRS-2 to PKC activation of NF-kB. 4PS signifies IL-4 induced phosphotyrosine substrate (same as IRS-2). {C} Cell *growth* promoted by shc/grb2/sos $\rightarrow$ Ras $\rightarrow$ MAPK and so activation of c-Myc and c-Fos. (i) IL-4 signalling uses Stat6. PC specific phospholipase C activates Stat6 [208]. (ii) IL-4 activates Stat6 dimers that bind E2F sites to aid T cell proliferation [209]. and Stat5 too aids T cell proliferation. (iii) Via the receptor IL-4R α /IL-13R α , IL-4 induces death of Th1 lymphocytes but skews cells (neonatally) to Th2 cells [210]

(IL-4-induced phosphotyrosine substrate) becomes responsible for mitogenic and antiapoptotic potential. Yet, an ITIM on the IL-4R associates with phosphatases and so regulates IL-4 induced T cell proliferation [205].

{C} The adaptor Shc recruits growth factor receptor-bound protein 2 (Grb2) and Son of Sevenless (Sos) and thus activates the Ras → MAPK pathway.

There is a unique role for Lyn as a negative regulator of signalling via IL-4 [206]. Since IL-4 is vital for IgE formation and promoting allergy in particular by promoting mast cells, Th2 cells and DCs, there is a need for such restraint. Negative regulation is also provided by Socs1 inhibition of Jak1 and by FRIP interaction (Fig. 14.8) with the IL-4R α . There is also a negative role for SHP-1 phosphatase.

As for the regulation of IL-4 gene, that is given in Fig. 4.13. The relevance of epigenetic changes of genes IL-4/5/13 was emphasized. It was found that PDK-1 activates protein kinase A; together with NFAT, that activates the IL-4 promoter [207].

14.12 Interleukin 13

IL-13R α I can substitute for common γ -chain [211, 212]. IL-13R is found on B cells, monocyte macrophages and vascular endothelial cells. It does not appear on Th0 cells and thus has no role in Th-2 cell differentiation. Binding of IL-13 induces tyrosine phosphorylation of IL-4R α (Jak2) and the IL-13R α 1-chain (Tyk2). Hence, Stat3 is activated and Stats5 and 6. IL-13 also activates the IRS-2 pathway{B}. SOCS, PIAS and SHP-1 downregulate IL-13 signalling by dephosphorylation. There is a decoy receptor for IL-13 in the form of the IL-13R α 2-chain [212]. Furthermore the transmembrane form of IL-13R α 2 can attenuate both IL-13 and IL-4 signalling [213].

IL-13 can activate secretion of PDGF-A and -C to effect lung fibroblast replication [214].

14.13 Decoy Receptors

Decoy receptors bind some inflammatory cytokines with high affinity and specificity and so prevent the agonist connecting with signalling systems. The first decoy to be identified was the IL-1 type II receptor produced by myelomonocytic cells under the influence of IL-4/13, glucocorticoid or aspirin. IL-1-RII in transmembrane or soluble form acts as a negative regulator of the IL-1 system, dampening florid local or systemic inflammatory reactions [215].

In an IL-10-dominated environment, decoy receptors are formed for the inflammatory chemokines CCR1/2/5 that affect DCs and monocytes. Thus, the actions of macrophage inflammatory proteins (MIPs) 1 α / β are thwarted.

Occasionally, soluble receptors confer on neighbouring cells the ability to respond to a ligand for which cells have an incomplete receptor system. So, the complex

formed by soluble IL-6R α and cytokine IL-6 activates signalling by the receptor gp130 glycoprotein. This process is called *trans-signalling* [216, 217]. It means that IL-6 can trigger cell responses in cell types that would be unresponsive to IL-6 itself [215], like stromal cells at sites of inflammation. IL-6R is able to promote leucocyte migration, activation and apoptosis. This mechanism operates in rheumatoid arthritis and in inflammatory bowel disease [218]. When there is fever, IL-6 trans-signalling controls the trafficking of lymphocytes across high endothelial venules [219].

Interestingly, soluble IL-6R trans-signalling stops the de novo induction of Treg [220].

References

1. ten Dijke P, Miyazono K, Heldin CH. Signaling inputs converge on nuclear effectors in TGF β signaling. *Trends Biochem Sci* 2000;25:64–70
2. Zhou S, Kinzler KW, Vogelstein B. Going MAD with Smads. *N Engl J Med* 1999;341:1144–1145
3. Blobel GC, Shiemann WP, Lodish HF. Role of transforming growth factor in human disease. *N Engl J Med* 2000;342:1350–1358
4. van Grunsven LA, Verstappen G, Huylebroeck D, Verschueren K. Smads and chromatin modulation. *Cytokine Growth Factor Rev* 2005;16:495–512
5. Choi K-C, Lee YS, Lim S et al. Smad6 negatively regulates IL-1 Receptor TLR signaling through direct interaction with the adaptor Pellino 1. *Nat Immunol* 2006;7:1057–1065
6. Izzi L, Attisano L. Regulation of the TGF β signaling pathway by ubiquitin-mediated degradation. *Oncogene* 2004;23:2071–2078
7. Whitman M. Signal transduction: feedback from inhibitory Smads. *Nature* 1997;389:549–550
8. Yamanaka M, Shegogue D, Pei H, Bu S et al. Sphingosine kinase-1 is induced by TGF β and mediates TIMP-1 upregulation. *J Biol Chem* 2004;279:53994–54001
9. Guo K. Ski and SnoN: negative regulators of TGF β signaling. *Curr Opin Genet Dev* 2004;14:65–70
10. Itoh S, ten Dijke P. Negative regulation of TGF β receptor/Smad signal transduction. *Curr Opin Cell Biol* 2007;19:176–184
11. Kajino T, Omari E. Tak1 mediates TGF β signaling by targeting Sno N oncoprotein for degradation. *J Biol Chem* 2007;282:9475–9481
12. Derynck R, Zhang YE. Smad dependent and independent pathways in TGF β family signaling. *Nature* 2003;425:577–584
13. Schiffer M, von Gersdorff G, Bitzer M et al. Smad proteins and TGF β signaling. *Kidney Int* 2000;58(Suppl 77):S45–S52
14. Massague J. TGF β signal transduction. *Annu Rev Biochem* 1998;67:753–791
15. Javeland D, Mavrić A. Mammalian transforming growth factor beta: Smad signaling and physiopathological roles. *Int J Biochem Cell Biol* 2004;36:1161–1165
16. Lu T, Tian L, Han Y, Vogelbaum M, Stark GR. Dose dependent cross-talk between TGF β and IL-1 signaling pathways. *Proc Natl Acad Sci U S A* 2007;104:4365–4370
17. Cordenonsi M, Montagner M, Adorno M et al. Integration of TGF β and MAPK signaling through p53 phosphorylation. *Science* 2007;315:840–844
18. Byfield SD, Roberts AB. Lateral signaling enhances TGF β response. *Trends Cell Biol* 2004;14:107–111
19. Liu S, Shi-Wen X, Kennedy L et al. FAK is required for TGF β induced JNK phosphorylation in fibroblasts. *Mol Biol Cell* 2007;18:2169–2178
20. Abecassis L, Rogier E, Vazquez A et al. Evidence for a role of MSK1 in TGF β 1 mediated responses through p38 and Smad signaling proteins. *J Biol Chem* 2004;279:30474–30479

21. Ventura JJ, Kennedy MJ, Flavell RA, Davis RJ. JNK regulates autocrine expression of TGF β . *Mol Cell* 2004;15:269–278
22. Lin X, Duen X, Liang Y-T et al. PPM1A functions as a Smad phosphatase to terminate TGF β signaling. *Cell* 2006;125:915–928
23. Remy I, Montmarquette A, Michnick SW. Protein kinase B/Akt modulates TGF β signalling through a distinct interaction with Smad3. *Nat Cell Biol* 2004;6:358–365
24. Conoy AR, Cao Y, Thompson EA et al. Akt interacts directly with Smad3 to regulate the sensitivity to TGF β induced apoptosis. *Nat Cell Biol* 2004;6:366–372
25. Koinuma D, Shinozaki M, Komura A et al. Arkadia amplifies the TGF β super-family signalling through degradation of Smad 7. *EMBO J* 2003;22:6458–6470
26. Suzuki C, Murakami G, Fukunchi M et al. Smurf1 regulates the inhibitory activity of Smad7 by targeting Smad 7 to plasma membrane. *J Biol Chem* 2002;277:39919–39936
27. Mishra L, Marshall B. Adaptor proteins and ubiquitinators in TGF β signaling. *Cytokine Growth Factor Rev* 2006;17:75–87
28. Wicks SJ, Liu S, Abdel Wahab N et al. Inactivation of Smad TGF β signaling by calcium-calmodulin dependent protein kinase II. *Mol Cell Biol* 2000;20:8103–8111
29. Gorelik L, Flavell RA. Transforming growth factor-beta in T cell biology. *Nat Rev Immunol* 2002;2:46–53
30. McKarns SC, Schwartz RH, Kaminski NE. Smad3 is essential for TGF-beta1 to suppress IL-2 production and TCR induced proliferation. *J Immunol* 2004;172:4275–4284
31. McKarns SC, Schwartz RH. Distinct effects of TGF-beta1 on CD4 and CD8 T cell survival and IL-2 production. *J Immunol* 2005;174:2071–2083
32. Li MO, Sanjabi S, Flavell RA. TGF β controls development, homeostasis and tolerance of T cells by regulatory T dependent and independent mechanisms. *Immunity* 2006;25:455–471
33. Li MO, Wen YY, Sanjabi S, Tobertson A-K, Flavell RA. TGF β regulation of immune responses. *Annu Rev Immunol* 2006;24:99–140
34. Veldoen M, Hocking RJ, Atkins C, Locksley R, Stockinger B. TGF β in the context of an inflammatory cytokine milieu supports differentiation of IL-17 producing T cells. *Immunity* 2006;24:179–190
35. Cua DJ, Kastein RA. TGF β , a double agent in the immune pathology war. *Nat Immunol* 2006;7:557–559
36. Lee K-Y, Ito K, Hayashi R et al. NF κ B and activator protein 1 response elements and the role of histone modifications in IL-1 β induced TGF β 1 gene transcription. *J Immunol* 2006;176:603–615
37. Lu T, Tion L, Han Y et al. Dose dependent crosstalk between the TGF β and IL-1 signaling pathways. *Proc Natl Acad Sci U S A* 2007;104:4365–4370
- 38a. Ganhi R, Andersen DE, Weiner HL. Immature DCs express latency associated peptide and inhibit T cell activation in a TGF β dependent manner. *J Immunol* 2007;178:4017–4021
- 38b. Annes JP, Munger JS, Rifkin DB. Making sense of latent TGF-beta activation. *J Cell Sci* 2003;116:217–224
39. Kretschmer A, Moepert K, Dames S et al. Differential regulation of TGF β signaling through Smad2, Smad3 and Smad4. *Oncogene* 2003;22:6734–6763
40. Remy I, Montmarquette A, Michnick SW. Protein kinase B/Akt modulates TGF β signalling through a direct interaction with Smad3. *Nat Cell Biol* 2004;6:358–365
41. Conery AR, Cao Y, Thompson EA et al. Akt interacts with Smad3 to regulate sensitivity to TGF β induced apoptosis. *Nat Cell Biol* 2004;6:366–372
42. Huang SS, Leal SM, Chen CL, Liu IH, Huang JS. Cellular growth inhibition by TGF β 1 involves IRS proteins. *FEBS Lett* 2004;565:117–121
43. Feng X-H, Derynck R. Specificity and versatility in TGF β signaling through Smads. *Annu Rev Cell Dev Biol* 2005;21:659–693
44. Ravitz MJ, Wenner CE. Cyclin dependent kinase regulation during G1 phase and cell regulation by TGF β . *Adv Cancer Res* 1997;171:165–207
45. Wolfrum LA, Walz TM, James Z et al. p21^{cip.1} and p27^{kip.1} act in synergy to alter sensitivity of naïve T cells to TGF β mediated G1 arrest through modulation of IL-2 responsiveness. *J Immunol* 2004;173:3093–3102

46. Nagata M, Goto K, Ehata S et al. Nuclear and cytoplasmic s-Ski differently modulate cellular functions. *Genes Cells* 2006;11:1267–1280
47. Krakowski AR, Laboureaux J, Mauviel A et al. Cytoplasmic SnoN antagonizes TGFbeta signaling by sequestration of Smad proteins. *Proc Natl Acad Sci U S A* 2005;102:12437–12442
48. Bright JJ, Sriram S. TGFβ inhibits IL-12 induced activation of Jak-Stat pathway in T lymphocytes. *J Immunol* 1998;161:1772–1777
49. Genestier L, Kasibhatla S, Brunner T, Green DR. TGFβ inhibits FasL expression and subsequent activation induced cell death in T cells via downregulation of c-Myc. *J Exp Med* 1999;189:231–239
50. Letterio JJ, Roberts AB. Regulation of immune responses by TGFβ. *Annu Rev Immunol* 1998;16:137–161
51. Huber S, Schwann C, Lehr HA et al. TGFβ signaling is required for expansion and immunosuppressive capacity of reg CD4⁺CD25⁺ T cells *J Immunol* 2004;173:6526–6531
52. ten Dijke P, Goumans M-J, Itoh F, Otoh S. Regulation of cell proliferation by Smad proteins. *J Cell Physiol* 2002;191:1–16
- 53a. Siafakas AR, Richardson DR. Growth arrest and DNA damage-45 alpha (GADD45a). *Int J Biochem Cell Biol* 2009;41:986–989
- 53b. Yoo J, Ghiassi M, Jirmanova L et al. TGFβ induced apoptosis is mediated by Smad dependent expression of GADD45b. *J Biol Chem* 2003;278:43001–43007
54. Dai C, Liu Y. Hepatocyte growth factor antagonises profibrotic action of TGFβ1 in mesangial cells by stabilizing Smad Transcriptional Corepressor TGFβ. *J Am Soc Nephrol* 2004;15:1402–1412
55. Iwano M, Neilson EG. Mechanisms of tubulointerstitial fibrosis. *Curr Opin Nephrol Hypertens* 2004;13:279–284
56. Yanagita M. Bone morphogenetic protein antagonists. *Cytokine Growth Factor Rev* 2005;16:309–317
57. Mochida Y, Parisuthiman D, Kaku M et al. Nephrocan is an inhibitor of TGFβ signaling. *J Biol Chem* 2006;281:36044–36051
58. Gupta S, Clarkson MR, Duggan J, Brady HR. Connective tissue growth factor: potential role in glomerulosclerosis and tubulointerstitial fibrosis. *Kidney Int* 2000;58:1389–1399
59. Zahner G, Disher M, Thaiss F et al. Effect of prostaglandin E2 on mRNA expression and secretion of collagens I, III and IV and fibronectin in cultured rat mesangial cells. *J Am Soc Nephrol* 1994;4:1778–1785
60. Mori T, Kawara S, Shinozaki M et al. Role and interaction of CTGF with TGFbeta in persistent fibrosis: a mouse fibrosis model. *J Cell Physiol* 1999;181:153–159
61. Runyon CE, Schnaper HW, Ponalet A-C. Smad3 and protein kinase C mediate TGFβ1 induced Coll I expression in human mesangial cells. *Am J Phys Renal Physiol* 2003;285:F413–F422
62. Flanders K. Smad 3 as mediator of fibrotic response. *Int J Exp Pathol* 2004;85:47–64
63. Chen L, Klass C, Woods A. Syndecan 2 regulates TGFβ signaling. *J Biol Chem* 2004;279:15715–15718
64. Meurer SK, Lahme B, Tihaa L et al. N-Acetyl-cysteine suppresses TGFβ signaling at distinct molecular steps. *Biochem Pharmacol* 2005;70:1026–1032
65. Zhou G, Li C, Cai L. AGEs induce CTGF mediated renal fibrosis predominantly through TGFbeta-independent pathway. *Am J Pathol* 2004;165:2033–2043
66. Abreu JG, Ketpura NI, Reversade B et al. CTGF modulates cell signalling by BMP and TGFbeta. *Nat Cell Biol* 2002;4:599–604
67. van Nieuwenhoven FA, Jensen LJN, Flyvberg A, Goldschmeding R. Imbalance of growth factor signaling in diabetic kidney disease. *Nephrol Dial Transplant* 2005;20:6–10
68. Brigstock DR. CCN family: a new stimulus package. *J Endocrinol* 2003;178:169–175
69. Asano Y, Ihn H, Yamone K et al. Impaired negative regulation of TGFβ signaling in scleroderma fibroblasts. *J Clin Invest* 2004;113:253–264
70. Zhou XD, Xiong MM, Tan FK et al. Sparc, an upstream regulator of CTGF in response to TGFβ stimulation. *Arthritis Rheum* 2006;54:3885–3889

71. Lozano E, Segarra M, Cid MC. Simulatory autoantibodies to the PDGF receptor in scleroderma. *N Engl J Med* 2006;355:1278–1279
72. Ulloa L, Doody J, Massague J. Inhibition of TGF β /Smad signaling by the interferon/Stat pathway. *Nature* 1999;397:710–713
73. Wynn TA. Fibrotic disease and the Th1/Th2 paradigm. *Nat Rev Immunol* 2004;4:583–594
74. Wang S, Wilkes MC, Peol EB, Hirschberg E. Imatinib mesylate blocks a non-Smad TGF β pathway and reduces renal fibrogenesis in vivo. *FASEB J* 2005;19:1–11
75. Daniels CE, Wilkes MC, Edens M et al. Imatinib mesylate inhibits the pro-fibrogenic activity of TGF β and prevents bleomycin lung fibrosis. *J Clin Invest* 2004;114:1308–1316
- 76a. Tridandapani S, Wardrop R, Baran CP et al. TGF β suppresses myeloid Fc γ receptor function by regulating the expression and function of the common γ subunit. *J Immunol* 2003;170:4572–4577
- 76b. Takeuchi O, Akira S. MyD88 as a bottle neck in Toll/IL-1 signaling. *Curr Top Microbiol Immunol* 2002;270:155–167
77. Ronger-Savle S, Valladeau J, Claudy A et al. TGF β inhibits CD1d expression on dendritic cells. *J Invest Dermatol* 2005;124:116–118
79. Nenci A, Becker C, Wullaert A et al. Epithelial Nemo links innate immunity to chronic intestinal inflammation. *Nature* 2007;446:557–560
80. Kelly D, Campbell JI, King TP et al. Commensal anaerobic gut bacteria attenuate inflammation by regulating shuttling of PPAR γ and RelA. *Nat Immunol* 2004;5:104–112
- 81a. Monteleone G, Pallone P, MacDonald TT. Smad 7 in TGF β mediated negative regulation of gut inflammation. *Trends Immunol* 2004;75:513–517
- 81b. Li MO, Flavell RA. Contextual regulation of inflammation: a duct by transforming growth factor-beta and interleukin-10. *Immunity* 2008;28:468–478
82. Broderick L, Bankert RB. Membrane associated TGF β inhibits human memory T cell signalling in malignant and non-malignant inflammatory microenvironments. *J Immunol* 2006;177:3082–3088
83. Posche P. Role of transforming growth factor in cancer. *J Cell Physiol* 2001;186:153–168
84. Pick E, Roberts AB. Suppressor and oncogenic roles of TGF β and its signaling pathways in oncogenesis. *Adv Cancer Res* 2001;83:1–54
85. Matsuura I, Denissova NG, Wang G et al. Cyclin dependent kinase regulates the antiproliferative function of Smads. *Nature* 2004;430:226–231
86. Kamarju AJ, Roberts AB. Role of Rho/Rock and p38 MAPkinase pathways in TGF β mediated Smad dependent growth inhibition of breast cancer cells. *J Biol Chem* 2005;280:1024–1036w
87. Friess H, Yamanaka T, Buchler M, Ebert M et al. Enhanced expression of TGF β isoforms in pancreatic cancer correlates with decreased survival. *Gastroenterology* 1993;105:1846–1856
88. Akhurst RJ. Large and small molecule inhibitors of TGF β signaling. *Curr Opin Invest Drugs* 2006;7:513–521
89. Wu K, Yang Y, Wang C et al. Dach1 inhibits TGF β signaling through binding Smad4. *J Biol Chem* 2003;278:51673–51684
90. Kretschmer M, Dooley J, Timokhina I, Massague JA. A mechanism of repression of TGF β /Smad signaling by oncogenic Ras. *Genes Dev* 1999;13:804–816
91. Gomis RR, Alarcon C, Nadal C et al. C/EBP β at the core of the TGF β cytostatic response and its evasion in metastatic breast cancer cells. *Cancer Cell* 2006;10:203–214
92. Yamagata H, Matsuzaki K, Moris S et al. Acceleration of Smad2 and Smad3 phosphorylation via c-Jun NH(2) terminal kinase during human colorectal carcinogenesis. *Cancer Res* 2005;65:157–165
93. Liu F. Smad3 phosphorylation by cyclin dependent kinases. *Cytokine Growth Factor Rev* 2006;17:9–16
94. Matsuura ID, Enissova NG, Wang G et al. CDKs regulate the antiproliferative function of Smads. *Nature* 2004;430:226–231
95. Dong M, Blobel GC. Role of transforming growth factor- β in hematologic malignancies. *Blood* 2006;107:4589–4596

96. Chen D, Zhao M, Mundy GR. Bone morphogenetic proteins. *Growth Factors* 2004;22:233–241
97. Bhatia M, Bonnet D, Wu D et al. BMPs regulate the developmental program of human hematopoietic stem cells. *J Exp Med* 1999;189:1139–1147
98. Shi W, Chang C, Nie S et al. Endofin acts as a Smad anchor for receptor activation in BMP signaling. *J Cell Sci* 2007;120(7):1216–1224
99. Chen B, Blair DG, Plisou S et al. BMP antagonists Dm/Gremlin and Don interact with Slits and Act as negative regulators of monocyte chemotaxis. *J Immunol* 2004;173: 5914–5917
100. Stark GR, Kerr IM, Williams BRG et al. How cells respond to interferons. *Annu Rev Biochem* 1998;67:227–264
101. Smith PL, Lombardi G, Foster GR. Type I interferons and the innate immune response-more than just antiviral cytokines. *Mol Immunol* 2005;42:869–877
102. Honda K, Takaoka A, Taniguchi T. Type I IFN gene induction by the IRF family of transcription factors. *Immunity* 2006;25:349–360
103. Reis L, Hanada H, Wolchok JD et al. Critical role of transcription factor IRF1 in the regulation of IFN γ inducible genes. *EMBO J* 1992;11:185–193
- 104a. Nguyen H, Hiscott J, Pitha PM. The growing family of interferon regulatory factors. *Cytokine Growth Factor Revs* 1997;8:293–312
- 104b. Hu X, Chakravarty SD, Ivashkiv LB. Regulation of interferon and Toll-like receptor signaling during macrophage activation. *Immunol Revs* 2008;226:41–56
105. Taniguchi T, Takaoka A. The IFN α/β system in antiviral responses: a multimodal machinery of gene regulation by IRF family of transcription factors. *Curr Opin Immunol* 2002;14:111–116
106. Ramana CB, Gil MP, Schreiber RD, Stark GR. Stat1 dependent and independent pathways in IFN γ dependent signaling. *Trends Immunol* 2002;23:96–101
107. Benedict CA, Ware CF. RIGing a virus trap. *Nat Med* 2005;11(9):929
- 108a. Creagh EM, O'Neill LAJ. TLRs, NLRs and RLRs. *Trends Immunol* 2006;27:352–356
- 108b. Kaur S, Sassano A, Joseph AM et al. Dual regulatory roles of phosphatidyl 3-kinase in IFN signaling. *J Immunol* 2008;181:7316–7323
109. Nurlan MA, Clemens HJ. Inhibition of cell proliferation by interferons. *Biochem J* 1986;237:871–876
110. Alsayed Y, Vadin S, Ahmad S et al. Interferon-gamma activates the C3G/Rap-1 signaling pathway. *J Immunol* 2000;164:1800–1806
- 111a. Epstein LB. The immunoregulatory importance of interferons in vivo. *Ann Inst Pasteur (Immunol)* 1985;135D:68–73
- 111b. Kelchtermans H, Billiau A, Matthys P. How interferon- γ keeps autoimmune diseases in check. *Trends Immunol* 2008;29:479–485
- 111c. Honma K, Kimura D, Tominaga N et al. IRF4 regulates the production of Th2 cytokines in naïve vs., effector/memory CD4+ T cells. *Proc Natl Acad Sci(USA)* 2008;105: 15890–15895
112. Duncan GS, Mittrucker H-W, Kagi D et al. Transcription factor IRF-1 is essential for NK cell function in vivo. *J Exp Med* 1996;184:2043–2048
113. Schindler C, Fu XY, Improta T et al. Proteins of transcription factor ISGF-3 that are activated by IFN- α . *Proc Natl Acad Sci U S A* 1992;89:7836–7839
114. Taniguchi T, Ogasawara K, Takaoka A, Tanaka N. IRF family of transcription factors as regulators of host defence. *Annu Rev Immunol* 2001;19:623–655
115. Jefferies CA, Fitzgerald KA. Interferon gene regulation: not all roads lead to Tolls. *Trends Mol Med* 2005;11:403–411
116. Han K-J, Su X, Xu L-G et al. Mechanisms of the TRIF induced interferon stimulated response element and NF κ B activation and apoptosis pathways. *J Biol Chem* 2004;279: 15652–15661
- 117a. Seya T, Oshiumi H, Sasai M et al. TICAM-1 and TICAM-2: toll like receptor adapters that participate in induction of type 1 interferons. *Int J Biochem Cell Biol* 2005;37:524–529;

- 117b. Tang X, Gao J-S, Gua Y-J et al. Acetylation dependent signal transduction for type I IFN receptor. *Cell* 2007;131:93–105
118. van Boxel-Dezaire AHH, Sandya Rani MR, Stark GR. Complex modulation of cell type specific signaling in response to type I interferons. *Immunity* 2006;25:361–372
119. Arorco T, Floyd-Smith G, Esp MJ, Jelinek DF. Dissociation between IFN α induced antiviral and growth signaling pathways. *J Immunol* 1999;162:3289–3297
120. Petricoin EF, Ito S, Williams BL. Antiproliferative action of IFN requires components of T cell receptor signalling. *Nature* 1997;390:629–639
121. O'Shea JJ, Visconti R. Type I IFNs and regulation of Th1 responses. *Nat Immun* 2000;1:17–19
122. Kawa T, Sato S, Ishii KJ et al. IFN- α induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. *Nat Immun* 2004;5:1061–1068
123. Kamath AT, Sheasby CE, Tough DF. Dendritic cells and NK cells stimulate bystander T cell activation in response to TLR agonists through secretion of IFN α/β and IFN γ . *J Immunol* 2005;174:767–776
124. Lohoff M, Mak TW. Roles of interferon-regulatory factors in T helper cell differentiation. *Nat Rev Immunol* 2005;5:125–135
125. Romieu-Mourez R, Solis M, Nardin A et al. Roles for IRF3 and IRF7 in the activation of antitumor human macrophages. *Cancer Res* 2006;66:10576–10585
126. Vankayalapati R, Klucar P, Wizel B et al. NK cells regulate CD8 $^{+}$ T cell effector function in response to an intracellular pathogen. *J Immunol* 2004;172:130–137
127. Colonna M. TLR pathways and IFN-regulatory factors. *Eur J Immunol* 2007;37:306–309
128. MacMicking JD. IFN-inducible GTPases and immunity to intracellular pathogens. *Trends Immun* 2004;25:601–608
129. Yoneyama M, Kikuchi M, Matsumoto K et al. Shared and unique functions on the DExD/H-box helicases in antiviral innate immunity. *J Immunol* 2005;175:2851–2858
130. Sen GC, Sarkar SN. Hitching RIG to action. *Nat Immun* 2005;6:1074–1076
- 131a. Takaoka A, Wang Z, Choi MK et al. DAI is a cytosolic DNA sensor and activator of the innate immune response. *Nature* 2007;448:501–505;
- 131b. van Boxel-Dezaire AHH Sandhya Rani MR, Stark GR. Complex modulation of cell type-specific signaling in response to type I interferons. *Immunity* 2006;25:361–372
132. Meylon E, Curran J, Hofmann K et al. Cardif is an adaptor protein in the RIG1 antiviral pathway and is targeted by hepatitis C virus. *Nature* 2005;437:1167–1172
- 133a. Yoshida R, Takaesu G, Yoshida H et al. TRAF6 and MEKK1 play a pivotal role in the RIG1 helicase anti-viral pathway. *J Biol Chem* 2008;283:36211–36220
- 133b. Bibeau-Poirier A, Gravel S-P, Clement J-F et al. Tank-binding kinase 1/IKKi and Cullin-based ubiquitin ligases in IRF3 degradation. *J Immunol* 2006;177:5059–5067
134. Matsui K, Kumagai Y, Kato H et al. Role of TBK1 and inducible I κ B kinase in IFN responses against viruses in innate immune cells. *J Immunol* 2006;177:5785–5789
135. Iwasaki A, Medzhitov R. Toll like receptor control of the adaptive immune responses. *Nat Immun* 2004;5:987–995
136. Balmelli C, Vincent IE, Rau H et al. Fc γ RII dependent sensitization of natural IFN producing cells for viral infection and IFN α responses. *Eur J Immunol* 2005;35:2406–2415
137. Greene JA, De Vecchio JL, Gould MP et al. Regulation of type I IFN synthesis by synergistic effects of CD40 and type II IFN. *J Immunol* 2006;176:5995–6003
138. Hoshino K, Sugiyama T, Matsumoto M et al. Ikappa B kinase α is critical for IFN α production induced by Toll-like receptors 7 and 9. *Nature* 2006;440:949–953
- 139a. Goutagny N, Severa M, Fitzgerald KA. Pin-ning down immune responses to RNA viruses. *Nat Immun* 2006;7:555–558
- 139b. Zeng W, Chen ZJ. MITA gating viral infection. *Immunity* 2008;29:513–515
- 139c. Huang J, Liu T, Xu LG et al. SIKE is an IKK epsilon/TBK1-associated suppressor of TLR3 and virus-triggered IRF3 activation pathways. *EMBO J* 2005;24:4018–4028
140. Carty M, Goodbondy R, Schroder M et al. The human adaptor SARM negatively regulates adaptor protein TRIF dependent TLR signaling. *Nat Immun* 2006;7:1074–1081

- 141a. An H, Zhao W, Hou J. SHP2 phosphatase negatively regulates TRIF adapter protein dependent type I interferon and proinflammatory cytokine production. *Immunity* 2006;25:919–928
- 141b. Wang H, Garcia CA, Rehani K et al. IFN-beta production by TLR4-stimulated innate immune cells is negatively regulated by GSK3-beta. *J Immunol* 2008;181:6797–6802
- 142. Sharif MN, Sosic D, Rothlin CV et al. Twist mediates suppression of inflammation by type I IFNs and Axl. *J Exp Med* 2006;203:1891–1901
- 143a. Costa-Mattioli M, Sonenberg N. RAPPing production of type I interferon in pDCs through mTOR. *Nat Immunol* 2008;9:1097–1099
- 143b. Blasius AL, Colonna M. Sampling and signaling in plasmacytoid dendritic cells: role of Siglec-H. *Trends Immunol* 2006;27:255–260
- 144. Cao W, Liu Y-J. OPN: key regulator of pDC interferon production. *Nat Immunol* 2006;7:441–443
- 145. Guiducci C, Ott G, Chan JH et al. Properties regulating the nature of the plasmacytoid dendritic cell response to TLR9 activation. *J Exp Med* 2006;203:1999–1908
- 146. Osawa Y, Iho S, Takauji R et al. NFkB and p38 MAPK are involved in CpG DNA-induced IFN α and chemokine production in human plasmacytoid dendritic cells. *J Immunol* 2006;177:4841–4852
- 147a. Fanning SL, George TC, Feng D et al. Receptor crosslinking on plasmacytoid dendritic cells leads to regulation of IFN α production. *J Immunol* 2006;177:5829–5839
- 147b. Gilliet M, Cao W, Liu Y-J. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. *Nat Rev Immunol* 2008;8:594–606
- 147c. Stout-Delgado HW, Yang X, Walker WE et al. Ageing Impairs IRF7 up-regulation in plasmacytoid dendritic cells during TLR9 activation. *J Immunol* 2008;281:6747–6756
- 148. Yasukawa H, Sasaki A, Yoshimura A. Negative regulation of cytokine signaling pathways. *Annu Rev Immunol* 2000;18:143–164
- 149. Shuai K, Liu B. Regulation of Jak-Stat signalling in the immune system. *Nat Rev Immunol* 2003;3:900–911
- 150. Chang MMW, Metcalf D, Jamieson E et al. Socs1 in T cells and macrophages is critical for preventing lethal inflammation. *Blood* 2005;106:1668–1675
- 151. Gonzalez-Leon MC, Soares-Schanoski A, del Fresno C et al. NO induces Socs1 expression in monocytes in a TNFa dependent manner. *J Endotoxin Res* 2006;12:296–306
- 152. Tassilus I, Park-Min KH, Hu Y et al. Apoptotic cells inhibit LPS induced cytokine and chemokine production and IFN responses in macrophages. *Hum Immunol* 2007;68:156–164
- 153. Watanabe H, Kubo M, Numata K et al. Overexpression of SOCS5 in T cells augments innate immunity during septic peritonitis. *J Immunol* 2006;177:8650–8657
- 154. Shuai K, Liu B. Regulation of gene-activation pathways by Pias proteins in the immune system. *Nat Rev Immunol* 2005;5:593–605
- 155. Alexander WS, Starr S, Fenner JE et al. Socs1 is a critical inhibitor of IFN γ signaling. *Cell* 1999;98:97–108
- 156. O'Sullivan LA, Lionque C, Lewis RS, Stephenson SEM, Ward AC. Cytokine receptor signaling through the Jak-Stat-Socs pathway in disease. *Mol Immunol* 2007;44(10):2497–2506
- 157. O'Keefe GM, Nguyen VT, Tang LP, Benevise EN. IFN γ regulation of class II transactivator promoter IV in macrophages and microglia: involvement of Socs1 proteins. *J Immunol* 2001;166:2260–2269
- 158. Berlato C, Cassatella MA, Kinjo I et al. Involvement of Socs3 as mediator of the inhibitory effects of IL-10 on LPS macrophage activation. *J Immunol* 2002;168:6404–6411
- 159. Qin H, Wilson CA, Roberts KL et al. IL-10 inhibits LPS induced CD40 gene expression through induction of Socs3. *J Immunol* 2006;177:7761–7771
- 160. Knisz J, Rothman PB. Suppressor of cytokine signaling in allergic inflammation. *J Allergy Clin Immunol* 2007;119:739–745
- 161. Bartz H, Avalos NM, Baetz A, Heeg K, Dalpke A H. Involvement of Socs in Toll-like receptor mediated block of dendritic cell differentiation. *Blood* 2006;108:4102–4108

162. Strengell M, Lehtonen A, Matikainen S, Jufferman I. Il-21 enhances SOCS gene expression and inhibits LPS induced cytokine production in human monocyte derived dendritic cells. *J Leukoc Biol* 2006;79:1279–1285
163. Bartz H, Avalos NM, Baetx A et al. Involvement of Socs in TLR-mediated block of dendritic cell differentiation. *Blood* 2006;108:4102–4108
164. Prele CM, Woodward EA, Bisley J et al. SOCS1 regulates the IFN but not NFkB pathway in TLR-stimulated human monocytes and macrophages. *J Immunol* 2008;181:8018–8026
165. Ilangumoran S, Ramanathan S, LaRose J et al. Socs1 regulates Il-15 receptor signaling. *J Immunol* 2003;171:2435–2445
166. Davey GM, Star R, Cornish AL et al. Socs.1 regulates Il-15 driven homeostatic proliferation of antigen-naïve CD8 T cells, limiting autoimmune potential. *J Exp Med* 2005;202:1099–1108
167. Croker BA, Krebs DL, Zhang JG et al. Socs3 negatively regulates Il-6 signaling in vivo. *Nat Immun* 2003;4:540–545
168. Johnston JA. Are SOCS suppressors, regulators and degraders. *J Leukoc Biol* 2004;75:743–748
169. Dennis VA, Jefferson A, Singh SR et al. Il-10 anti-inflammatory response to *Borrelia burgdorferi*: role for SOCS 1 and 3. *Infect Immunol* 2006;74:5780–5789
170. Piessevaux J, Lavens D, Montoye T et al. Functional cross-modulation between Socs proteins can stimulate cytokine signalling. *J Biol Chem* 2006;281:32953–32966
171. Ilangumaran S, Ramanathan S, Rottapel R. Regulation of the immune system by SOCS family adapter proteins. *Semin Immunol* 2004;16:351–365
172. Gomez-Guerrero C, Lopez-Franco O, Sanjuan G et al. Suppressors of cytokine signaling regulate Fcγ receptor signaling and cell activation during immune renal injury. *J Immunol* 2004;172:6969–6977
173. Stevenson NJ, Haan S, McClurg AE et al. Attractants Il-8 and FMLP regulate G-CSF signaling by inducing SOCS-1 expression. *J Immunol* 2004;173:3243–3249
174. Marine LC, McKay C, Wang D, Topham DJ et al. Socs3 is essential in the regulation of fetal liver erythropoiesis. *Cell* 1999;98:597–608
175. O'Shea JJ. Targetting the Jak/Stat pathway for immunosuppression. *Ann Rheum Dis* 2004;63(Suppl 11):1167–1171
176. Heller NM, Matsukura S, Georas SN et al. IFN-gamma inhibits Stat6 signal transduction and gene expression in human airway epithelial cells. *Am J Resp Cell Mol Biol* 2004;31:573–582
177. Li Y, Chu N, Rostami A, Zhang GX. Dendritic cells transduced with Socs3 form tolerogenicDC2 phenotype that directs Th2 cell differentiation. *J Immunol* 2006;177:1679–1688
178. Banerjee A, Banks AS, Nawijn ML et al. Suppressor of cytokine signaling 3 inhibits activation of NFATp. *J Immunol* 2002;168:4277–4281
179. Sharrocks AD. PIAS proteins and transcriptional regulation—more than just SUMO E3 ligases. *Genes Dev* 2006;20:754–758
180. Mabb AM, Wuerzberger-Davis SM, Miyaneh S. PIASy mediates Nemo sumoylation and NFkB activation in response to genotoxic stress. *Nat Cell Biol* 2006;8:986–993
181. Econimides AN, Carpenter LR, Rudge JS et al. Cytokine traps: multicomponent high affinity blockers of cytokine action. *Nat Med* 2003;9:47–52
182. Borish LC, Nelson HS, Lanz MJ et al. Il-4 receptor in moderate atopic asthma. *Am J Respir Crit Care Med* 1999;160:1816–1823
183. Hayashi S, Piura Y, Nishiyama T et al. DR3 expressed in rheumatoid synovial fluid fibroblasts protects cells against Fas-induced apoptosis. *Arthritis Rheum* 2007;56:1067–1075
184. Rakesh K, Agrawal DK. Controlling cytokine signaling by constitutive inhibitors. *Biochem Pharmacol* 2005;70:649–657
185. Tegedeer I, Pfeilschifter J, Geisslinger G. Cyclooxygenase independent action of cyclooxygenase inhibitors. *FASEB J* 2001;15:2057–2072
186. Jimenez JL, Punzon C, Nararro J et al. PDE4 inhibitors prevent cytokine secretion by T lymphocytes by inhibiting NFkB and NFAT activation. *J Pharmacol Exp Ther* 2001;299:753–759

187. Yang J, Castle BE, Honidu A et al. Sphingosine kinase 1 is a negative regulator of CD4⁺ Th.1 cells. *J Immunol* 2005;175:6580–6588
188. Sengupta TK, Schmitt EM, Ivashkiv LB. Inhibition of cytokines and Jak-Stat activation by distinct signalling mechanisms. *Proc Natl Acad Sci U S A* 1996;93:9499–9504
189. Cook SJ, McCormick F. Inhibition by cAMP of Ras dependent activation of Raf. *Science* 1993;262:1069–1072
190. Tamier A, Granot Y, Isakar N. Inhibition of T lymphocyte activation by cAMP is associated with regulation of two MAPK kinase pathways. *J Immunol* 1996;157:1514–1521
191. Barry CGN, Mackman N. Role of cAMP response element binding protein in a cAMP inhibition of NFkB mediated transcription. *J Immunol* 1997;159:5450–5456
192. Aronoff DM, Canetti C, Serezani C et al. Macrophage inhibition by cAMP: differential roles of protein kinase A and exchange protein directly activated by cAMP. *J Immunol* 2005;174:595–599
193. Lappas CM, Tieger JM, Linden J. A2A adenosine receptor induction inhibits IFN γ production in murine CD4⁺ T cells. *J Immunol* 2005;174:1073–1080
194. Watanabe M, Watanabe S, Hara Y et al. ICOS mediated costimulation on Th2 differentiation is achieved by the enhancement of Il-4 receptor mediated signaling. *J Immunol* 2005;174:1989–1996
195. Meyer S, Tenne C, Wahle E. Messenger RNA turnover in eukaryotes: pathways and enzymes. *Crit Rev Biochem Mol Biol* 2004;39:197–216
196. Yarovinsky TO, Butler NS, Manick MM, Hunninghake GW. Early exposure to Il-4 stabilizes Il-4 mRNA in CD4 T cells via RNA binding protein HuR. *J Immunol* 2006;177:4426–4435
197. Casolaro V, Fang X, Tancowny B et al. Post-transcriptional regulation of IL-13 in T cells: role of RNA binding protein HuR. *J Allergy Clin Immunol* 2008;121:853–859
198. Zhu J, Guo L, Min B, Watson CJ et al. Growth factor independent-1 induced by Il-4 regulates Th2 cell proliferation. *Immunity* 2002;16:733–744
199. Takeda K, Tanaka T, Si W, Matsumoto M. Essential role for Stat6 in Il-4 signalling. *Nature* 1996;380:627–628
200. Martin P, Villares R et al. Control of T helper 2 cell function and allergic airway inflammation by PKC zeta. *Proc Natl Acad Sci U S A* 2005;102:9866–9871
201. Huang Z, Xin J, Coleman J, Huang H. IFN γ suppresses Stat6 phosphorylation by inhibiting its recruitment to the Il-4 receptor. *J Immunol* 2005;174:1332–1337
202. Mora AL, Stephenson LM, Enerson B et al. New programming of Il-4 receptor signal transduction in activated T cells. *J Immunol* 2004;171:1891–1900
203. Stephenson LM, Park D-S, Mora AL, Goenka S, Boothby M. Sequence motifs in Il-4R α mediating cell-cycle progression of primary lymphocytes. *J Immunol* 2005;175:5178–5185
204. Veno H, Sasaki K, Honda H et al. C-cbl is tyrosine phosphorylated by Il-4 and enhances mitogenic and survival signals of Il-4R by linking with the PI3-kinase pathway. *Blood* 1998;91:46–53
205. Kashiwoda M, Gialburakis CC, Pan P-Y, Rothman PB. ITIM of the Il-4 receptor associates with SH2 containing phosphatases and regulates Il-4 induced proliferation. *J Immunol* 2001;167:627–638
206. Janas ML, Hodgkin P, Hibbs M, Tarlinton D. Genetic evidence for Lyn as a negative regulator of Il-4 signaling. *J Immunol* 1999;163:4192–4198
207. Nirula A, Ho M, Phee H, Roose J, Weiss A. PDK1 targets protein kinase A in a pathway that regulates Il-4. *J Exp Med* 2006;203:1733–1744
208. Zamoro J, Rivas MD, Garcia-Trinidad A et al. Phosphatidylcholine specific phospholipase C activity is necessary for the activation of Stat 6. *J Immunol* 2003;171:4203–4205
209. McDonald C, Vanscoy S, Hearing P, Reich NC. Induction of genes involved in cell cycle progression by interleukin 4. *J Interferon Cytokine Res* 2004;24:729–738
210. Li L, Lee H-H, Bell JJ et al. Il-4 utilises an alternative receptor to drive apoptosis of Th1 cells and skews neonatal immunity toward Th2. *Immunity* 2004;20:429–440
211. Kelly-Welch AE, Hanson EM, Boothby MR, Keegan AD. Interleukin 4 and interleukin 13 signaling connection maps. *Science* 2003;300:1527–1528

212. Hershey K. Interleukin 13 and signaling pathways. *J Allergy Clin Immunol* 2003; 111:677–690
213. Andrews A-L, Nasir T, Bucchieri F et al. A regulator of Il-13 and Il-4 signal transduction in primary human fibroblasts. *J Allergy Clin Immunol* 2006;118:858–865
214. Ingram JL, Antao-Menzes A, Mangum JB et al. Opposing actions of Stat1 and 6 on Il-13 induced upregulation of egr-1 and PDGF ligands in pulmonary fibroblasts. *J Immunol* 2006; 177:4141–4148
215. Mantovani A, Locati M, Vecchi A et al. Decoy receptors: a strategy to regulate inflammatory cytokines and chemokines. *Trends Immunol* 2001;21:328–334
216. Peters M, Muller AM, Rose-John S. Il-6 and soluble Il-6 receptor: stimulation if gp130 and hematopoiesis. *Blood* 1998;92:3495–3504.
217. Rose-John S, Scheller J, Elson G, Jones SA. Il-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer. *J Leukoc Biol* 2006;80:227–236
218. Jones SA. Directing transition from innate to acquired immunity: defining a role for Il-6. *J Immunol* 2005;175:3463–3468
219. Chen Q, Fisher DT, Clancy KA et al. Fever range thermal stress promotes lymphocyte trafficking via Il-6 trans-signaling. *Nat Immunol* 2006;7(12):1299–1308
220. Dominitzki S, Fantini MC, Neufert C et al. Trans-signaling via soluble Il-6R abrogates induction of Foxp3 naïve CD4⁺CD25⁻ T cells. *J Immunol* 2007;179:2041–2045

Chapter 15

Natural Killer Cells

Abstract This chapter on natural killer cells is an introduction to signalling in cells that exert control over an amazing number of systems. Getting started requires concentration.

15.1 Receptors for HLA Class I

Natural killer (NK) cells are large granular lymphocytes that do not have T or B cell antigen receptors but instead immunoglobulin (Ig)-like killer-inhibitory receptors (KIRs) or killer-activating receptors (KARs) that recognise HLA class I molecules on target cells. The engaging NK cell is inhibited when its KIRs are involved but is activated by KARs, which detect weak or absent HLA class I molecules, as is the case on virus-infected or cancer cells. Then, NK cells will attack and kill the target cells by releasing granzyme esterases and the membrane pore-forming perforin. Thus, NK cells form a temporary defence for the innate immune system, whilst adaptive immunity is mounted. Since NK cells release $\text{IFN}\gamma$, and often $\text{IFN}\alpha$ and $-\beta$, they help to promote subsequent immunity (Table 15.1).

The KIR/KAR molecules are either C-type lectins or molecules of the Ig superfamily [1, 2]. Each KAR has immunoreceptor tyrosine activation motifs (ITAMs), whilst a long-tailed KIR has its immunoreceptor tyrosine inhibitory motifs (ITIMs). KIRs limit NK cell production of cytokines. The target ligands are not identified [3], especially those for the natural cytotoxicity receptors (NCRs) such as NK p46, p44 or p30 [4], but NCRs do have a major role in NK-mediated lysis of various tumours. On NK cell surfaces, the NCRs are associated with anchored polypeptides bearing ITAMs, like $\text{CD3}\zeta$, $\text{Fc}\epsilon\text{RI}\gamma$ or Dap12 (DNAX-activation protein 12) [5]. Human NKG2D is important for antiviral and antitumour defence [6]. NKG2D is upregulated likewise on CD8 cytotoxic T lymphocytes [7]. Activating receptor NKG2D is a lectin-like homodimeric receptor, linked to Dap10, that recognises MHC I-like molecules such as MICA and MICB, and the unique long 16-binding proteins (ULBPs) on target cells [6]. NK cells also have surface LFA-1, which is triggered by ICAM1 on targets, so that Vav1 directs actin organisation [8]. Conversely human NKG2A is an ITIM-containing inhibitory receptor, whose activation disrupts the actin network of

Table 15.1 Classification of killer-inhibitory receptors (KIRs) and killer-activating receptors (KARs)

KIR C-type lectins are type II transmembrane proteins

Murine homodimer Ly49 (a, c, e, g)	Reacting with H2K, H2D molecules
Human NKG2A/B	Reacting with HLA-A, B, C
NKG2A or NKG2C with CD94	Reacting with HLA-E

KAR C-type lectins

Murine Ly40d, Ly49h	Reacting with MHC class I molecules
Human homodimer NKG2D (Dap12/10)	Reacting with MICA or MICB (tumours)
Human homodimer NKG2C	Reacting with HLA-E

KIRs of immunoglobulin superfamily are type I TM glycoproteins

Human KIR2DL4 p49	Reacting with HLA-G molecules
Human KIR2D p58	Reacting with HLA-C molecules
Human KIR3D p70	Reacting with HLA-Bw4
Human KIR3D dimer	Reacting with HLA-A3

KARs of immunoglobulin superfamily

Natural cytotoxicity receptors NK p46, p44, p30 with as-yet-unknown ligands

KIR nomenclature is based on the number of extracellular immunoglobulin (Ig) domains, 2D for two domains or 3D for three domains, and the lengths of the cytoplasmic tails, L for long or S for short.

the immune synapse [9]. Other coreceptors include CD69, CD56, CD16, DNAM-1 (DNAX), 2B4 and NTB-A [10], and cytokine (IL-18) is costimulatory [11]. 2B4 and NTB-A belong to the signalling lymphocyte activation molecule (SLAM)-related receptors. NK cells express chemokine receptors and migrate in response to CXCL12 and CXC3L1.

NK cells that are quite positive for NCAM (CD56), together with CCR7 and L-selectin, predominate in the lymphoid organs, and they proliferate in response to cytokines IL-15, IL-16 and IL-2. In contrast, there are CD56-dim NK cells in the blood. The last are familiar cytolytic cells, as shown by their expression of CD107 LAMP and their production of IFN γ [12]. Yet, MHC class I-specific KIRs are expressed on subsets of CD56-dim cytolytic NK cells.

Human CD8 α protects NK cells against apoptosis and increases their release of cytokines [13]. CD8 α induces CD69, activation inducer molecule, which can evoke TGF β . TGF β is recognised to inhibit the expression of NK receptors like NK p30 and NKG2D [14]. Since NKG2Ds are present on both NK cells and cytotoxic lymphocytes, one will not be surprised that NKG2D blockade prevents autoimmune diabetes in NOD mice [15]. NK cells can inhibit T lymphocyte proliferation by induction of p21-mediated cell cycle arrest in those cells [16].

Detailed description of NK cell synapses has been provided recently by Krzewski and Strominger [17a]. One might expect some features of NK cells that are not found in T lymphocytes. At the immunological synapse of the NK cell, there is cdc42-interacting protein 4 (CIP4), which is an adaptor protein that arranges tubulin and microtubules in accordance with the microtubule organising centre [17b]. Secondly so as to support production of IFN γ , the cells utilize the protein phosphatase PP2A inhibitor SET [18a]. Thirdly HS1 homolog of cortactin enables stable adhesion to the target cell [18b].

With regard to the control of pathogens, NK cells are activated by IL-12 and the type I IFNs that arise from activated macrophages and dendritic cells (DCs). One might call these “accessory cells” for NK cell activation [19, 20], and they act by direct contact or by cytokine release. DC-derived IFN α/β , IL-12 and IL-18 and TNF α are all crucial in NK cell activation [21a]. Neutrophils also activate NK cells [21b]. The NK cells (a) secrete chemokines and IFN γ and TNF α and (b) become cytotoxic via upregulation of FasL and via NF- κ B induction of perforin. How NF- κ B is activated in NK cells using the lymphoid CBM complex (Carma-1/Bcl-10/Malt-1) will be of interest to many readers [21c]. KIR3D-S1 which associates with Dap12 on NK cells, has been shown to trigger both formation of IFN γ and cytotoxicity [22]. IL-21 aids cytotoxicity and so does IL-15 via its enhancement of LFA-1 on NK cell surfaces. Conversely, 1-kynurenine derived from L-tryptophan inhibits upregulation of receptors for NK cell killing like NK p46 and the NKG2D [23a]. IL-2 is needed to enable expression of perforin. Dynamin 2 localizes with lytic granules and enables exocytosis [23b].

With regard to recognition of tumours, it is unfortunate that soluble MIC from MICA/B-expressing tumours blocks recognition of that ligand, downregulating the controlling capacity of NKG2D-bearing NK cells [24]. CD1d molecules on target cells protect them from NK cell cytolysis [25]. Furthermore, TGF β secretion by cancers will downregulate the NKG2D molecules [26]. Nevertheless, we continue to produce lymphokine-activated LAK cells that express NK p44, Fas ligand and TRAIL in the hope of learning how to kill cancer cells [27].

With regard to autoimmune diseases, there are NKG2D ligands on target tissues to interact with NKG2D on NK and T cells. NKG2D associates with Dap10, which enabled Horng et al. [28] to reduce NK cell numbers in mice. They found that such cells are insensitive to IL-15, but via activation of Jak3, IL-15 is able to prime Dap10 signalling.

Akin to these NK cell recognition molecules, there are on myeloid or DCs activating or inhibitory signal regulatory proteins (SIRPs) [29]. There are also immunoglobulin-like transcripts (ILTs) [2], which bind various HLA class I molecules, independent of their locus or allelic specificity. TREMs (triggering receptors expressed on myeloid cell) of myeloid cells are related to NK p44. There are TREMs on DCs and on neutrophils TREM1. They amplify cytokine responses in sepsis [30]. Such TREMs are activated on the myeloid cells when there is pneumonia.

How macrophages are educated to respect self-tissues is important. It is done by ligation of macrophage SIRP α by integrin-associated protein CD47 so that phosphatases are recruited. Hence, there is decreased production of cytokine TNF α [31].

15.1.1 Hyporesponsiveness of NK Cells

Missing self-recognition refers to the hyporesponsiveness of NK cells that fail to bind self MHC class I molecules. In normal C57BL/6 mice, 10–15% of NK cells lack the expression of MHC class I specific receptors. Furthermore, NK cells that

lack inhibitory receptors for self MHC class I molecules can show diminished sensitivity to stimulatory ligands. It is not known whether the hyporesponsive phenotype is conferred actively [32]. The missing self concept means that NK cells do not attack until inhibitory signals are removed.

Missing self recognition is ensured by inhibitory receptors such as KIRs in humans and Ly49 molecules in mice. Inhibitory NK cell receptors dampen NK cell activation on interaction with their MHC class I ligands. Furthermore, recognition of self MHC class I molecules by inhibitory NK cell receptors contributes to the maturation of NK cells [12].

15.2 ITAMs and NK Cell Receptors

The ITAMs (Fig. 15.1) (cf. Fig. 13.1) have a duplicate YXXL sequence, where L is leucine, X is any amino acid and Y is tyrosine. When ITAMs are phosphorylated, they provide docking sites for adaptor proteins. In the NK cells, there are the adaptors LAT (Linker of Activated T cells), SLP76 and 3BP2, which present multiple docking sites for SH2 domain signalling proteins [32].

In the case of mouse KARs such as NKG2C/D, they have to associate for signalling purposes with adaptor proteins DAP12 or DAP10, which recruit Syk or PI3 kinase (Fig. 15.2) [2, 33]. The human NKG2D that is expressed for cytotoxicity associates only with adaptor protein Dap10. Dap10 has no ITAM, and instead there is an YXXM motif homodimer; when phosphorylated, it binds the p85 subunit of PI3K. DAP12 is a Y-T motif homodimer; when phosphorylated, it connects with Syk and then Zap70. Dap12 is the signal module that mediates the effects of a large group of receptors: NK p44, NKG2C-CD94, TREM1/2, SIRPβ1, CD200R.

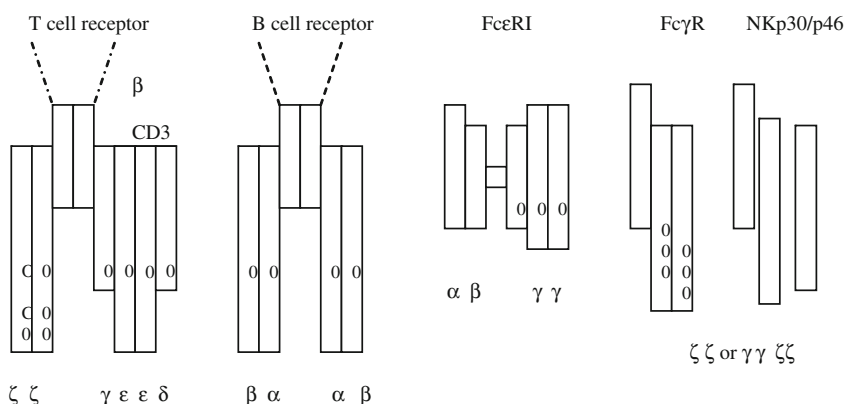


Fig. 15.1 Location of immunoreceptor tyrosine activation motifs (ITAMs; = 0) on signalling units. *TCR* T cell receptor for antigen, *BCR* B cell receptor for antigen, *FcεRI* high-affinity receptor for immunoglobulin E (*IgE*), *FcγR* Fc receptor for *IgG* (e.g. CD64)

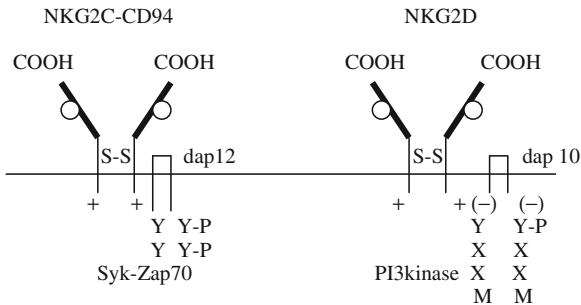


Fig. 15.2 Signal transduction by killer-activating receptors (KARs). The stimulatory molecules associate with adapter molecules Dap12 or Dap10, which recruit protein tyrosine kinases

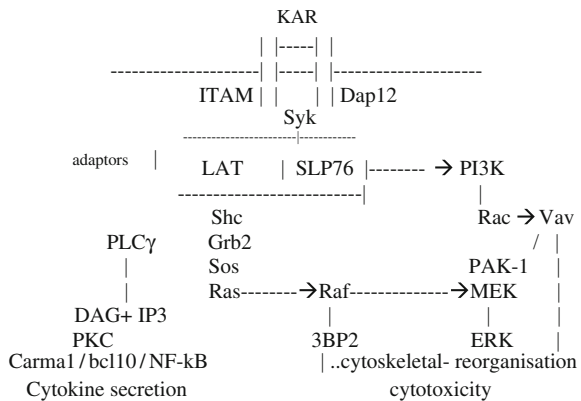


Fig. 15.3 Signal transduction in natural killer (NK) cells. The adaptors LAT and SLP-76 form scaffolds for attachments of SH2-binding proteins like PLCγ and Vav. LAT is tyrosine phosphorylated following contact with target cells and stimulation of the KARs/KIRs [34,35]. The ITAM bearing receptors use either PLCγ1 or 2 and NKG2D via Dap 10 only uses the PLCγ2 [36]. 3BP2, a positive regulator of NK cytotoxicity, couples to Vav1 and Vav2 [37]. Independently of its role in acting rearrangement, in NK cells WASp regulates NFAT2 and NF-κB[38]. As in T cells, Carmal-Bcl10-Malt 1 activation of NF-κB is used for cytokine and chemokine generation [21c, 39]

The DAP12/10 association means that the KAR can effect NK cell cytotoxicity and production of cytokines. NK cell cytotoxicity exerted by Dap10 requires Vav1 protein, whilst that via Dap12 requires Vav2 [19]. As an aside, Dap12 in macrophages acts conversely to control the activation of Th1 lymphocytes [33].

15.3 ITIMs and NK Cell Receptors

As seen in Fig. 15.4, each KIR has its corresponding KAR. Coligation of activating receptors with inhibitory receptors results in a dominant inhibitory effect. The actin cytoskeleton at the immune synapse is involved in this balance [40]. Immune

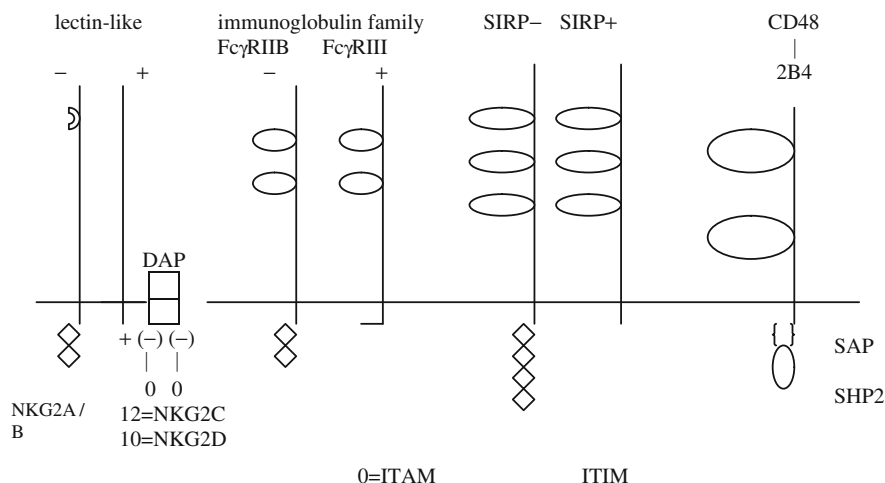


Fig. 15.4 Natural killer cell killer-inhibitory receptors (*KIRs*) (–) and killer-activating receptors (*KARs*) (±). The diamond inhibitory receptors recruit phosphatases SHP-1 and -2 and *SHIP*

inhibitory receptors, the ITIMs, have the consensus sequence (I/V/L/S)xYxx(L/V), which on tyrosine phosphorylation recruits SHP-1, SHP-2 and SHIP phosphatases. Accordingly, cell activation is suppressed.

Each KIR with its ITIMs (e.g. NKG2A/B) has a homologous KAR (e.g. NKG2C/D) that needs an ITAM. ITIMs recruit tyrosine phosphatases like SHP-1/2 to curtail the signal. The ITAM for NKG2C is Dap12, and that for NKG2D is Dap10 [2, 41]. The natural cytotoxicity receptor NK p44 associates with Dap12. Dap12 is an integral membrane protein with homology to CD3ζ. It has two cytoplasmic ITAMs that are phosphorylated by Src kinases. Conversely, Dap10 has a PI3K-binding motif similar to that found on CD28. 2B4 recognises CD48, which is upgraded during virus infections.

15.4 NK Cells in Action

In humans, NKG2D is expressed by NK cells, by CD8⁺ T lymphocytes and by intraepithelial lymphocytes. Mice differ since NKG2D is on their NK cells, on CD8 T cells following TCR triggering and on activated macrophages. Obviously, expression of NKG2D ligands on target cells induces NK cell toxicity toward the targets [42a]. Often, an NK cell is activated by the KAR NKG2D interacting with MHC class I-like MICAs on the target cells [2, 3, 43]. NTB-A enhances NK cell cytotoxicity and the production of IFNγ [44]. Conversely, KIR receptors thwart any action by the NK cell owing to involvement of ITIMs and the phosphatases SHP-

1/2 [45]. Actually, need for inhibition can evoke the SHP phosphatases and PTEN. In particular SHP-1 dephosphorylates tyrosines in Vav1, and phosphorylation of the adaptor Crk leads to inhibition [42b].

NK cells migrate in response to various C-C and C-X-C chemokines. NK cells express constitutive chemokine receptors like CCR4, CCR7, CXCR4, CX3CR1, and there is low-grade expression of inflammatory chemokine receptors like CCR1 and CXCR3. One should not be put off by the nomenclature: key tables are available [42c]. At sites of inflammation, there is attachment of those NK cells with high expression of adhesion molecules to vascular endothelium, followed by their extravasation. The NK cells become activated by IL-2 along with IFN γ . Their cytolytic activity is enhanced by chemokines CCL2–5 (i.e. MCP-1, MIPs and Rantes) and by fractalkine, which is a molecule that sits on the endothelium on a mucin stalk.

As emphasised, activation of NK cells via their KARs (Table 15.1), or by adhesion molecules, cytokines, immune complexes, pathogens and tumour cell ligands, leads to formation of a signalling scaffold within lipid rafts [46], as is the case for T cells. Use of NKG2D-Dap 10 (Fig. 15.2) leads to (a) Jak2-Stat activation, (b) PI3K activation of Akt, and (c) PI3K activation of ERK1:2 [47]. The signal for the release of granules operates via PI3K-Rac1-MEK $\rightarrow$ ERK [48, 49]. As is usual, Vav, Rac and Rho guanosine triphosphatase (GTPase) reorganize the actin cytoskeleton. NF- κ B activation helps the expression of perforin [49], and platelet-activating factor (PAF) release aids perforin killing of the target. For NK cells to mount effective cytotoxicity, there is at the plasma membrane the 3BP2 adaptor protein, which connects the human receptor CD244 with Vav and with the isoforms of PLC γ [50]. Of course, NK cells work most effectively against tumour cells when those display distinct antigens, but often such antigens can be hidden.

When microbial agents are encountered, resident DCs show early changes in gene expression, so that IL-2 and IFN γ are produced as part of the innate immune response, and these cytokines activate NK cells [51]. Interestingly, IL-2 helps survival of NK cells by means of PI3K reduction of acid sphingomyelinase (Fig. 7.4), so that any production of ceramide is reduced [52]. Importantly, IL-12 bridges the synapse between DCs and NK cells, so that the latter are activated to secrete IFN γ [53].

A vital link between the innate and adaptive immune responses is provided by the interaction of activated NK cells with CD4 $^{+}$ T cells, which are induced to secrete IFN γ and to proliferate [54]. OX40L, adjacent to NKG2D on the primed NK cells, stimulates OX40 of T cells. As one might anticipate, viruses can evade activated NK cells. Human cytomegalovirus (CMV) protein pp65 interacts with the NK p30 to suppress cytotoxicity (Fig. 15.3).

Just as the HLA class I molecules do, nonclassical immunomodulatory HLA-G molecules inhibit NK cell-mediated cytotoxicity (Table 15.1) and antigen-specific CD8 T cell-mediated cytotoxicity [55a]. HLA-G is the unique ligand of the NK cell inhibitory receptor KIR2DL4 [55b]. The soluble form of HLA-G induces apoptosis of activated T cells. Hence, it is suspected that expression of HLA-G on tissues like the trophoblast can protect against aggression by NK cells.

15.5 Signalling Lymphocyte Activation Molecule and Adapter SAP

The SLAM family of surface receptors fine-tune leucocyte responses [56]. Included are 2B4 (CD244), SLAM (CD150), NTB-A (Ly108), CD48, CD229 (Ly-a) and CD319. The 2B4 (Fig. 15.4) coreceptor of NK cells, in association with adapter molecule SLAM-associated protein (SAP), aids cytotoxicity and production of IFN γ [57]. Akin to it is SLAM (Fig. 15.5) of T lymphocytes, which also uses adapter SAP and activates Src family kinase Fyn so that IL-4 is produced.

In X-linked lymphoproliferative (XLP) disease, there is deficiency of SAP on T and NK cells, explaining the severe response if a patient develops mononucleosis [58–61]. Indeed, SLAM receptors with SAP adapters are involved [58] in (a) NK and CD8 T cell cytotoxicity, (b) NK T cell (NKT) development, (c) memory B cell generation and antibody production [61, 62] and (d) Th2 cell priming. They are also involved in neutrophil defences against bacteria [63]. Originally, SLAM was thought to direct a signal cascade that enhances Th1 lymphocyte responses in intra-cellular infections [64]. There may still be uncertainty about this. Certainly, when SLAM works with TCRs to signal via SAP and Fyn (Fig. 15.5), there is activation of PKC θ , activating Bcl-10 (Fig. 13.6), and thence NF- κ B, and cytokine IL-4 that promotes Th2 lymphocytes is formed [57, 65]. In SLAM-deficient allergic mice, both Th2 and Th1 cytokines are decreased [66].

In response to SLAM receptors (Fig. 15.5), the unconventional adapter protein SAP mediates activation of lymphocytes by recruiting the Src family kinase Fyn by means of an untypical SH2-SH3 domain interaction. SAP will prevent the attraction of phosphatases SHP-1 and SHP-2. Yet, observe that in T cells when the TCR is colligated to SLAM, SHIP slips in and connects via DOK1/2 to Ras-GAP, and IFN γ formation is thus curtailed [67]. However, SLAM and SAP expression is a feature

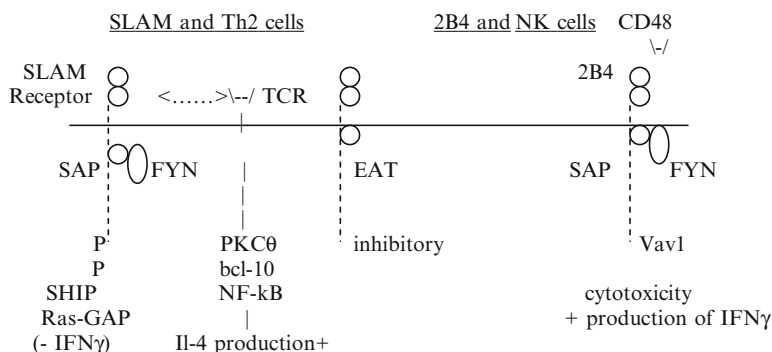


Fig. 15.5 The workings of signalling lymphocyte activation molecule (SLAM) and SLAM-associated protein (SAP). In Th2 cells, SLAM with adaptor SAP stimulates Fyn(T), and interleukin 4 (IL-4) is produced. Ewings sarcoma-associated transcript (EAT) couples SLAM to inhibitory signals [70]

of cytokine-directed killer cells [68], as it is of neutrophils [62], and then IFN γ is produced. Hence, the 2B4/SAP path is the key to CD8 cytotoxic T cell activity against Epstein-Barr-positive target cells [69]. Likewise, NTB-A associated with SAP triggers NK cell cytotoxicity, cytokine production and proliferation. NTB-A-induced IFN γ production is greatly reduced in the absence of SAP [70]. In lipopolysaccharide (LPS)-activated monocytes, SLAM signalling is enhanced fourfold [71].

Since SLAM operates on so many different leucocytes [54], there are various additional considerations, like the fact that 2B4 stimulation releases peroxidase from eosinophils. Most of its interactions result in enhanced formation of IL-12 and of IFN γ [63]. However, CD229 (Ly9) on T cells reduces their proliferation and IFN γ formation. Tetraspanin molecules are known to effect activation of T cells but reduce activity of NK cells [72].

As indicated, both humans and mice deficient in SAP exhibit profound defects in the generation of long-term antibody production in response to T cell-dependent antigen. In some NK cells, SAP is replaced by a homologue called EAT-2 (Ewings sarcoma-associated transcript 2). Those cells show reduced cytotoxicity and impaired formation of IFN γ [56] for EAT is associated with SHP-1/2 phosphatases or with Csk (C-terminal Src kinase), which negatively regulates other kinases.

Human 2B4 is mainly an activating receptor that interacts with CD48 on other NK cells, and T cells might seem simply to stimulate cell proliferation [73]. Furthermore, macrophages activate NK cell cytokine production and cytotoxicity through 2B4 [74]. Yet, mouse 2B4 is usually an inhibitory receptor, but sometimes it is excitatory. In reality, according to the situation, 2B4 might be inhibitory for human NK cells [75].

Apart from the 2B4/CD48 interaction, SLAM receptors are involved in homotypic self-self ligand interactions. Thus, interactions of immature DP thymocytes lead to the selection of NKT cells [76]!

15.6 NKT Cells

NKT cells express both NK and T cell markers such as NK1.1 and an invariant TCR, whose activity is restricted to interactions with glycolipids presented by CD1d antigens on antigen-presenting cells. These cells are activated by α -Gal-ceramide. The NKT cells produce high levels of a broad spectrum of cytokines (IL-2, IFN γ , TNF α or IL-4), and they are cytotoxic.

15.7 KIRs and KARs on T Cells

Several members of the KIR family are expressed on CD4⁺ and CD8⁺ T cells, as observed in a chronic inflammatory disease like rheumatoid arthritis. In fact, suboptimal TCR activation will necessitate costimulation by means of a molecule like

KIR2DS2 [77], so that the T cell can produce IFN γ and be cytotoxic. Alternatively, a KIR2DS2 molecule can function alone if it is associated with DAP12/KARAP (killer cell-activating receptor-associated protein). The KAR called NKG2D is now recognised as a costimulatory receptor for human CD8 $^{+}$ T cells [78].

15.8 ILT Molecules on Regulatory Cells

Immunoglobulin-like transcripts, mentioned as inhibitory molecules [2] since they recruit SHIP-1, are found on tolerogenic DCs and, in the context of transplant tolerance, on endothelial cells. Tolerogenic DCs bearing ILT3 and ILT4 molecules can give rise to CD8 $^{+}$ and CD4 $^{+}$ regulatory T cells. ILT3 is associated with the inhibition of NF- κ B.

References

1. Lanier LL. NK cell receptors. *Annu Rev Immunol* 1998;16:359–394
2. Moretta A, Bottino C, Vitale M et al. Activating receptors and coreceptors involved in human NK cell mediated cytotoxicity. *Annu Rev Immunol* 2001;19:197–121
3. Bottino C, Castriconi R, Moretta L, Moretta A. Cellular ligands of activating NK receptors. *Trends Immunol* 2005;26:221–226
4. Moretta A, Biassoni R, Bottino C et al. Natural cytotoxicity receptors that trigger human NK cell mediated cytotoxicity. *Immunol Today* 2000;21:228–234
5. French AR, Sjolín H, Kin S et al. Dap12 signaling directly augments proliferative cytokine stimulation of NK cells during viral infections. *J Immunol* 2006;177:4981–4990
6. Eagle RA, Trowsdale J. Promiscuity and the single receptor: NKG2D. *Nat Rev Immunol* 2007;7:737–744
7. Verneris MR, Karani M, Baker J et al. Role of NKG2D signaling in the cytotoxicity of activated and expanded CD8 T cells. *Blood* 2004;103:3065–3072
8. Graham DB, Cella M, Giurisato E et al. Vav1 controls Dap10 mediated natural cytotoxicity by regulating actin and microtubule dynamics. *J Immunol* 06;177:2349–2355
9. Masilamani M, Nguyen C, Kabat J, Borrego F, Coligan JE. CD94/NKG2A inhibits NK cell activation by disrupting the actin network at the immunological synapse. *J Immunol* 2006;177:3590–3596
10. Moretta L, Bottino C, Pende D et al. Different checkpoints in human NK cell activation. *Trends Immunol* 2004;25:670–676
11. Ortaldo JR, Young HA. IL-18 as critical costimulatory molecules modulating the immune response of ITAM bearing lymphocytes. *Semin Immunol* 2006;18:193–196
12. Vivier E. What is natural in NK cells? *Immunol Lett* 2006;107:1–7
13. Addison EG, North J, Bakhsh I et al. Ligation of CD8 α on human NK cells prevents activation induced apoptosis and enhances cytotoxicity. *Immunology* 2005;116:354–361
14. Castriconi R, Cantoni C, Della Chiesa M et al. TGF β 1 inhibits surface expression of natural killer NKp30 and NKG2D triggering NK receptors: consequences for the NK-mediated killing of dendritic cells. *Proc Natl Acad Sci U S A* 2003;100:4120–4125
15. Ogasawara K, Hamerman JA, Ehrlich LR et al. NKG2D blockade prevents autoimmune diabetes in NOD mice. *Immunity* 2004;20:757–767
16. Trivedi PP, Roberts PC, Walf NA, Swanborg RH. NK cells inhibit T cell proliferation via p21 mediated cell cycle arrest. *J Immunol* 2005;174:4590–4597

- 17a. Krzewski K, Strominger JL. The many functions of NK cell immunological synapses. *Curr Opin Cell Biol* 2008;20:597–605
- 17b. Banerjee PP, Pandery R, Zheng R et al. Cdc42 interacting protein-4 links actin and microtubule networks at the cytolytic NK cell immunological synapse. *J Exp Med* 2007;204:2305–2320
- 18a. Trotta R, Ciarlariello D, DalCol J et al. The PP2A inhibitor SET regulates NK cell IFN γ production. *J Exp Med* 2007;204:2397–2405
- 18b. Butler B, Kastendieck DH, Cooper JA. Forms of the cortactin homolog HS1 mediate distinct functions in NK cells. *Nat Immunol* 2008;9:887–897
19. Newman KC, Riley EM. Whatever turns you on: accessory cell dependent activation of NK cells by pathogens. *Nat Rev Immunol* 2007;7:279–291
20. Lodoen MB, Lanier LL. NK cells as an initial defense against pathogens. *Curr Opin Immunol* 2006;18:391–398
- 21a. Ferlazzo G, Munz C. NK cell compartments and their activation by dendritic cells. *J Immunol* 2004;172:1333–1339
- 21b. Sporri R, Joller N, Hilbi H, Oxenius A. A novel role for neutrophils as critical activators of NK cells. *J Immunol* 2008;181:7121–7130
- 21c. Hara H, Ishihara C, Takeuchi A et al. Cell-type-specific regulation of ITAM-mediated NF κ B activation by adaptors CARMA1 and CARD9. *J Immunol* 2008;181:918–930
22. Carr WH, Rosen DB, Lanier LL. KIR3D on NK cells triggers cytotoxicity and IFN γ production. *J Immunol* 2007;178:647–651
- 23a. Chiesa MD, Carlomagno S, Frumento G et al. L-kynurenine inhibits the surface expression of NKP46 and NKG2D activating receptors. *Blood* 2006;108:4118–4125
- 23b. Arneson LN, Segovis CM, Gomez TS et al. Dynamin 2 regulates granule exocytosis during NK cell mediated cytotoxicity. *J Immunol* 2008;181:6995–7001
24. Groh V, Wu J, Yee C, Spies T. Tumour derived soluble MIC ligands impair expression of NKG2D and T cell activation. *Nature* 2002;419:734–738
25. Campos Martin Y, Gomez de Moral M, Gazalbo-Lopez B et al. Expression of human CD1d molecules protects target cells from NK cell mediated cytotoxicity. *J Immunol* 2004;172:7297–7302
26. Lee J-C, Lee K-M, Kim D-W, Heo DS. Elevated TGF β 1 secretion and modulation of NKG2D underlies impaired natural killer cytotoxicity in cancer patients. *J Immunol* 2004;172:7335–7340
27. Zamai L, Ponti C, Mirandola et al. NK cells and cancer. *J Immunol* 2007;178:4011–4016
28. Horng T, Bezbradica JS, Medzhitov R. NKG2D is coupled to the interleukin 15 receptor signaling pathway. *Nat Immunol* 2007, Dec;8(12):1345–1352
29. Dietrich J, Cella M, Seiffert M et al. Signal regulatory protein-1 is a Dap12 associated activating receptor expressed in myeloid cells. *J Immunol* 2000;164:9–12
30. Radsah MP, Salih HR, Rammensee H-G, Schild H. TREM1 in neutrophil inflammatory responses. *J Immunol* 2004;172:4956–4963
31. Takizawa H, Manz MG. Macrophage tolerance. *Nat Immunol* 2007;8:1287–1289
32. Ravlet DH, Vance RE. Self tolerance of NK cells. *Nat Rev Immunol* 2006;6:520–531
33. Divangahi M, Yang T, Kugathasan K et al. Critical negative regulation of type 1 T cell immunity by signaling adaptor DAP12. *J Immunol* 2007;179:4015–4026
34. Vivier E, Nunes JA, Vely F. Natural killer cell signaling pathways. *Science* 2004;306:1517–1519
35. Cella M, Fujikawa K, Tassi I et al. Differential requirement for Vav proteins in Dap10 and ITAM mediated NK cell cytotoxicity. *J Exp Med* 2004;200:817–823
36. Upshaw JL, Schoon RA, Dick CJ, Billadeau DD, Leibson PJ. The isoforms of phospholipase- γ are differentially used by distinct human NK activating receptors. *J Immunol* 2005;175:213–218
37. Jevremovic D, Billadeau DD, Schoon RA, Dick CJ, Leibson PJ. Regulation of NK cell cytotoxicity by adapter protein 3BP2. *J Immunol* 2001;166:7217–7222

38. Huang W, Ocho HD, Dupont B, Vyas Y M. The Wasp regulates nuclear translocation of NFAT2 and NFkB(RelA). *J Immunol* 2005;174:2602–2626
39. Malarkannan S, Regunathan J, Chu H et al. Bcl10 plays a divergent role in NK cell mediated cytotoxicity and cytokine generation. *J Immunol* 2007;179:3752–3762
40. Standeven LJ, Carlin LM, Borszcz P et al. The actin cytoskeleton controls the efficiency of killer-Ig like receptor accumulation at inhibitory NK cell immune synapses. *J Immunol* 2004;173:5617–5625
41. Lanier LL, Bakker ABH. The ITAM bearing transmembrane adapter Dap12 in lymphoid and myeloid cell function. *Immunol Today* 2000;12:611–614
- 42a. Diefenbach A, Raulet DH. Innate immune recognition by stimulatory immunoreceptors. *Curr Opin Immunol* 2003;15:37–44
- 42b. Peterson ME, Long EO. Inhibitory receptor signaling via tyrosine phosphorylation of the adaptor Crk. *Immunity* 2008;29:578–588
- 42c. Zlatnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. *Immunity* 2000;12(2):121–127
43. Leibson PJ. The regulation of lymphocyte activation by inhibitory receptors. *Curr Opin Immunol* 2004;16:328–336
44. Flaig RM, Stark S, Watzl C. NTB-A activates NK cells via homophilic interaction. *J Immunol* 2004;172:6524–6527
45. Yusa S-I, Catina TL, Campbell KS. KIR2DL5 can inhibit human NK cell activation via recruitment of SHP-2. *J Immunol* 2004;172:7385–7392
46. Colucci F, DiSanto JP, Leibson PJ. NK cell activation in mice and men. *Nat Immun* 2003;3:807–813
47. Jiang J, Zhang B, Gilvary DL et al. Pivotal role of PI3-kinase in regulation of cytotoxicity by NK cells. *Nat Immun* 2000;1:419–425
48. Wei S, Gilvary DL, Corliss BC et al. Direct tumor lysis by NK cells uses a Ras independent MAP kinase signal path. *J Immunol* 2000;165:3811–3819
49. Zhou J, Zhang J, Lichterheld MG, Meadows GG. A role for NFkB activation in perforin expression of NK cells upon Il-2 receptor signaling. *J Immunol* 2002;169:1319–1325
50. Sabarit-Villarroya I, Del Valle JM, Romero X et al. The adapter protein 3BP2 binds human CD244 and links this receptor to Vav signaling, ERK activation and NK cell killing. *J Immunol* 2005;175:4226–4235
51. Degli-Esposti MA, Smyth MJ. Close encounters: dendritic cells and NK cells take centre stage. *Nat Rev Immunol* 2005;5:112–124
52. Taguchi Y, Kando T, Watanabe M et al. Interleukin 2 induced survival of NK cells involving reduction of ceramide. *Blood* 2004;104:3285–3293
53. Borg C, Jalil A, Laderach D et al. NK cell activation by dendritic cells requires formation of a synapse leading to Il-12 polarization in DCs. *Blood* 2004;104:3267–3275
54. Zingani A, Sornasse T, Cocks BG et al. Crosstalk between activated human NK cells and CD4 T cells via OX40-OX40 ligand interactions. *J Immunol* 2004;173:3716–3724
- 55a. Carosella ED, Favier B, Rouas-Freiss N et al. Beyond the increasing complexity of the immunomodulatory HLA-G molecule. *Blood* 2008;111:4862–4878
- 55b. Gonnens-Gross T, Achdout H, Gazit E et al. Complexes of HLA-G protein on the cell surface are for leukocyte-Ig-like receptor-1 function. *J Immunol* 2003;171:1343–1351
56. Bhat R, Eissmann P, Endt J et al. Fine tuning of immune responses by SLAM-related receptors. *J Leukoc Biol* 2006;79:417–424
57. Chuang SS, Kumarssen PR, Mathew PA. 2B4(CD244) mediated activation of cytotoxicity and IFN γ release by human NK cells. *J Immunol* 2001;167:6210–6216
58. Veillette A. Immune regulation by SLAM family receptors and SAP-related adapters. *Nat Rev Immunol* 2006;6:56–66
59. Dupre L, Andolfi G, Tangye SG et al. SAP controls the cytolytic activity of CD8+ T cells against Epstein-Barr virus infected cells. *Blood* 2005;105:4383–4389
60. Engel P, Eck MJ, Terhorst C. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nat Rev Immunol* 2003;3:813–821

61. Cannons JL, Yu LJ, Jankovic D et al. SAP regulates T cell mediated help for humoral immunity by a mechanism distinct from cytokine regulation. *J Exp Med* 2006;203:1551–1565
62. Kamperschroer C, Dibble JP, Meents DL et al. SAP is required for T helper cell functions and for immunity to influenza. *J Immunol* 2006;177:5317–5327
63. Howie D, Laroux FS, Morra M et al. The SLAM family receptor Ly108 controls T cell and neutrophil functions. *J Immunol* 2005;174:5931–5935
64. Quiroga MF, Martinez GJ, Pasquinelli V et al. Activation of SLAM triggers a signaling cascade that enhances Th1 responses in intracellular infection. *J Immunol* 2004;173:4129–4133
65. Cannons JL, Yu LJ, Hill B et al. SAP regulates Th2 differentiation and PKC theta mediated activation of NFkB1. *Immunity* 2004;21:693–706
66. Wang N, Campo M, Ting L et al. The costimulatory molecule SLAM is critical for pulmonary allergic responses. *Am J Resp Cell Mol Biol* 2006;35:206–210
67. Ma CS, Nichols KE, Tangye SG. Regulation of cellular and humoral immune responses by SLAM and SAP families of molecules. *Annu Rev Immunol* 2007;25:337–379
68. Mehrle S, Frank S, Schmidt J. SAP and SLAM expression in anti-CD3 activated lymphocytes correlates with cytotoxic activity. *Immunol Cell Biol* 2005;83:33–39
69. Veillette A. SLAM family receptors regulate immunity with and without SAP-related adaptors. *J Exp Med* 2004;199:1175–1178
70. Eissmann P, Watzl C. Molecular analysis of NTB-A signaling: role for EAT2 in NTB-A mediated activation of human NK cells. *J Immunol* 2006;177:3170–3177
71. Jiang H, Van de Ven C, Satwani P et al. Gene expression patterns by oligonucleotide microarray of basal versus LPS activated monocytes of blood. *J Immunol* 2004;172:5870–5879
72. Crotta S, Ronconi V, Olivieri C et al. Cytoskeletal rearrangement induced by tetraspanin engagement modulates the activation of T and NK cells. *Eur J Immunol* 2006;36:919–929
73. Assarson E, Kambayashi T, Schatzle JD et al. NK cells stimulate proliferation of T and NK cells through 2B4/CD48 interactions. *J Immunol* 2004;173:174–180
74. Nedvetzki S, Sowinski S, Eagle RA et al. Reciprocal regulation of human NK cells and macrophages associated with distinct immune synapses. *Blood* 2007;109:3776–3785
75. Kumar V, McNerney ME. A new self MHC class I independent natural killer cell self-tolerance. *Nat Rev Immunol* 2005;5:363–374
76. Veillette A, Dong Z, Latour S. SLAM-SAP signaling. *Immunity* 2007;27:698–709
77. Snyder MR, Nakajima T, Leibson PJ et al. Stimulatory killer Ig-like receptors modulate T cell activation through DAP12 mechanisms. *J Immunol* 2004;173:3725–3731
78. Maasho K, Opuko-Anane J, Marusina AI et al. NKG2D is a costimulatory receptor for human naïve CD8+ T cells. *J Immunol* 2005;174:4480–4484

Chapter 16

B Lymphocytes

Abstract B lymphocytes and their development and the formation of antibodies are clearly important in immune defence. So is the role of Epstein-Barr virus in B cell proliferation and relevant aspects of chronic lymphatic leukaemias, the lymphomas and myeloma.

16.1 B Cell Receptor Signalling

The B cell receptor (BCR) for binding antigen is formed by a Y-shape membrane immunoglobulin (Ig) and the associated signal transducing CD79 α /CD79 β (Ig α /Ig β) that bear immunoreceptor tyrosine activation motifs (ITAMs) (Fig. 16.1). The ITAMs associate with Src family phosphotyrosine kinases (PTKs) like Lyn, Fyn and Blk so that there can be tyrosine phosphorylation in the cytoplasm domain of the Ig α / β [1a]. This facilitates binding of Syk (splenic tyrosine kinase). All this occurs in glycosphingolipid GSL micro domains enriched by these PTKs, once CD45 has removed the negative regulatory phosphate groups from the Fyn, Lyn and Blk tails. Extracellular calcium helps B cell activation [1b].

A system for looking at these signal transduction pathways is needed [2] (cf. Fig. 13.2). One will discern three pathways for activation of the genes of B lymphocytes: (a) activation of phospholipase C γ 2 (PLC γ 2), leading to activation of protein kinase C (PKC); (b) activation of p21Ras and hence Ras $\rightarrow$ MAPK signalling; (c) activation of PI3 kinase, and so PKC β and thereby NF- κ B. BCR signal strength is important for it is critical in guiding B cell precursors from bone marrow into the B1, follicular or marginal zone (MZ) compartments of B cell development. The strength of BCR signalling is controlled by an amplification loop between Ca²⁺ ion influx and locally generated reactive oxygen species (ROS), which of course inhibit phosphatases [3]. Actually, SHP-1 phosphatase keeps the resting BCR in check but dissociates following BCR ligation. Thereafter, SHP1 may attenuate the signal in conjunction with negative regulator CD22.

The lipid rafts of mature B cells contain Syk, Lyn, Fyn, Vav, PI3K and PLC γ 2. As in Fig. 16.1, there are (a) protein tyrosine kinases Syk, Zap70, Bruton's tyrosine kinase (Btk), Blk, Lck, Fyn, Lyn, as in Fig. 16.1 (b) Ser-Thr kinases PKC and Akt;

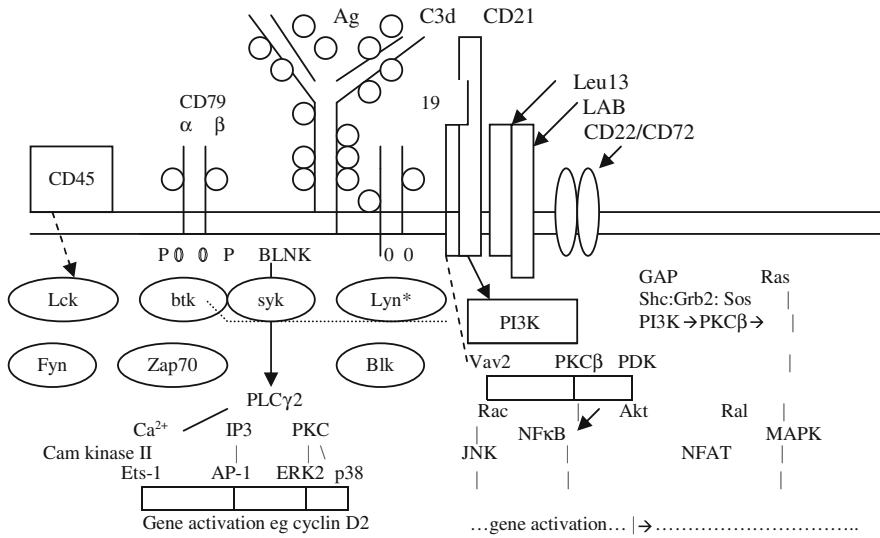


Fig. 16.1 Structure of the B cell receptor (BCR). Antigen binding leads to phosphorylation of the BCR Igα/β Lyn* and phosphorylation of (Syk) by Lyn. The Igβ ITAM tyrosines aid setting of the BCR signalling threshold by regulating receptor internalization [1]. Syk or Zap70 phosphorylate downstream effector proteins like BLNK and Btk, which phosphorylates PLCγ2, and adaptor BCAP, which activates PI3-kinase. PI3K dependent activation of ERK1/2 and of NFκB activates cyclin D2. Vav2 (a GEF) activates Rac1, causing cytoskeletal actin reorganization, and Vav2 links BCR signalling to JNK and p38 MAP kinase. The BCR signal leads to NF-κB activation

and (c) phosphoinositide kinases PI3K and PI5K. The phosphatase CD45 controls the Src-PTKs of the signalosome. Maximum receptor signalling requires the binding of ITAMs to Src family kinase Src-homology domain 2 (SH2) domains. Hence, there is progressive amplification of ITAM phosphorylation. Syk phosphorylates B cell linker protein (BLNK; the same as SLP65), which is a molecular scaffold for several signalling pathways. It is the docking site for PLCγ2. Phosphorylated BLNK binds the SH2 domain of Btk, so bringing Btk up to the PLCγ2. When Btk is deficient, there is a γ-globulinaemia. Btk activates PI5K, so that PI(4,5)P2 is formed as substrate for both PI3K and PLCγ [4, 5]. Btk controls actin cytoskeleton dynamics [5]. BCR-mediated activation of NF-κB occurs in a Btk-PI3K-Akt sequence and via PLCγ2 activity that generates IP3 and diacylglycerol (DAG), involving PKCβ. Curiously, Btk is also implicated in integrin-mediated adhesion and migration of B lymphocytes [6].

LAB (Linker for Activation of B cells) is another adaptor protein, the equivalent of LAT, Linker of Activated T cells [7]. It recruits BLNK and growth factor receptor-bound protein 2 (Grb2) to lipid rafts [5]. Grb2 with non-T cell activation linker (NTAL) helps the influx of Ca²⁺ ions [8]. Other adaptors (Bam32 and Carma1) (Fig. 13.6) help to establish downstream pathways to ERK, Jun N-terminal kinase (JNK), and NF-κB [9], so that B cell proliferation can occur [10]. The MAPK family (ERK, JNK, p38 MAPK) phosphorylate different sets of transcription factors (TFs), as do activator protein 1 (AP-1), NF-κB and calmodulin (CaM) kinase II [1b].

Table 16.1 Molecules that moderate B lymphocytes

SHP-1	Controlling phosphatases
CD45	Moderating phosphatase
Lyn	+/- 2 edge kinase deficiency causes SLE-like syndrome
PTP	Inhibits cell proliferation, limits B cell expansion
CD22, CD72	A lectin and stimulator of membrane Ca^{2+} ATPase
PD-1	A CD28 homologue that inhibits Ca^{2+} mobilization
PECAM1 (CD31)	Negatively regulates BCR signaling
Fc γ RIIb	Controls autoantibody production
BANK	Adaptor that attenuates CD40-mediated Akt activation

BCR B cell receptor, *PD-1* programmed death receptor, *PTP* = a phosphatase

The CD19 that is associated with CD21 (CR2) lowers the threshold for B cell activation [11]. Significantly, it sequesters Lyn, preventing its negative regulation of CD22 [12]. CD19 recruits Vav, PI3K and PI5K, so it determines levels of PIP2. The cytoplasmic tail of CD19, bearing Y-X-X-M sequences, is phosphorylated by Lyn to create binding sites for the SH2 domains of the p85 adaptor unit of PI3K. The PI3K association with CD19 increases the activity of the p110 catalytic unit of PI3K. Since CD21 binds the C3d fragment of activated C3, which is an opsonin for antigen, the CD21/CD19 complex couples innate immune recognition of microbial agents to B lymphocyte activation [11].

Lyn is a double edged kinase that shows positive and negative regulation of the BCR [13, 14]. In its early activation role, Lyn phosphorylates tyrosine residues of the ITAMs of Ig α / β and CD19 and leads to an alternate pathway of pERK activation, which is inhibited by rottlerin [15]. Yet, Lyn then phosphorylates tyrosine residues on the ITIMs of negative BCR coreceptors such as Fc γ RIIb, programmed death 1 (PD-1), PECAM1, and CD22 [14]. Inhibitory receptors are important for reducing the chance that autoimmunity will emerge [16] (see Table 16.1). CD22 is a sialic acid-binding Ig-like lectin (Siglec) that recognizes α 2–6-linked sialic acid, a common structure on the N-glycans. Thus, CD22 contacts CD4 and CD45 molecules on T cells. Essentially, CD22 is a tunable membrane Ca^{2+} ATPase that mediates Ca^{2+} efflux from B cells [17, 18]. It stimulates the pump plasma membrane Ca-ATPase 4 (PMCA4). On the positive side, CD22 activates Syk, and so PLC γ and PI3K [19, 20], and in its negative role it activates Lyn more than SHP-1 and SHIP phosphatases with their ITIMs, so that there is inactivation of Vav1 and Vav2.

The lipid rafts that contain mature BCRs and relevant molecules may actually be assembled in the *cis*-Golgi, helped by PKC β and tetraspanins such as CD81 and by raftlin raft linking protein and Syk. Syk action helps other molecules to gain access to the rafts. Association of Lyn with rafts is helped by its myristoylation/palmitoylation.

Interestingly, nicotinamide stops the coengagement of Toll-like receptors (TLRs) and the BCRs on B cells, disrupting MAPK activation and stopping cell proliferation [21].

16.2 Negative Signalling on B Lymphocytes

Negative signalling in B cells is mediated by IgG when it is bound to specific antigen, and its Fc domain interacts with Fc γ RII receptors on B lymphocytes. Those receptors are then cross-linked with the BCRs (Fig. 16.2), forming a negative signalosome that suppresses antibody formation [22]. On SLE B cells, the Fc γ RIIb are reduced in number.

As in Fig. 16.2, the SH2 domain adaptor protein Shc associates with the ITAMs of the BCR. In the germinal centres, follicular B cells express both BCRs and their Fc γ RIIB. The ITIM, negative regulator, of the Fc γ receptors attracts the SH2 domain of SHIP, phosphatase. Those negative signals cause phosphorylation of SHIP and a reduction of the Shc-Grb2 interaction [22, 23]. SHIP hydrolyses PIP3 to PIP2 and impedes recruitment to the membrane of survival factor Akt, so B cells may undergo apoptosis. Another negative signal to the PLC γ 2 pathway arises from the paired immunoglobulin-like receptor B (PIR-B). Both PIR-B and Fc γ RII inhibit Ca²⁺ signals. PIR-B uses SHP-1 and SHP-2 phosphatases [4, 24]. There are also the related myeloid-associated immunoglobulin-like receptors (MAIRs) [16].

In B cell development, there are central and peripheral checkpoints to prevent generation of autoantibodies. Gene deletion studies in mice have identified several proteins, including the Fc γ RIIB, that are involved in this. In fact, impaired Fc γ RIIB expression occurs frequently in autoimmune-prone mouse strains.

Plasma cells in bone marrow express Fc γ RIIB receptors that are in contact with immune complexes that can cause apoptosis, so this is a mechanism for homeostatic control of the numbers of long-lived plasma cells [25].

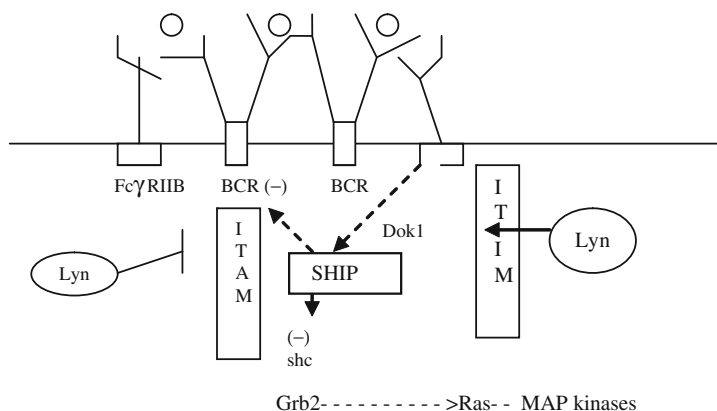


Fig. 16.2 Scheme for negative signalling of B lymphocytes. Cross-linking Fc γ RIIB receptors/B cell receptors (BCRs) via immunoglobulin G (IgG) immune complexes. First, *SHIP1* is recruited to Lyn by means of its adaptor Dok (downstream of kinase). Second, *SHIP1* is also recruited to coaggregated Fc γ RIIB

16.3 CD40 Signalling

CD40 signalling was described in Sect. 12.12. CD40 action is required for germinal centre formation, for isotype switching and for formation of antibodies. CD40 engagement even eliminates the need for Btk when the BCR signals to NF- κ B [26]. When CD40 ligand (CD154) acts on the CD40 coreceptors, that entails activation of TRAF2 and TRAF6, with the result that there is CD40-BCR synergy, activation of NF- κ B and of JNK, secretion of IgM and production of IL-6 [27]. Furthermore, kinase MEKK1 (MAP3K) is required for CD40-dependent activation of JNK and p38 and hence germinal centre formation [28]. It has been mentioned that CD40 induces B cells to produce TNF α , which binds to the TNFR2 and thus creates an amplification loop of B cell activation. CD40 stimulation of NF- κ B and GADD45 (growth arrest and DNA damage-inducible gene 45) will protect B cells from Fas-mediated apoptosis. Yet, the adaptor BANK must attenuate CD40-mediated Akt activation for BANK^{null} mice show enhanced germinal centre formation and production of IgM [29]. Also, since Cbl-b (casitas B lineage lymphoma protein b) associates with TRAF2, it stops recruitment of this TRAF2 to the CD40 and thereby attenuates activation of NF- κ B and of JNK [30].

16.4 Sustaining B Cells with B Cell-Activating Factor (BLys)

CD40 mainly activates the rapid NF- κ B1 (p50, p65) pathway. Yet, there is always some sustained NF- κ B2 (p52, RelB) activation [27, 31a]. The sustained noncanonical (p52, relB) pathway is the one that is stimulated by B cell-activating factor (BAFF; BLys), albeit it also makes some input to the canonical pathway to p50, p65 via Btk action. BAFF of the TNF α family ligands is essential for the development of long-lived B cells, to which it can bind by three receptors: BAFF-R, TAC1, and BCMA (B cell maturation antigen) [32–34]. BCR ligation upregulates BAFF-R expression on B cells, which promotes increased sensitivity to BAFF-mediated survival signals as B cells mature (Fig. 16.3). Since stimulation of the BAFF-Rs helps CD21 and CD19 signalling, there is support for B cell activation, proliferation and differentiation. Obviously, antiapoptotic molecules Bcl-2 and Mcl-1 will be promoted to explain BAFF-dependent B cell survival. The anti-apoptotic drive originates from Pim-2 and Akt kinases.

At the earliest stage, canonical NF- κ B is boosted, and B cell survival then allows BAFF to act to generate noncanonical NF- κ B2 and hence the BCR/CD19/CD21 coreceptor complex. Yet, BAFF also stimulates Btk and hence the classical NF- κ B1 pathway. When there are activated T cells in spleen, CD40 induces both NF- κ B forms. The maturing B cells either turn into plasma cells or migrate back into lymphoid follicles to generate germinal centres. Bcl-6 inhibition of p53 protein helps.

Since BAFF promotes B cell survival by modulating nuclear accumulation of PKC δ [35], it is involved in various B cell-driven autoimmune diseases [36] and in B cell chronic lymphatic leukaemia (CLL) [36]. BAFF also promotes

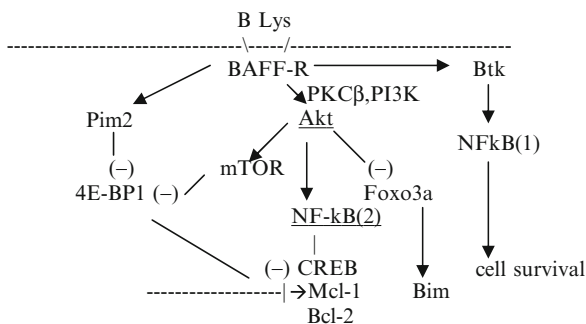


Fig. 16.3 B cell-activating factor (BAFF)-dependent antiapoptotic proteins and survival of B cells. *Bcl-2* is an antiapoptotic protein that is boosted. *Mcl-1* is a short-lived antiapoptotic protein. *Bim* and *Bad* are proapoptotic proteins that are reduced. Activation of CD21 lowers the BCR threshold. BAFF stops the normal tendency of TRAF3+2 to negatively regulate NF- $\kappa\beta^2$. [31b, 31c].

T cell activation and survival [37], as in Th1 cell-driven delayed-type hypersensitivity (DTH) reactions, and it supports monocyte activation [38]. Elevated BAFF levels via the activation of BCMA enable the presentation of self-antigen by B cells [39].

16.5 Apoptosis of B Lymphocytes

B lymphocytes are driven to apoptosis by signals through death receptors on Fas or TNF receptors, via the BCR itself and by glucocorticoids or γ -radiation. Activation of mature B lymphocytes upregulates their Fas expression, but that process is offset when there is antigen receptor signalling [40]. Survival of B cells is helped by antiapoptotic *Bcl-2* family members supported by Akt and the Pim kinases and by Rel/NF- κ B [41, 42], by inhibitor of apoptosis proteins (IAPs), by Flice inhibitor protein (c-FLIP), and by Fas apoptosis inhibitor molecule (FAIM). As an aside, NF- κ B-induced expression of cellular FLIP is important for rheumatoid arthritis synovial fibroblasts since it ensures their protection from TNF α -induced apoptosis [43]. With regard to lymphoid homeostasis, antiapoptotic *Bcl2* and *Mcl-1* in opposition to *Bax* and *Bak* actively help to maintain the lymphocyte population [44].

Interestingly, CD5 on B1 cells promotes B cell survival via stimulation of auto-crine IL-10 [45]. CD5 works in association with SHP-1 to reduce BCR signalling, and there may be other means [46a]. Natural antibodies produced by B1-a (CD5⁺) cells are produced in response to infection by *Streptococcus pneumoniae* or the vaccine Pneumovax.

The B1 repertoire is skewed toward reactivity with common bacterial epitopes and to self-antigens. Such B1 cells are able to recirculate to the spleen.

16.6 Control Over B Cell Proliferation

IL-4 promotes B cell proliferation, leading to antibody production, and it protects B cells from apoptosis, largely by boosting their glycolysis [46b]. IL-4 works via the p38 MAPK. In theory, IL-4 could provoke serious autoimmune disease [47]. So might BAFF (B lymphocyte stimulator protein) acting via NF- κ B2. Conversely, transforming growth factor- β (TGF β) will cause apoptosis of B cells, thwarting the emergence of autoimmune disease. Unfortunately, the B cells of CLL patients are resistant to TGF β . There is Pim kinase overexpression in CLL and in B cell lymphoma. Remember that Akt and Pim phosphorylate and inactivate proapoptotic Bad. Akt (with SGK1, serum and gc-inducible kinase 1) phosphorylate and inactivate FKHRL1, a TF that normally upregulates proapoptotic proteins like Bim. Interestingly, the balance of pro/antiapoptotic proteins (e.g. Bim vs. Bcl-2) affects cell cycle progression along with apoptosis in B cells. Use of Bim knockout mice has shown that Bim regulates BCR-induced entry of B cells to the cell cycle in response to BCR or TLR3/4 engagement [48].

Kruppel-like factor 4 (KLF4) operates to control B cell numbers and activation-induced B cell proliferation. It does this by binding to the promoter of cyclin D2 [49].

Many cells depend on phosphatidyl-inositol products PI3,4P2 and PI3,4,5P3, which are generated by PI3K (Fig. 2.4). Mice with knockout of the controlling phosphatase PTEN (Fig. 2.6) show selective expansion of B1 lymphocytes and marginal zone splenic B cells. The latter are those that respond to blood-borne antigens. It is clear that PI3K (class Ia) is required for those B cells [50].

Whereas in mature B cells BCR ligation induces B cell survival, proliferation and differentiation, in immature B cells BCR signals arising from self-antigens lead to apoptosis, anergy or receptor editing. Interaction with self-antigens accounts for the fact that only 5% of precursors originating from the bone marrow eventually become mature B lymphocytes.

16.7 B Cell Development

For mature B cells to produce their specific antibodies, there will be three forms of Ig gene alteration. First, the variable region exons of heavy and light chains are assembled from germline variable (V), diversity (D) and joining (J) gene segments by a recombinase enzyme that is determined by RAG 1:2 genes. Second, there is IgH (heavy chain) class switch recombination (CSR) using enzyme activation-induced cytosine deaminase (AID). Third, within the germinal centres late immature B cells undergo receptor editing via somatic hypermutation (SHM) [51].

In development, pro-B cells become pre-B cells, which turn into immature B cells when they meet (auto)antigen. All these B cells express antigen B220, which is the phosphatase CD45. Immature B cells respond to low antigen levels with enhanced Ca²⁺ signal and PtdIns turnover. Their levels of BLNK, Btk and PLC γ are enhanced threefold [52]. Yet, now apoptosis may ensue because BCR action leads to Myc

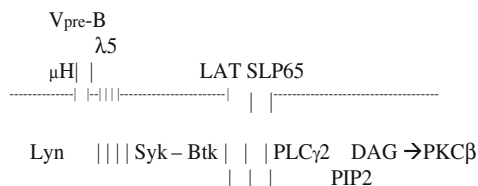


Fig. 16.4 Pre-BCR signalling

expression, conferring that liability [53]. In the germinal centres, BAFF promotes B cell differentiation and survival since Bcl-2 is induced [54]. In due course, plasma cells in bone marrow or spleen produce high-affinity antibody. This is helped by IL-21 stimulation of c-Myc and Blimp1. Blimp1 is required for production of immunoglobulin. IL-21 is produced by germinal centre follicular Tfh cells [55].

The pre-BCR of early B cells is able to induce proliferation in a ligand-independent manner (i.e. without antigen). A pre-BCR has a heavy μ -chain and surrogate light chains called V-preB and $\lambda 5$. The pre-BCR is a checkpoint in B cell development [56]. It seems to serve as its own ligand [57] for the amino terminal of $\lambda 5$ creates signal transduction (Fig. 16.4). Interestingly, a gain-of-function mutation of Blk will induce pre-BCR activation [58]. BLNK (SLP76) and Btk act as feedback inhibitors of pre-BCR signalling, so they limit cell proliferation. Such proliferation, as there is, is promoted by Syk leading to the Ras $\rightarrow$ MAP kinase. Ras leads to Bim degradation and so protection against apoptosis. Whenever Syk is deregulated, there is growth factor-independent proliferation of pre-B cells and liability to leukaemia [59] or lymphoma [60]. In follicular lymphoma, there is Syk-dependent activation of mTOR [61].

As noted, KLF4 is relevant to B cell proliferation. The quiescence of naïve and memory B cells is explained by their expression of Kruppel-like factors KLF4 and KLF9, which means that they do not respond to BCR or CD40 stimuli. However, when required, downregulation of the KLFs ensures a rapid ability to form antibodies [62].

No doubt the reader will be interested in how plasma cells arise [63]. B cell lineage-specific activator protein (BSAP) is essential for early B cell development. When BSAP falls, the transcription factor XBP1 becomes dominant [64], and Blimp1 and XBP1 together determine plasma cell development. The change follows inhibition of Bcl-6, which has the critical function of repressing Blimp1 gene, which would stop germinal centre B cell development. Bcl-6 is the major regulator of B cell development [51]. It allows GC B cells to sustain the genotoxic stress that is associated with their high rate of proliferation.

BSAP determines early B cell development at a stage when repressive transcription factors Bach2 and Bcl-6 restrain Blimp1 [64b]. Later when unrestrained Blimp1 and XBP1 together determine development of plasma cells (Fig. 16.5). On the one hand, Blimp1 cancels development of mature B cell phenotypes by inhibiting expression of Pax5 regulator of B cell differentiation, and Blimp1 inhibits c-Myc. On the

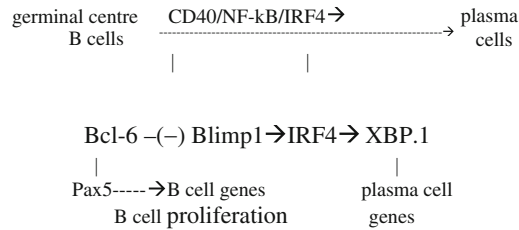


Fig. 16.5 XBP1 upregulates plasma cell genes. Pax5 is a B cell linear activator, Blimp1 = B lymphocyte induced maturation protein-1, XBP-1 = X box binding protein-1

other hand, Blimp1 enables IRF4 to augment the expression of immunoglobulin genes in plasma cells. Blimp1, IRF4, and XBP1 all activate Ig secretion.

There are additional points. First maturation of B cells is marked by loss of MHC class II antigen expression by silencing of the class II transcriptional co-activator CIITA [65]. In mature B cells lipid rafts help to amplify signal transduction. Oligomerization of the BCRs by multivalent antigen results in their association with membrane rafts and so proximity to Src family kinases [66a]. Yet B cells require continuous signalling through the BCRs and associated BAFF-receptors (Fig. 16.3) for their survival. Cross-talk between these receptors ensures that B cells gain a resistance to negative selection as they mature. Additionally crosstalk with TLRs (such as 4, 7, 9) can enhance antibody formation [66b].

16.8 B Cell Tolerance

Elimination of B cells by apoptosis before they progress to the mature B cell compartment will create tolerance. Yet, in fact “receptor editing” that creates a new B cell antigen receptor with innocuous specificity is the normal way [67a]. Interestingly Foxo transcription factors are regulators of receptor editing by activating the RAG recombination activating genes [67b].

In the process of development, there is a switch in B cell propensity to positive selection by self-antigens. In foetal and neonatal life, even self-antigens can cause positive selection of B cells. However, in adult bone marrow self-antigens cause negative selection of B cells; that is, such signals are tolerogenic [68]. In normal development, a high percentage of B cells actually become anergic. They are T3 B cells, and they show reduced NF-κB activation. Anergic B cells have a high basal intracellular free Ca^{2+} . These cells do not mobilize calcium; they do not perform tyrosine phosphorylation and do not proliferate [69]. The chronic binding of self-Ag is thought to cause poor phosphorylation of BCR ITAM tyrosines with consequent limited activation of Syk and yet Lyn activation that allows recruitment of SHIP-1 (cf. Fig. 16.2), dampening BCR signaling [70]. Complement C3d-linked antigens will break B cell anergy [71].

Anergic B cells do have antigen-binding capacity. Yet, they show only mono-phosphorylation of the C-terminal ITAMs of Ig α and Ig β , with the result that Lyn phosphorylates the CD22 ITIM [64b]. So, they are inert, but potentially they can be rescued.

16.9 Epstein-Barr Virus and B Cell Proliferation

Epstein-Barr (EB) virus promotes B lymphocyte proliferation and is a cause of malignancies like Burkitt’s lymphoma, Hodgkin’s disease and non-Hodgkin lymphoma (NHL) (Table 16.2). So, considerable effort [72] has been directed at understanding the EB virus antigens and what they are capable of doing to cell cycle control. EBNA2 induces transition of resting B cells from Go to G1. It upregulates c-fgr, c-myc, CD21, and CD23. It transcribes antiapoptotic genes, and it immortalizes cells by complexing with a DNA binding cellular protein called RBP-J [73]. EBNA1 is also a transcriptional regulator that provides for survival, but it destabilizes p53 protein. The activity of the viral activator EBNA2 is enhanced by Pim-1/2 kinases, which favour antiapoptosis [74]. EBER1 binds to the interferon (IFN)-inducible PKR and inhibits its proapoptotic activity [75]. EBER, which is actually EB virus-encoded RNAs, is recognized by cell Rig1 as double-stranded RNA, and it thus stimulates NF- κ B and IRF3 to induce type I IFN [76]. EBER helps growth potential of cells [77]. A posttranscriptional effect of EB virus is to cause phosphorylation of Bim, which leads to its degradation, so infected cells are protected from apoptosis [78].

LMP1 is present constitutively in lipid rafts, where it signals and associates with the actin cytoskeleton through TRAF3, and it colocalises with IRF7 [79a]. LMP1 recruits several TRAF adaptor proteins to induce NF- κ B and AP-1. LMP1 synergises with CD40 for activation of B cells [72]. In fact, LMP1 enhances production of cellular IL-10 [79b], an autocrine growth factor for B cell lymphomas. Furthermore, LMP1 binds TRAF3, which would be an inhibitor, so it promotes B cell signalling [80]. LMP1 cooperates with BAFF and April to ensure DNA class switch recombination and formation of IgA, IgG or IgE [81]. LMP1 mimics an activated CD40 receptor [74].

Table 16.2 The potential of Epstein-Barr virus antigens

EBNA1	Provides for survival of B lymphocytes Regulates transcription: destabilizes p53
EBNA2	Effects B cell transformation Transcription of antiapoptotic genes
EBER	Binds to PKR to inhibit its proapoptotic activity
LMP1	is antiapoptotic Activates PI3 kinase, NF- κ b, and MAP kinase Activates IL-10 production (i.e. growth factor for B cells)
LMP2A	Increases the sensitivity of B cell TLRs 4/7/9 A B cell receptor mimic that is antiapoptotic and transforming It activates NF- κ B and Bcl-2 and enhances BAFF expression

EBNA = Epstein Barr Virus nuclear antigen; LMP = latent membrane protein; EBER = Epstein Barr Virus

LMP2A is a scaffold for recruitment of BCR-associated tyrosine kinases. Actually, LMP2A is a BCR mimic with antiapoptotic and transforming capabilities [82]. It activates the MAPKs and the PI3K-Akt pathway [82b]. As shown in transgenic mice, LMP2A can drive development of B cells with bypass of normal developmental checkpoints [83]. LMP2A allows autoreactive B cells to overcome the regulatory checkpoint [84]. It sets the scene for autoimmunity [85]. Indeed, at the same time it heightens sensitivity to TLRs [84]. LMP2A can replace signal functions of the pre-BCR in early B cell development. By residing in lipid rafts, LMP2A blocks BCR-raft association [86].

Infection of human B cells with EB virus results in the expression of SHM-inducing molecules like AID and the polymerase- η gene, so that there can be oncogenic mutations/translocations and so lymphomagenesis [87].

16.10 B Cells of Chronic Lymphatic Leukaemia

The typical phenotype of B-CLL includes the expression of CD19, CD20, CD21, CD23 and CD5 [88]. IL-15 via Stat5 phosphorylation promotes proliferation. The B cells survive on account of decreased apoptosis, for which there are several reasons [89]. The double-negative lymphoid progenitor has deregulation of c-myc, p53 and antiapoptotic bcl-2. There is high expression of TNF α and of BLys (BAFF) and April, so that NF- κ B is activated. CD40 action induces the antiapoptotic protein survivin, and in lymph nodes there is follicular dendritic cell induction of Mcl-1 via a CD44-dependent pathway [90]. Furthermore, CLL cells are resistant to CD95 (Fas)-induced apoptosis after CD40 ligation owing to expression of IAPs like XIAP. An anomalous Lyn also contributes [91].

There are two groups of B-CLL. When the Vh gene receptors are mutated, prognosis is fair, but when receptor mutations are lacking and the BCRs connect to Zap70, those patients have a poor prognosis [88, 92, 93]. In CLL cells both Zap70 and Syk become phosphorylated on BCR activation, which could explain their aggression. There is enhanced CLL cell migration in response to stromal cell-derived factor 1 (SDF1). Interestingly, CLL cells induce altered gene expression in normal CD4 and CD8 T cells when there is cell-cell contact. Furthermore, there are mutations in microRNA that confer prognostic significance [94]. Another feature of CLL B cells is their expression of CD5, which can imply that there is cell anergy and reduced proliferation; certainly, this matches expression of IL-10 [95].

16.11 The Lymphomas

NF- κ B plays essential roles in the development of lymphocytes, in their proliferation and in their activation. Indeed, NF- κ B induces cell cycle regulators like cyclins D1 and D2 and c-myc and c-myb [96], and it induces survival factors such as bcl-2 family members, cIAP and c-Flip. In Hodgkin lymphoma, several distinct means of

aberrant NF- κ B activation have been identified [97]. There is activation of both canonical and alternative NF- κ B signalling. (a) In Reed-Sternberg (RS) cells, there is self-oligomerization of CD30 receptor molecules that recruit TRAF2 and TRAF5 so that there is activation of unit I-kappa B kinase (IKK). (b) RS cells express both RANKL (receptor activator of NF- κ B ligand) and the receptor RANK, and via TRAFs 2, 5 and 6, it activates NF- κ B. (c) CD40 is activated on local CD4 T cells, leading to canonical and alternative activation of NF- κ B. (d) Epstein-Barr virus LMP1 antigen often usurps the CD40 signalling pathway.

Malt lymphomas arise in extranodal sites by at least three site-specific chromosomal translocations [98]. The t(11;18)(q21;q21) results in fusion of the cIAP2 of 11q21 with the Malt1 gene on 18q21. The cIAP2 normally consists of baculovirus IAP repeat (BIR)-BIR-BIR-Card-Ring, and the Ring has E3 ligase activity. The fusion protein cIAP2-Malt1 can still use its BIR domains to bind Bcl-10, but it is not able to ubiquitylate it. Hence, synergistic action of Carma1, bcl-10, and Malt1 (Fig. 13.6) lead to strong activation of NF- κ B [97].

In diffuse large B cell lymphoma (DLBCL), genomic alterations of the NF- κ B2 locus on chromosome 10q24 led to a decrease of inhibitory p100 function and increased activity of p52 of the alternative signal path. There is activation of IKK via bcl-10 [99]. In any case, translocations place various genes alongside Bcl-6.

In B cell NHLs, somatically mutated IgV genes indicate their origin from GC B cells. There can be either aberrant somatic hypermutation (i.e. mistakes in Ig gene remodelling mechanisms) or chromosomal translocations that involve the Ig locus [51, 100]. Other translocations are the t(14;18), involving the Ig and Bcl2 loci in follicular lymphoma, and t(8;14), implicating the immunoglobulin and Myc loci in Burkitt lymphoma.

It is so evident that chronic antigenic stimulation and chronic inflammation are linked to the induced risk of malignant lymphomas [101a].

16.12 Plasma Cells in Multiple Myeloma

The plasma cells of multiple myeloma (MM) have Ig VDJ genes that have undergone somatic hypermutation and isotype switch recombination in the germinal centres of lymph nodes before migrating to and expanding in the bone marrow. The immunoglobulin heavy-chain region on chromosome 14q32 is involved in translocations that result in activation of oncogenes FGFR3 and cyclins D1/D3. Maf of the AP-1 superfamily is over-expressed [101b]. As the disease progresses, there are activating mutations of proliferation factors like N- and K-Ras and c-Myc [102]. There is inactivation of antiproliferation factors like p53, Rb and PTEN. Possession of CD200 is a bad prognostic factor. The Ras-Raf-MEK-ERK pathway is stimulated by cytokines IL-6, insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor (VEGF) provoked by interaction of MM cells with bone marrow stromal cells. The proteasomal inhibitor bortezomib (Velcade) prevents NF- κ B activation, and so IL-6 production, and it inhibits MAPK signalling [103]. There is now a specific MEK1/2

inhibitor AZD6244 that blocks MAPK ERK1/2 signalling to stop the proliferation of MM cells. The same agent downregulates osteoclast-activating factors [104].

Downstream of FGFR3 is the gene for macrophage inflammatory protein 1 α (MIP-1 α) (CCL3) that aids bone erosion [105]. MM cells produce large amounts of hepatocyte growth factor (HGF) [106], which promotes formation of new blood vessels. It is inhibited by NK4, which is homologous to angiostatin.

References

- 1a. Gazamyan A, Reichlin A, Nussenzweig MC. Ig β tyrosine residues contribute to the control of BCR signaling by regulating receptor internalization. *J Exp Med* 2006;203:1785–1794
- 1b. Hammond CM, White D, Tomic J et al. Extracellular calcium sensing promotes human B cell activation and function. *Blood* 2007;110:3985–3995
2. DalPorto JM, Gauld SB, Merrell KT, Mills D, Pugh-Bernard E, Cambier J. B cell antigen receptor signaling. *Mol Immunol*. 2004;41:599–613
3. Singh DK, Kumar D, Siddiqui Z et al. The strength of receptor signalling is controlled through a cooperative loop between Ca²⁺ and an oxidant signal. *Cell* 2005;121:281–293
4. Kurosaki T, Maeda A, Ishiai M, Hashimoto A et al. Regulation of the phospholipase C γ 2 pathway in B cells. *Immunol Rev* 2000;176:19–29
5. Sharma S, Orlowski G, Song W. Btk regulates BCR-mediated antigen processing by controlling actin cytoskeleton dynamics. *J Immunol* 2009;182:329–339
6. de Gorter DJ, Beuling EA, Kersseboom R et al. Bruton's tyrosine kinase and PLC γ 2 mediate controlled B cell migration and homing. *Immunity* 2007;26:93–104
7. Janssen E, Zhu M, Zhang W et al. LAB, a new membrane associated adaptor molecule in B cell activation. *Nat Immun* 2003;4:117–123
8. Stork B, Engelke M, Frey J et al. Grb2 and NTAL constitute a calcium regulatory signal circuit in B lymphocytes. *Immunity* 2004;21:681–685
9. Niro H, Clarke H. Branches of the B cell antigen receptor pathway are directed by protein conduits Bam32 and Carma 1. *Immunity* 2003;19:637–640
10. Ren H, Schmolstieg A, Yvon D, Gaynor RB. I-kB kinase is critical for B cell proliferation and antibody response. *J Immunol* 2002;168:577–587
11. Carter RH, Fearon DT. CD19 lowering the threshold for antigen receptor stimulation of B lymphocytes. *Science* 1992;256:102–105
12. Fujimoto M, Poe JC, Hasegawa M, Tedder TF. CD19 amplification of B lymphocyte Ca²⁺ responses: a role for Lyn sequestration in extinguishing negative regulation. *J Biol Chem* 2001;276:44820–44827
13. Nishizumi H, Horikawa K, Milmaric-Rascan I, Yamamoto T. A double edged kinase Lyn: a positive and negative regulator for antigen receptor mediated signals. *J Exp Med*. 1998;187:1343–1348
14. Zouali M, Sarmay G. B lymphocyte signaling pathways in systemic autoimmunity. *Arthritis Rheum* 2004;50:2730–2741
15. Guo B, Blair D, Chiles TC et al. B cell receptor cross-talk: the IL-4 induced alternate pathway for BCR signaling. *J Immunol* 2007;178:4726–4730
16. Pritchard NR, Smith KGC. B cell inhibitory receptors and autoimmunity. *Immunology* 2003;108:263–273
17. Chen J, McLean PA, Neel BG et al. CD22 attenuates calcium signaling by potentiating plasma membrane calcium-ATPase activity. *Nat Immun* 2004;5:651–657
18. Nitsche L, Tsubata T. Molecular interactions regulate BCR signal inhibition by CD22 and CD72. *Trends Immunol* 2004;25:543–550

19. Otipoby KL, Draves KE, Clark EA. CD22 regulates B cell receptor mediated signals via two domains that recruit Grb-2 and SHP-1. *J Biol Chem* 2001;276:44315–44322
20. Marth JD. Unmasking connections in transmembrane immune signaling. *Nat Immun* 2004;5:1008–1010
21. Daniel J, Marechal Y, van Gool F et al. Nicotinamide inhibits B lymphocyte activation by disrupting MAPK signal transduction. *Biochem Pharmacol* 2007;73:831–842
22. Tridandapani S, Kelley T, Cooney D et al. Negative signaling in B cells. *Immunol Today* 1997;18:424–427
23. Ono M, Bullard S, Temsh Ravetch JV. Role of inositol phosphate SHIP in negative regulation of the immune system by FcγRIIB. *Nature* 1996;383:263–266
24. Takai T. Paired immunoglobulin-like receptors and their MHC class I recognition. *Immunology* 2005;115:433–440
25. Xiang Z, Cutler AJ, Brownlie RJ et al. FcγRIIB controls bone marrow plasma cell persistence and apoptosis. *Nat Immunol* 2007;8:419–429
26. Mizumo T, Rothstein TL. CD40 engagement eliminates the need for Bruton's tyrosine kinase in B cell receptor signalling for NF-κB. *J Immunol* 2003;170:2806–2810
27. Bishop GA. The multifaceted roles of TRAFs in the regulation of B cell function. *Nat Rev Immunol* 2004;4:774–786
28. Gallagher E, Enzler T, Matsuzawa A et al. MEKK1 is required for CD40 dependent activation of the kinases JNK and p38. *Nat Immunol* 2007;8:57–63
29. Aiba Y, Yamazaki T, Okada T et al. BANK negatively regulates Akt activation and subsequent B cell responses. *Immunity* 2006;24:259–268
30. Quiao G, Lei M, Li Z et al. Negative regulation of CD40 mediated B cell responses by E3 ubiquitin ligase Cbl-b. *J Immunol* 2007;179:4473–4479
- 31a. Zarnegar B, He JQ, Oganessian G et al. Unique CD40 mediated biological program in B cell activation requires both type 1 and type 2 NF-κB activation pathways. *Proc Natl Acad Sci U S A* 2004;101:8108–8113
- 31b. Cancro MP. Living in context with the survival factor BAFF. *Immunity* 2008;28:300–301
- 31c. Benson MJ, Elgueta R, Noelle RJ. B cell survival: An unexpected mechanism of lymphocyte vitality. *Immunol Cell Biol* 2008;86:485–486
32. Melchers F. Actions of BAFF in B cell maturation and its effects on the development of autoimmune disease. *Ann Rheum Dis* 2003;62(Suppl II):ii25–ii27
33. Cancro MP. The BLys family of ligands and receptors: an archetype for niche-specific homeostatic regulation. *Immunol Rev* 2004;202:237–249
34. Zhang X, Park CS, Yoon SO et al. BAFF supports human B cell differentiation in the lymphoid follicles through distinct receptors. *Int Immunol* 2005;17:779–788
35. Mecklenbrauker I, Kolled SL, Leitaes M, Mackay F, Tarakhovsky A. Regulation of B cell survival by BAFF dependent PKCδ mediated nuclear signalling. *Nature* 2004;431:456–460
36. Mackay F, Schneider P, Rennert P, Browning J. BAFF and APRIL: tutorial in B cell survival. *Annu Rev Immunol* 2003;21:231–264
37. Haiat S, Billard C, Quiney C et al. Role of BAFF and APRIL in human B cell chronic lymphatic leukemia. *Immunity* 2006;118:281–292
38. Chang SK, Arendt BK, Darce JR et al. A role for BLys in the activation of innate immune cells. *Blood* 2006;108:2687–2694
39. Yang M, Hase H, Legarda-Addison D et al. B cell maturation antigen induces antigen presentation in B cells. *J Immunol* 2005;175:2814–2824
40. Rothstein TL, Zhong X, Schram BR et al. Receptor specific regulation of B cell susceptibility to Fas mediated apoptosis and a novel Fas apoptosis inhibitory molecule. *Immunol Rev* 2000;176:116–122
41. Owyang AM, Tumang JR, Schram BR et al. c-Rel is required for protection of B cells from antigen receptor mediated apoptosis. *J Immunol* 2001;167:4948–4956
42. Gerondakis S, Grumot RJ, Banerjee A. Regulating B cell activation and survival in response to TLR signals. *Immunol Cell Biol* 2007;85:471–475

43. Bai S, Liu H, Chen K-H et al. Expression of cellular FLIP protects rheumatoid synovial fibroblasts from TNF α mediated apoptosis. *Arthritis Rheum* 2004;50:3844–3855
44. Khaled AR, Durum SK. Cytokines and the control of lymphoid homeostasis. *Nat Rev Immunol* 2002;2:817–830
45. Gary-Guy H, Harriaque J, Bismuth G et al. Human CD5 promotes B cell survival through stimulation of autocrine IL-10. *Blood* 2000;100:4537–4543
46. (a) Frances R, Tumang JR, Rothstein TL. Defective B cell receptor signal transduction in B-1 cells. *J Immunol* 2005;175:27–31 (b) Dufort FJ, , Bleiman BF, , Gumina MR et al. IL-4 mediated protection of primary B lymphocytes from apoptosis via Stat6 dependent regulation of glycolytic metabolism. *J Immunol* 2007;179:4953–4957
47. Foote LC, Evans JW, Cifuni JM et al. Interleukin 4 produces a breakdown of tolerance in vivo with autoantibody formation and tissue damage. *Autoimmunity* 2004;37:569–577
48. Craxton A, Draves KE, Clark EA. Bim regulates B cell receptor induced entry of B cells into the cell cycle. *Eur J Immunol* 2007;37:2715–2722
49. Klaewongkram J, Yang Y, Golech S et al. KLF4 regulates B cell number and activation induced B cell proliferation. *J Immunol* 2007;179:4679–4684
50. Anzelon AN, Wu H, Rickert TC. PTEN inactivation alters peripheral B lymphocyte fate and reconstitutes CD19 function. *Nat Immunol* 2003;4:287–294
51. Klein U, Dalla-Favera R. Germinal centres: role in B cell physiology and malignancy. *Nat Rev Immunol* 2008;8:34–47
52. Benscop RJ, Brandl E, Chan AC, Cambier JC. Unique signaling properties of B cell receptor in mature and immature B cells. *J Immunol* 2001;167:4172–4179
53. Leider N, Melamed D. Differential c-Myc responsiveness to B cell receptor ligation in B cell negative selection. *J Immunol* 2003;171:2446–2452
54. Mackay F, Mackay CR. The role of BAFF in B cell maturation, T cell activation and autoimmunity. *Trends Immunol* 2002;23:113–117
55. Vinuesa CG, Tangye SG, Moser B, Mackay CR. Follicular B helper T cells in antibody responses and autoimmunity. *Nat Rev Immunol* 2005;5:853–865
56. Hendriks RW, Middendorp S. The pre-BCR checkpoint as a cell autonomous proliferation switch. *Trends Immunol* 2004;25:249–255
57. Ohnishi K, Melchers F. The nonimmunoglobulin portion of I δ mediates cell autonomous preB cell receptor signaling. *Nat Immunol* 2003;4:849–856
58. Tretter T, Ross AE, Dordai DI, Desiderio S. Mimicry of preB cell receptor signaling by activation of tyrosine kinase Blk. *J Exp Med.* 2003;198:1863–1873
59. Wossning T, Herzog S, Kohler F et al. Deregulated Syk inhibits differentiation and induces growth factor independent proliferation of pre-B cells. *J Exp Med* 2006;203:2829–2840
60. Gururajan M, Dasu T, Shahidain S et al. Spleen tyrosine kinase, target of curcumin, is required for B lymphoma growth. *J Immunol* 2007;178:111–121
61. Leseux L, Hamdi SM, al Saati T et al. Syk dependent mTOR activation in follicular lymphoma cells. *Blood* 2006;108:4156–4162
62. Good KL, Tangye SG. Decreased expression of Kruppel-like factors in memory B cells induces the rapid response typical of type 2 antibody responses. *Proc Natl Acad Sci USA* 2007;104:13420–13425
63. Brewer JW, Hendershot LM. Building antibody factory. *Nat Immunol* 2005;6:23–29
64. (a) Calame KL, Lin K-I, Tunyaplin C. Regulatory mechanisms that determine the development and function of plasma cells. *Annu Rev Immunol* 2003;21:205–230 (b) Igarishi K, Ochiai K, Muto A. Architecture and dynamics of the transcription factor network that regulates B to plasma cell differentiation. *J Biochem* 2007;141:783–789
65. Green MR, Yoon H, Boss JM. Epigenetic regulation during B cell differentiation controls CIITA promoter accessibility. *J Immunol* 2006;177:3865–3873
- 66a. Gupta N, De Franco AL. Lipid rafts and B cell signalling *Semin Cell & Develop. Biol.* 2007;18:616–626
- 66b. Meyer Bahlburg A, Rawlings D J.B cell autonomous TLR signalling and autoimmunity. *Autoimmune Rev* 2008;7:333–336

- 67a. Halverson R, Torres RM, Pelanda R. Receptor editing is the main mechanism of B cell tolerance toward membrane antigens. *Nat Immunol* 2004;5:645–650
- 67b. Rowh MAW, Bassing CH. Foxos around make B cells tolerable. *Nat Immunol* 2008;9:586–588
68. Ferry H, Crockford TL, Leung J, Cornall RJ. Signals from self-antigen induce positive selection in early B cell ontogeny but are tolerogenic in adults. *J Immunol* 2006;176:7402–7411
69. Merrell KT, Benschop RJ, Gauld SB et al. Identification of anergic B cells with a wild type repertoire. *Immunity* 2006;25:953–962
70. Cambier JC, Gauld SB, Merrell KT, Vilen BJ. B cell anergy. *Nat Rev Immunol* 2007;7:633–643
71. Lyubchenko T, Dal Porto J M, Holers V, Cambier J C. Complement C3d linked antigens break B cell anergy. *J Immunol* 2007;179:2695–2699
72. Clemens MJ. Epstein Barr virus: inhibition of apoptosis as a mechanism of cell transformation. *Int J Biochem Cell Biol* 2006;38:164–169
73. Dolcetti R, Masucci MG. Epstein-Barr virus induction and control of cell transformation. *J Cell Physiol* 2003;196:207–217
74. Hatzivassiliou EG, Kieff E, Mosialos G. Constitutive CD40 phenocopies the transforming function of the Epstein-Barr virus oncoprotein LMP1 in vitro. *Leukoc Res* 2007;31:315–320
75. Rainio EM, Ahlfors H, Carter KL et al. Pim kinases are upregulated during EB virus infection and enhance EBNA2 activation. *Virology* 2005;333:201–206
76. Samonta M, Iwakiri D, Kanda T et al. EB virus encoded RNAs are recognized by RIG1 and activate signaling to induce type I IFN. *EMBO J* 2006;25:4207–4214
77. Felton-Edkins ZA, Kondrshov A, Karoli D et al. EB virus induces allows active expression of EBER genes by RNA polymerase III. *J Biol Chem* 2006;281:33871–33880
78. Clybourn C, Mchichi B, Muuhamed S et al. EB virus infection of human B lymphocytes leads to downregulation of Bim expression. *J Immunol* 2005;175:2968–2973
- 79a. Kim I-W, Park H-S. Colocalization of IRF7 with LMP1 of EB virus. *J Korean Med Sci* 2006;21:379–384
- 79b. Lambert SL, Martinez OM. LMP1 of EBV activates phosphatidylinositol 3-kinase to induce production of IL-10. *J Immunol* 2007;179:8225–8234
80. Uchida J, Yasui T, Takaoka-Shichijo Y et al. Mimicry of CD40 signals by Epstein Barr virus LMP-1 in B lymphocyte responses. *Science* 1999;286:300–303
81. Xie P, Histager BS, Bishop GA. Requirement for TRAF3 in signaling by LMP1 but not CD40 in B lymphocytes. *J Exp Med* 2004;199:661–671
82. (a) Mancao C, Hammerschmidt W. Epstein Barr virus LMP2A is a B cell receptor mimic and essential for B cell survival. *Blood* 2007;110:3715–3721; (b) He B, Raab-Traub N, Casali P, Cerutti A. Epstein-Barr virus encoded LMP-1 cooperates with Baff and April to induce T cell independent immunoglobulin heavy chain class switching. *J Immunol* 2003;171:5215–5224
83. Keda AI, Merchant M, Lev L et al. Latent membrane protein 2A, a viral B cell receptor homologue, induces CD5⁺ B1 cell development. *J Immunol* 2004;172:5329–5337
84. Wang H, Nicholas MW, Conway KL et al. EB virus LMP-2A induces autoreactive B cell activation and TLR hypersensitivity. *J Immunol* 2006;177:2793–2802
85. Swanson-Mungerson M, Longnecker R. Epstein-Barr virus LPM2A and autoimmunity. *Trends Immunol* 2007;28(5):213–216
86. Dykstra ML, Longnecker R, Pierce SK. EB virus coopts lipid rafts to block the signaling and antigen transport functions of the BCR. *Immunity* 2001;14:57–67
87. Epeldegui M, Hung YP, McQuay A et al. Infection of human B cells with Epstein-Barr virus results in oncogene mutations. *Mol Immunol* 2007;44:934–942
88. Ciorazzi N, Rai KR, Ferrarini M. Chronic lymphatic leukaemia. *N Engl J Med* 2005;352:804–815
89. Pedersen IM, Kitada S, Leoni LM et al. Protection of CLL B cells by follicular dendritic cell line is dependent on induction of Mcl-1. *Blood* 2002;100:1795–1801

90. Contri A, Brunati AM, Trentin L et al. CLL B cells contain anomalous Lyn tyrosine kinase: contributions to defective apoptosis. *J Clin Invest* 2005;115:369–378
91. Lanham S, Hamblin T, Oscier D et al. Differential signaling via surface IgM is associated with Vh gene mutational status. *Blood* 2003;101:1087–1093
92. Chen L, Apgar J, Huynh L et al. Zap70 directly enhances IgM signaling in chronic lymphatic leukaemia. *Blood* 2005;105:2036–2041
93. Calin GA, Ferracin M, Cimmino A et al. A microRNA signature associated with prognosis and progression in chronic lymphocytic leukaemia. *N Engl J Med* 2005;353:1793–1801
94. Guipaud O, Deriano L, Salin H et al. B cell CLL. *Lancet Oncol* 2003;4:506–514
95. Gary-Gouy H, Sainz-Perez A, Marteau J-B et al. Natural phosphorylation of CD5 in chronic lymphatic leukemia B cells. *J Immunol* 2007;179:4335–4344
96. Hinz M, Krappmann D, Eichten A et al. NF-kappa B function in growth control: regulation of cyclin D1 expression and Go/G1 to S-phase transition. *Mol Cell Biol* 1999;19:2690–2698
97. Jost PJ, Ruland J. Aberrant NF-kB signaling in lymphoma. *Blood* 2007;109:2700–2707
98. Bertoni F, Zucca E. MALT lymphoma biology. *J Clin Invest* 2006;116:22–26
99. Lucas PC, Yonezumi M, Inohara N et al. Bcl-10 and Malt1, independent targets of chromosomal translocation in malt lymphoma, cooperate in a novel NF-kappa B signaling pathway. *J Biol Chem* 2001;276:19012–19019
100. Kuppers R. Mechanisms of B cell lymphoma. *Nat Rev Cancer* 2005;5:251–262
- 101a. Smedby KE, Askling J, Mariette X, Baecklund E. Autoimmune and inflammatory disorders and risk of malignant lymphomas – an update. *J Intern Med* 2008;264:514–527
- 101b. Eychène A, Rocques N, Pouponnot C. A new MAFia in cancer. *Nat Revs Cancer* 2008;8:683–693
102. Hideshima T, Bergsagel P, Kuehl WM, Anderson KC. Advances in biology of multiple myeloma: clinical applications. *Blood* 2004;104:607–618
103. Hideshima T, Chauhan D, Hayashi T et al. Proteasome inhibitor PS341 abrogates Il-6 triggered signaling cascades via caspase dependent downregulation of gp130 in multiple myeloma. *Oncogene* 2003;22:8386–8393
104. Tai YT, Fuciniti M, Hideshima T et al. Targeting MEK induces myeloma cell cytotoxicity and inhibits osteoclastogenesis. *Blood* 2007;110:1656–1663
105. Masih-khon E, Trudel S, Heise C et al. MIP1 α (CCL3) is a downstream target of FGFR3 and Ras-MAPK signaling in myeloma. *Blood* 2006;108:3465–3471
106. Du W, Hattori Y, Yamada T et al. NK4, an antagonist of HGF, inhibits growth of multiple myeloma cells: molecular targeting of angiogenic factor. *Blood* 2007;109:3042–3049

Chapter 17

The Cell Cycle

Abstract Considered here is the cell cycle, which is driven by mitogen growth factors and dampened by cytokine TGF β . Surface integrins help cell proliferation and, of course, c-Myc oncogene. How cell cycle arrest or apoptosis are achieved is to be appreciated. One can consider the proliferation of T lymphocytes, and one will have tumors in mind. Hence, the embryonic hedgehog and Wnt signaling pathways have to be considered as means to tumourigenesis.

17.1 Control of the Cell Cycle

In normal cells, the cycle of growth followed by cell division may take 10–30 h. Quiescent cells in G₀ must first move into gap G₁ phase, in which there is protein synthesis, then comes the S phase of DNA synthesis, which is followed by the G₂ phase, at which mitotic priming factor acts, and finally mitosis occurs in the M phase. Cells respond to DNA damage by arresting the cycle at the G₁/S-phase checkpoint, by preventing progress to replication at the intra-S checkpoint or by stopping mitosis at the G₂ checkpoint (Fig. 17.1). One can verify cell proliferation in tissue sections by immunostaining for proliferating cell nuclear antigen (PCNA), Ki67 or the p57 CKI (cyclin-dependent kinase [CDK] inhibitor). PCNA trimer actually forms a ring clamp for the two DNA polymerases δ that form the two new strands of DNA. Ki67 immunostaining can distinguish between cells that are quiescent, in early G₁ or proliferating since it changes its nuclear distribution.

Orderly progression through the phases of the cell cycle [2] is controlled by the action of cyclins D, E, A and B and their catalytic partners the CDKs. CDKs are serine-threonine kinases that are activated by their replicative cyclin partners and by phosphorylation. Regulation of the cyclins and of cdks occurs at multiple levels (i.e. at transcription of their messenger RNA) by regulation of translation, by post-translational controls and by their association with CKIs. Cell proliferation is determined by cyclin activation of CDKs. On the other hand, cell hypertrophy results when there is G₁ arrest via inhibitors p21/p27, and mTOR is in control. We now

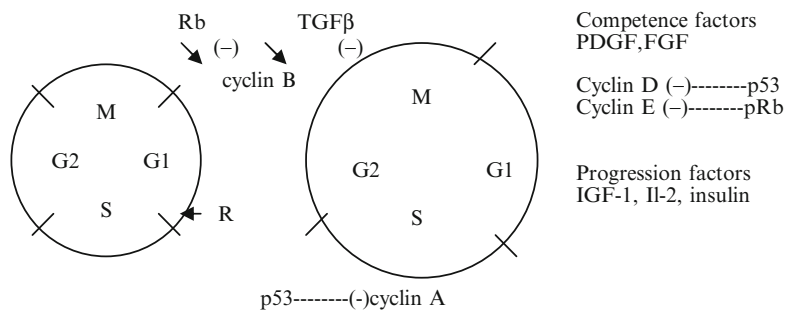


Fig. 17.1 The cell cycle and its control. Note the restriction point R during late G1. Beyond R, cells no longer require growth factors to promote mitosis. Note also that there are “competence” growth factors like platelet-derived growth factor (*PDGF*) that act early in G1 and the “progression” factors that act later [1]. A cell is maintained in G1 phase when the retinoblastoma protein (*pRb*) is in its active hypophosphorylated state. Rb controls cell proliferation, whilst p53 (**Fig. 8.5**) mediates cell cycle arrest or cell apoptosis. Reduced cell proliferation will result from (a) low DNA synthesis due to G1/S checkpoint arrest or (b) reduced mitosis arising from G2/M checkpoint arrest

Table 17.1 Cell cycle regulatory proteins

Phase	Cyclin	Partner CDK	Associated CKI
G1	D1, D2, D3	cdks 4, 6 and 2	p15–19, p21, p27, p57
G1/S	E	cdk2	p21, p27, p57
S	A	cdk2	p21, p27, p57
M	B	cdc2 (CDK1)	

CDK cyclin-dependent protein kinase, **CKI** CDK inhibitor

know a great deal about the role of CDKs in T cell development, proliferation and functioning [3a] but there is new knowledge from gene targeting of CDKs [3b].

During cell cycle progression, newly formed CDK-cyclin complexes initially accumulate in an inactive state since the CDK is phosphorylated on two residues. Dephosphorylation by the cell division cycle 25 (*cdc25*) phosphatases leads to activation of CDK-cyclin complexes and cell cycle progression. Unexpectedly, all three *cdc25s* contribute to the G1-S and G2-M transitions (Table 17.1).

Activated integrins or the growth factor receptor tyrosine kinases activate Ras-Raf → MKK1/2 → mitogen-activated protein kinase (MAPK) (**Fig. 4.3**) and so cyclin D1, which determines cell cycle progression. They also downregulate the CDK inhibitory proteins p21^{cip1} and p27^{kip1}.

Growth factors induce persistent activation of p42/p44 MAPKs during the G1 period. Jun N-terminal kinase (JNK) activates c-Jun and JunB. Variation in the ratio of transcription factors (TFs) c-Jun to JunB is critical for modulation of the expression of cyclin D1 [4, 5]. c-Jun induces cyclin D1, but JunB represses it. During mitogenic stimulation, cyclin D genes are induced by nuclear factor kappa B (NF-κB) or activator protein 1 (AP-1) or by c-Myc oncogene. The duration of the extracellular signal-related kinase (ERK) signal determines induction of cyclin D1

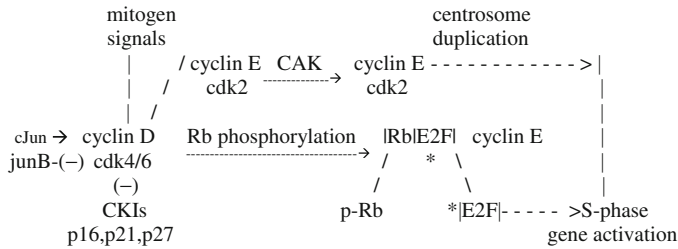


Fig. 17.2 Regulation of the G1- to S-phase transition. (a) cyclin-dependent protein kinase (CDK) activating kinase (CAK). (b) *The transcription factor (TF) E2F1 regulates S-phase genes. (c) Regulating the G1/S checkpoint are p16^{INK4a}-cyclin D-cdk4-Rb, p27^{kip1}-cyclin E-cdk2, p14^{ARF}-murine double minute 2 (*mdm-2*)-p53-p21^{cip1}. With mitogen stimulation, accumulation of D-type cyclins allows formation of active cdk4/6-cyclin D complexes, which phosphorylate and inactivate Rb. (e) Phosphorylated Rb releases E2F TF. (f) In cancer cells, inactivation of p53 leads to absence of p16, p21, and so on, so there is Rb phosphorylation and release of E2F factor [7, 8a, 8b]. (g) Progression of the cell cycle requires cdc25 family phosphatases, which remove the inhibitory phosphorylations by Cdk's and thus allow S phase (cdk2) and mitosis (cdk1)

[6]. PI3 kinase (PI3K) affects the translation and stability of cyclin D1. Yet, transforming growth factor- β (TGF β), acting through the p38 MAPK, exerts restraint. There is autoregulation of the MAPK module by MAPK phosphatases MKP-1 and -2. This negative regulatory loop is (MAPK phosphatase) enhanced by p42/44 phosphorylation of MKP-1/-2.

Figure 17.2 depicts the regulation of the G1- to S-phase transition. Mitogens promote formation of active cyclin D-dependent kinases cdk4 or cdk6. Unphosphorylated retinoblastoma protein Rb binds E2F factor [7, 8a, 8b]. However, after cyclin E with cdk2 is activated by CAK (cdk-activating kinase) phosphorylation, so that CKIs are released, cyclin D- and E-dependent kinases contribute sequentially to Rb phosphorylation, allowing release of E2F. E2F then regulates transcription of genes required for entry into S phase [8b].

In a normal cell cycle in the mid- to late G1, cyclin D/cdk4, 6 phosphorylate Rb protein, and the pRb loses its growth inhibitory ability. When Rb is hyperphosphorylated, transcription of cyclin E occurs. Stimulation of cdk2-cyclin E/A or cdk1-cyclin B complex kinase activity is essential for progression through the cell cycle G1/S and G2/M transitions, respectively (Figs. 17.1, 17.2). Cyclin A is synthesized close to the G1/S phase boundary and forms complexes with cdk2 when it becomes available following degradation of cyclin E. Cdk2 activity is inhibited by phosphorylation of Thr14 and Tyr15 by the dual-specificity Myt1 kinase [9]. But, these residues are dephosphorylated by cdc25A phosphatase (G1/S phase), cdc25B (late S phase) and cdc25c phosphatases (G2/M phase) to stimulate cdk activity [10]. The change to mitosis is effected by cyclin B/cdc2 (CDK1). Cyclin B-cdc2 kinase leads to breakdown of nuclear envelopes by phosphorylation of the lamins; thus, cyclin E can move into the nuclei. Exit from mitosis occurs when the cell ubiquitination machinery targets cyclin B for destruction. Ubiquitination of the various cyclins shows a tight dependence on the cell cycle [11].

Table 17.2 Human tumours and checkpoint component aberrations

Gene protein	Molecular abnormality	Hereditary syndrome or tumor	Sporadic tumour
*ATM	Mutation, deletions	Ataxia-telangiectasia	Breast cancer
*chk1	Frameshift mutation		Colorectal, endometrial
*chk2	Missense mutations	Li-Fraumeni	Breast, lung, colon
*BRCA1	Mutations, deletions	Familial breast, ovary	Breast, ovary
*p53	Missense mutations	Li-Fraumeni	Many cancers
**cdc25A	Overexpression	Breast	
Rb	Deletions, mutations	Retinoblastoma	Many cancers
*p16	Deletions, mutations	Familial melanoma	Many cancers
*CyclinD1			Many cancers
amplification/ translocation			
*Cyclin E			Breast, ovary
amplification/ overexpression			

BRCA breast cancer, *Rb* retinoblastoma
 *Tumor suppressor
 **Protooncogene

The CKIs (Table 17.1) that regulate cell cycle checkpoints are named according to their molecular weights[11]:

CIP/KIP family: p21^{cip1}, p27^{kip1}, p57^{kip2}

INK4 inhibitors: p15 (INK4a), p16 (INK4b), p18 (INK4c), p19 (INK4d)

INK refers to inhibitor of cdk4 and is typified by the tumour suppressor p16^{INK4a}. The ability of INKs to arrest cells in G1 and to inhibit cdc4/6 is dependent on functional Rb [8].

Since p21 is promoted by suppressor gene p53 [12a], its expression results in G1/S arrest and inhibition of DNA synthesis. The tumour suppressor protein tyrosine phosphatase (PTEN) promotes p27^{kip1} expression. p27 has to fall before there can be any proliferation of cells. In human osteosarcoma, use of the agent KN-93 inhibits CaM (calmodulin) kinase II [12b], as a result of which p21 is elevated and cellular proliferation is controlled. Elevated p21 stops cyclin D and cdk4/6 operation.

Consider haemopoietic cells. When those cells adhere by their integrins to fibronectin (Fig. 9.10), p27^{kip} is elevated. Growth-promoting cytokines override this, and cells can then progress into S phase. Thus, stem cell factor and interleukin 3 (IL-3) work like this on haemopoietic progenitor cells [13]. The growth-restraining action of TGFβ occurs by promotion of inhibitors p15 and p21, with the result that there is inhibition of the actions of cdk4/cyclin D and of cdk2/cyclin E. TGFβ acts to keep Rb protein (Fig. 17.1) in its unphosphorylated state [14, 15] (Fig. 17.3).

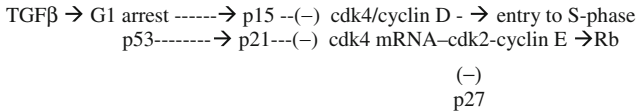


Fig. 17.3 Transforming growth factor- β (*TGF β*) inhibition of retinoblastoma (*Rb*) protein phosphorylation

There is a difference between cell cycle arrest created by autocrine *TGF β* and exogenous therapeutic *TGF β* . The former raises p21 and decreases cdk2 activity, whilst the latter decreases cdk4 and pRB phosphorylation [16].

17.2 PI3K and Cell Proliferation

PI3K is described in Chap. 2 (**Fig. 2.8**). It helps to promote nutrient uptake by cells, and thus cell growth and proliferation. Levels of cyclin D must rise for the G1-S transition to occur. If it is phosphorylated by glycogen synthase kinase 3 (GSK3), cyclin D1 undergoes proteasomal degradation. However, when PI3K stimulates protein kinase B (Akt), GSK3 is inhibited by phosphorylation by PKB, so cyclin rises. Moreover, PKB-induced phosphorylation of Forkhead TFs causes repression of transcription of CDK inhibitors like p27^{kip1}, so there is entry to the cell cycle. PI3K regulates the binding of RNA pol II to a group of Myc target genes involved in cell proliferation.

17.2.1 Notch and Cell Proliferation

To achieve cell proliferation, the CKIs p21 and p27 have to be removed. The ligands for Notch and other mitogens activate S-phase kinase-associated protein 2 (SKP2), with the result that there is proteasomal degradation of p21 and p27 [17]. We are beginning to realise the importance of Notch for lymphopoiesis [18].

17.3 Integrins and Cell Proliferation

Both growth factor and integrin receptors are necessary for cell proliferation. Their combined signalling results in appropriate ERK activation. **Figure 9.11** depicts a scheme for integrin signalling. Cell attachment and cell cycle signalling are actually interdependent [19]. Signals from integrins or growth factor receptors converge on Ras-MAPK (**Fig. 4.3**), so they affect cyclins D1 and E and cell cycle progression (Fig. 17.4). Ras is implicated in mitogen-induced passage

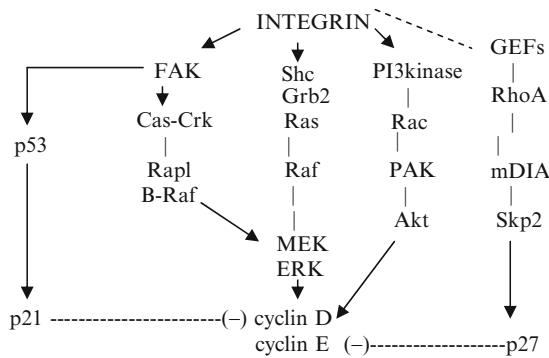


Fig. 17.4 How surface integrins affect cyclins D/E control of the cell cycle. By now you are familiar with all this. The role of mDia-skp2-p27 is found in Ref. [20]

through G1 and entry into the S phase. Furthermore, studies in Swiss 3T3 cells revealed that introduction of dominant interfering forms of Rac1 and RhoA inhibited growth factor-induced cell cycle progression, whilst a dominant active form of each small GTPase enabled cell cycle progression [21]. Note that RhoA, Rac and Rap1 appear in Fig. 17.4.

17.4 C-Myc and Cell Proliferation

C-myc, helix–loop–helix leucine zipper TF, promotes cell proliferation (**Fig. 4.18**). One has to be cautious about descriptions of c-Myc action because its effects are cell-type and context dependent [22a]. Myc induces cell cycle genes like *cdk4*, *Id2*, *cdc25A*; the cyclins D1, D2, A and E; and ornithine decarboxylase and E2F2. Myc negatively regulates the inhibitor *p27^{kip1}* and other growth arrest genes like *GADD45*, *p15^{Ink4b}* and *p21^{cip1}*, and it represses *gas-1* (growth arrest-specific) cell cycle inhibitory gene. It also downregulates N-cadherins and integrins.

The c-myc is subject to complex transcriptional and posttranscriptional regulation [22a]; it can induce cell mortalization. However, high c-myc levels result in induction of p19 ARF, which then sequesters mdm-2 (murine double minute 2), leading to activation of p53-mediated arrest or apoptosis (Fig. 17.5). p19 ARF can also downregulate c-myc. A recent article on mesangial cells proliferation [22b] indicates how growth factor PDGF via Ras→ERK stimulation activates TFs like C-Myc and cyclin D1.

17.5 The Cell Cycle and DNA Damage

Small nucleotide changes occur at the rate of 25,000 per day in every single cell [24]. When there is cell DNA damage, transcription is switched off at the G1 check-point, and there will be cell apoptosis (**Fig. 8.4**). DNA damage leads to upregulation

of protein phosphatase PP1 α , which is a regulator of the pRb pathway, there is then clearance of pRb, and apoptosis occurs. At the G2 checkpoint, activation of p53 leads to overproduction of protein 14-3-3, and that retains cdc2/cyclin B complexes in the cytosol so that they cannot oscillate into nuclei. Therefore, mitosis is prevented [25, 26]. The cdc25 isoforms play major roles in cell cycle arrest [27].

In the meantime, p53 is retained within the nuclei, and its exonuclease activity is involved in DNA repair (**Fig. 8.4**). p53 often determines cell apoptosis. With regard to cell cycle arrest, observe that Rb functions downstream of p53 (Figs. 17.3,17.6). p53 also induces p21 CKI, which inactivates cdk2-cyclins E/A or cdk4-cyclin D complexes. The p53 suppresses expression of cyclin A [28], and Rb suppresses the p34 cdc2.

In healthy cells, p53 is maintained below its functional threshold since it is inhibited by protein mdm-2 (**Fig. 17.5**). In response to DNA damage, p53 is phosphorylated on serine 1 by DNA-dependent protein kinase (DNA-PK), ATM (**Fig. 8.4**) and Chk1/2. This phosphorylation impairs interaction between mdm-2 and p53. Hence, p53 becomes activated [29–31a]. SOCS3 promotes p53-dependent p21 expression and so cell cycle arrest [31b].

In resting cells, Rb is hypophosphorylated, so it sequesters the TF complex E2F-DP1 (**Fig. 17.6**). When E2F is bound, there is elevation of p21^{cip1}, so there is cell cycle arrest [32].

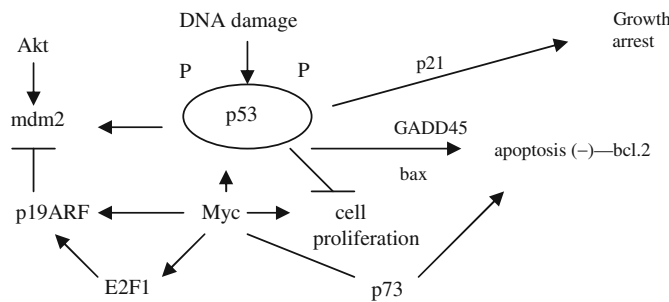


Fig. 17.5 Interactions of c-Myc $\rightarrow$ p53. p53 mediates G1 arrest by inducing genes that inhibit cell cycle progression. The major mediator of G1 arrest is the p21. p53 will repress cdc2, cyclin B and cdc25c. (a) p53 is regulated by its acetylation by Sir, a sirtuin. (b) Murine double minute 2 (*mdm-2*) is an E ligase that leads to proteasomal degradation of p53. (c) Inhibition of mdm-2 by *ARF* activates p53. (d) Furthermore, p19 ARF can downregulate c-myc. (e) Note growth arrest and DNA damage-inducible gene (*GADD45*), which in haemopoietic cells may act to ensure cell survival [23]

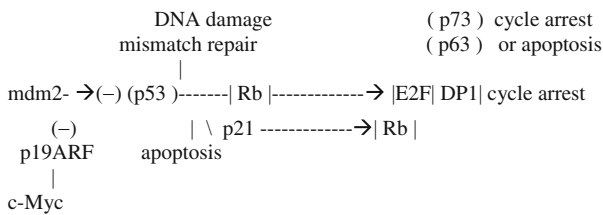


Fig. 17.6 Suppressors p53 and retinoblastoma (*Rb*) in tandem

A second pathway for p53 activation is via the expression of viral oncogenes so that there is transcription of p19^{ARF} gene. That protein sequesters mdm-2 within nucleoli, keeping it away from the p53 [30]. Since viruses produce proteins that will inhibit p53, cells can become cancerous. As an example, consider that Abelson murine leukaemia virus causes transformation of fibroblasts. That entails loss of p53 and of p19 ARF, and there is enhanced c-myc and Stat3 signalling, which explains cell proliferation, and there is enhanced bcl-XL, accounting for cell survival.

17.6 p53 Tumour Suppressor

p53 is a TF that binds to specific DNA sequences, where it induces target genes that mediate tumour suppression. The p53 responsive element consists of two decamers (RRRC A/T A/T GYYY) separated by a spacer of 0–13 bp. p53 mediates cell cycle arrest, DNA repair and apoptosis (Fig. 17.6). p63 and p73 have an additional sterile alpha motif domain (Fig. 17.7).

DNA damage creates a stress signal leading to induction of p21 and inhibition of cyclins D/E and so G1 arrest and to inhibition of cdc25c, CAK, Gadd45 and 14-3-3σ, so that there is inhibition of cdc2/cyclin B and thus G2 arrest. When tumours grow, there must be neovascularization, and that is inhibited by p53 since p53 represses vascular endothelial growth factor (VEGF) expression and promotes thrombospondin 1 (TSP-1). At the stage of metastasis, normal p53 opposes that process since it induces PAI-1 (plasminogen activator inhibitor 1), which stops fibrinolysis, and it induces the serpin maspin, which enhances cell adhesion.

17.7 Control of the Cell Cycle Checkpoints

The DNA tumour viruses that cause tumours in humans or experimental animals encode proteins that inactivate both Rb and p53 tumour suppressor proteins. It is impossible for a cancer to emerge until p53 and Rb have been inactivated. Clearly,

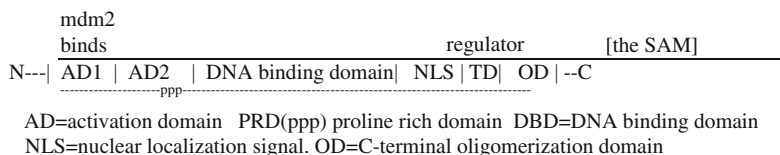


Fig. 17.7 Domains of the p53 protein. The N-terminus responsible for transactivation contains acidic amino acids and many prolines. The C-terminus has three nuclear localization signals (NLS) and is hydrophilic. The central portion is highly hydrophobic. Most mutations in tumours occur here. The sites are modified by phosphorylation, acetylation, sumoylation or neddylation. The SAM of p63/p73 is involved in protein–protein interactions

there has to be inactivation of (a) the sequence p16 (Ink4a)-cyclin D/cdk-pRb-E2F (Fig. 17.2) and (b) of the sequence p19ARF-mdm2-p53, as in Fig. 17.5. One is now sure of the controls over the cell cycle checkpoints (Table 17.2):

- 1. DNA damage acting via ATM/ATR-chk2/chk1-p53-p21^{cip1/waf1} induces cell cycle arrest at the G1.
- 2. The S-phase checkpoint is put into effect (a) by chk2/chk1 inactivation of cdc25A (**Fig. 8.4**) and (b) by the sequence through cohesion protein SMC.
- 3. At the G2 checkpoint, the target is the cyclin B/cdk1 kinase, and that is controlled by (a) the p21 inhibitor, (b) GADD45A and (c) 14-3-3 protein [33].

No doubt other controls will be uncovered. The SNIP1/Brahma-related gene 1 (BRG1) complex regulates other genes associated with the cell cycle. BRG is a component of SWi/SNF(**Fig. 4.12**). Here, there is SNIP1, which interacts with transcriptional coactivators. Reduction of SNIP1 by short interfering RNAs results in reduced cyclin D1, a reduction of cell proliferation, and accumulation of cells in the G1 phase of the cell cycle [34]. Nitric oxide (NO) has an antiproliferative action for it induces p21. NO causes G1 arrest, and it represses the G2/M genes [35].

17.8 The Cell Cycle of T Lymphocytes

At the Go of the T cell cycle, p57^{kip2} promotes the assembly of cyclin D and cdk6, and this leads to hypophosphorylation of Rb, so that there will be sequestration of E2F family members, and transcription is prevented (Fig. 17.8). As the T cell becomes activated in G1, cyclin E/cdk2 are activated so that E2F dissociates from Rb and is ready to promote the S phase. Levels of p57 do not fluctuate with the cell cycle. Yet, p57^{kip2} modulates cyclin E/cdk2 and controls the activity of cyclin A/cdk2 by regulating cyclin A production. In lymphoma cells, p57^{kip2} is lost, leading to inappropriate activity of cdk2 and elevated cyclin A levels, and expansive growth is allowed [36].

p27^{kip1} is important as an inhibitor of T cell proliferation, which is degraded rapidly in proliferating cells. It can act as a scaffold and as a nuclear chaperone.

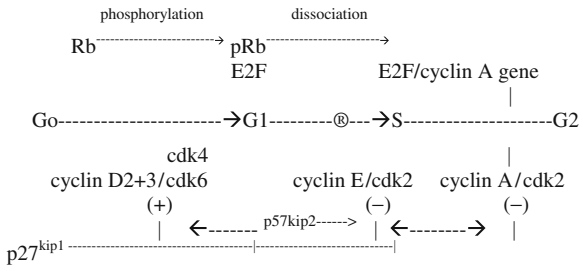


Fig. 17.8 T lymphocyte cycle as controlled by p57-kip2

It actually promotes the early assembly of cyclin D-cdk4/6 complexes [37]. T cell receptor (TCR)/CD28 costimulation is linked to p27 expression by the action of Vav1 [38]. p27 limits clonal expansion. With the onset of cdk2 activity, p27 is phosphorylated and degraded.

How does IL-2 affect T cell proliferation? At the induction of competence in T cells, there is an IL-2-independent expression of cdk4 and cyclin D expression, and thereafter IL-2 stimulates a second phase of cdk4 and cyclin D2 expression [39]. IL-2, acting via PI3K and Akt, also stimulates mTOR and thus protein synthesis. In fact, IL-2 stimulates the kinase aurora B, which works with survivin/mTOR to regulate the G1/S checkpoint [40].

L-Arginine helps cell cycle progression of T cells. When it is absent, T cells are arrested at G0-G1. There is inability to upregulate cdk4 and cyclin D and decreased phosphorylation of protein Rb [41]. Arginase released by cancer cells causes dysfunction of local T lymphocytes.

Prostacyclin inhibits G1-S phase progression of T lymphocytes [42]. It inhibits the expression of cyclin A gene, and it influences the amount of p27 inhibitor.

17.9 Liver Regeneration

The mammalian liver can completely regenerate after damage by toxins, virus-induced hepatitis or resection. There is reentry of terminally differentiated hepatocytes into the cell cycle and proliferation in response to tumour growth factor TGF α , the hepatocyte growth factor (HGF), and cytokines tumour necrosis factor- α (TNF α) and IL-6. Coincidentally, there is activation of TFs like cJun/cFos, c-Myc, NF- κ B, Stat3 and the C/EBP proteins [43a]. GADD45b helps cell survival [43b].

Increased hepatic expression of TF Fox M1B occurs at the G1/S transition of the cell cycle and remains high for the period of proliferation [44]. Fox M1B boosts the cyclins and cdc25B, and it stops inhibitors p21^{cip1} and p27^{kip1}.

17.10 Tumours and the Cell Cycle

In almost all cancers, there is malfunctioning of the retinoblastoma protein (pRb), of cdk4, of cyclin D or of p16^{INK4}. p53 activates pRb. pRb is substrate for the protein phosphatase PP1 α . This PP1 α can override oncogenic signalling, causing cell cycle arrest or apoptosis [45].

As one might expect, aberrations of G1 and S-phase checkpoint controls are often associated with cancers [46] (Table 17.2). The first set of suppressors (indicated by a single asterisk in Table 17.2) are seen in **Fig. 8.4** of checks on DNA strand breaks. Rb, p53 and ARF regulate apoptosis. Other suppressors (*) or oncogenes (**) are part of the cell cycle. The typical tumour suppressor proteins include not only p53 and pRb, but also breast cancer genes (BRCA1/2) (for breast or

ovarian cancers), Kruppel-like factor 6 (KLF6; for prostate cancer), APC (for adenomatous polyposis coli), NF1 (neurofibromatosis), INK4A (melanoma) and WT1 (for Wilms' tumour). One can find tables of tumour suppressor genes [47].

Breast cancer is discussed more thoroughly in Chap. 18. Gene Efp produces a ubiquitin E3 ligase that targets protein 14-3-3 for proteolysis. G2 arrest is then abrogated [48], and cells grow and proliferate. In tumours with a normal pRb-encoding gene, there can be increased levels of cyclins D and E, amplification of the genes for cdk4 and cdk6 and deletion of p16. Often, methylation deletes p15 and p16. Aggressive breast tumours show low concentrations of p27.

Interestingly, aggressive breast cancer cells utilise activated Abl kinases for their cell proliferation, anchorage-independent growth and survival [49].

Many cancers overexpressing cyclin D1 do not show high levels of the canonical E2F target gene product cyclin E. Cyclin D1 levels do not correlate with the presence of Ki67, marker for proliferating cells. This tells us that cyclin D1 can act independently of cdk4 and cdk6. In fact, cyclin D1 can directly affect genes through C/EBP β (Fig. 4.3).

Overexpression of the Rho GTPase family members leads to dysregulation of their normal capacity to form cell–cell contacts and adherens junctions [50]. At the same time, there is upregulation of enzymes that digest the extracellular matrix [51], like collagenases I and IV. Add this to the fact that Rho members promote cell cycle progression by their influence on NF- κ B, cyclin D1 and inhibitors p21 and p27, and one can appreciate how Rho GTPases help induce tumour progression and spread [52].

The ERM protein ezrin connects cell surface receptors like that for HGF (the Met receptor) and CD44 to the Rho GDI (guanine nucleotide dissociation inhibitor) with the result that Rho is activated [53].

17.11 Cell Renewal and Hh Signalling

Transient hedgehog (Hh) and Wnt activities promote the renewal of stem or progenitor cells in adult tissues like skin, blood, gut, prostate, muscle and the nervous system. The skin, the gut mucosa and the lungs are exposed continuously to environmental insults [54]. Unfortunately, evidence suggests that stem cells often give rise to cancers, especially when chronic injury to a tissue means that there is continuous generation of activated stem cells. One third of total cancer deaths are caused by forms of cancer in which the Hh or Wnt pathway activity is implicated [55a]. Even activation of the Hh pathway in the tumor stroma environment is relevant [55b].

The very rare Gorlin's syndrome is associated with basal cell carcinoma, medulloblastoma and rhabdomyosarcoma. With it, there are heterozygous mutations affecting Patch (PTCH), which should act to restrain activation of the seven-transmembrane protein Smo (protooncogene smoothened) (Fig. 17.9). The binding of Hh to Patch liberates Smo, so that there is activation of Gli TFs. In turn, the Gli proteins are regulated by Suppressor of Fused (Su-Fu) protein [54, 55a]. As a

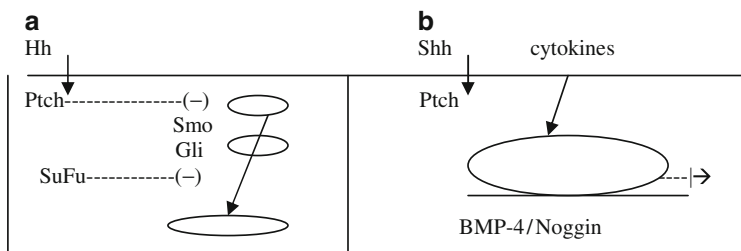


Fig. 17.9 The hedgehog signalling pathway. *Hh* hedgehog, *Ptch* Patch, *Shh* sonic hedgehog, *Smo* Smoothened, *BMP4* bone morphogenetic protein. *Gli* transcription factors (*TFs*) promoting genes like *Noggin* inhibit *BMP4* [46]. In thymocytes, *Gli2* activation of *Hh* reduces T cell receptor (*TCR*) signal strength and alters thymocyte development. In peripheral T cells, *Gli2* attenuates T cell activation and proliferation [63]

general rule, cell surface heparan sulphates regulate various developmental signalling pathways like Wnt and Hh, and likewise TGF β - and fibroblast growth factor-directed pathways [56].

Whereas the Hh family members *Shh* (sonic hedgehog) and *Ihh* (Indian hedgehog) are expressed in normal endodermal tissues, high level activation of Hh targets occurs only in cancer cells. The Hh pathway antagonist cyclopamine can be used to assess cancers in tissues of endodermal origin (gastrointestinal tract, prostate, lungs). Cyclopamine causes regression of basal cell carcinoma of skin [57].

Shh treatment induces the expansion of pluripotent haemopoietic cells in immunodeficient mice [58]. Cytokine-induced proliferation of human haemopoietic cells can be inhibited by antibodies to hedgehog. *Noggin*, the inhibitor of bone morphogenetic protein 4 (*BMP4*) can inhibit *Shh*-induced proliferation in a similar manner to anti-Hh (cf. Fig. 17.9). Although *BMP4* can maintain repopulating stem cell [59], *BMP4* does not induce stem cell expansion as does *Shh*. Hedgehog causes cell growth and proliferation by inducing cyclins D and E [60]. So, Hh is important for repair of epithelia. Hh is implicated also in small cell lung cancer [61] and at multiple stages of pancreatic tumorigenesis [62]. Stroma-derived Hh ligands help survival of B cell malignancies like lymphoma and plasmacytoma [63].

17.12 Wnt Signalling

Wnt is an acronym for wingless genes of the fruitfly and *Int-1* from the mouse *Mus musculus* as a determinant of embryonic development and, by support of mesenchymal stem cells, adult tissue self-renewal. Its actions are less apparent in immunology, although it aids thymocyte development. Continuous Wnt exposure in the absence of *Klotho* antagonist leads to cell senescence [65]. Wnt signals are implicated in various types of cancer. Wnt-1 signalling in mouse mammary tumours is a consequence of mouse mammary tumour virus (MMTV) insertion.

Wnt ligands bind to their frizzled (Fz) receptors or to LRP4/5 [66] (lipoprotein receptor-related proteins 4 and 5). Fz coreceptors use a heparin sulphate coreceptor Dally. To inhibit Wnt signalling, there can be binding of five mammalian sFRPs (frizzled-related proteins). This is the case in normal colon epithelial cells [67]. In unstimulated cells in the absence of signalling from Wnt, β -catenin is complexed (along with APC, axin and GSK3) and so is targeted for proteasomal degradation. However, when Wnt signals by its canonical (β -catenin-dependent) route (Fig. 17.10), β -catenin is released from the degradation complex, and it enters nuclei to regulate gene transcription via association with TCFs (T cell factors) [68, 69]. Wnt signalling stabilizes β -catenin. The intranuclear β -catenin induces genes that stimulate cell proliferation, such as cyclin D1 and Myc. Should PTEN be expressed, GSK3 β is activated, there is degradation of the β -catenin, and cyclin D1 falls. However, mutations of the PTEN suppressor gene are common in the cancers.

Phosphatases help to control the β -catenin signal pathway. Thus, PP2A affects the negative influence of the APC/Axin/GSK3 β , and PP2A directly affects the β -catenin path and the c-Myc that is so induced. Then, the path β -catenin - Myc-cyclin D $\rightarrow$ Rb is affected at the final step by phosphatase PP1A. There is now good evidence that the TGF β and Wnt/ β -catenin signal pathways are closely intertwined in cancers [70]. Breast cancer is considered in Chap. 18. It has been clarified how Wnt signalling triggers the oncogenic conversion of human breast epithelial cells by

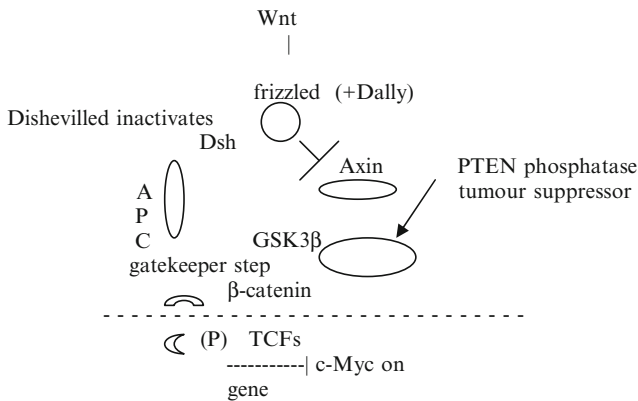


Fig. 17.10 Wnt signalling via nuclear β -catenin (the canonical route). Canonical Wnt signalling leads to activation of β -catenin-ternary complex factor (TCF) complexes. In the absence of Wnt, the scaffold protein Axin and the tumour suppressor adenomatous polyposis coli (APC) phosphorylate β -catenin. This is then recognised by E3 ubiquitin ligase for proteasomal degradation. When Wnt is bound, glycogen synthase kinase 3 (GSK3) is inactivated by phosphorylation. Hence, β -catenin becomes stabilized, and it moves into the nucleus to bind to TCF. C-myc stops cell cycle arrest by p15 INK4B and p21 cip1. Starting at the dishevelled branch point, there is a noncanonical path by which the original Wnt-Frizzled complexes lead via heterotrimer G proteins to release of Ca^{2+} and to activation of *GTPases* and hence cytoskeletal rearrangements. There is also activation of Jun N-terminal kinase (*JNK*) $\rightarrow$ activator protein 1 (*AP-1*) $\rightarrow$ target genes. The non-canonical path does not make use of β -catenin

liaison with Notch [71, 72]. Notch signalling in oncogenesis involves complicated scenarios [73]. Notch can promote cell cycle cdk2 and cyclin D1. The Wnt gradient also controls expression of Ephrin B. In high-grade ovarian neoplasms, ephrin B expression signifies formation of microvessels and a poor prognosis [74].

There is non-canonical Wnt signalling in which expression of LRPS/6 (LDL-receptor-related protein) and β -catenin are not required [75]. So there is a Wnt/ Ca^{2+} signal path [76a] and a Wnt cell polarity signal [76b]. The Wnt/Fz pathway turns out to be implicated in cardiac hypertrophy. In embryogenesis Wnt/ β -catenin signalling has both positive and negative actions on the development of the heart and the haemopoietic system [77].

The extent of Wnt-Fz-induced activation of β -catenin, and hence of TFs TCF-1, determines the extent of T lymphocyte proliferation in foetal thymic organ cultures [78]. Wnt signalling aids DN to DP transition of thymocytes [75]. In peripheral T cells Wnt signalling keeps cells in an undifferentiated state and it helps survival of Treg cells [75]. Wnt signalling, as revealed by Tcf reporter activity and accumulation of nuclear β -catenin occurs in blast cells in chronic myeloid leukaemia (CML) crisis. Wnt signals are involved too in acute lymphoblastic leukaemia. Wnt mutations are only rarely a cause of cancer [79], but the situation is mimicked by activating β -catenin mutations or inactivating APC mutations or mutation of Axin2 (all causing familial adenomatous polyposis, Chap. 18).

Now, there is information on Wnt5a, which is a macrophage effector molecule that triggers inflammation [80]. When there is Toll-like receptor stimulation by pathogens on dendritic cells (DCs) or macrophages, NF- κ B activity leads to Wnt5a formation, and it then acts on Fz-5 receptors to stimulate production of cytokines that sustain inflammation. This actually occurs via a noncanonical β -catenin-independent pathway [81]. Wnt5a upregulates the microbial-induced IL-12 response of antigen-presenting cells [82]. Levels of Wnt5a are high in persons with sepsis. Well surely Wnt-Frizzled signalling will turn out to be involved in various aspects of inflammation [83]. Already we know of ROR receptor tyrosine kinases that act as receptors for Wnts [84].

References

1. Prem Veer Reddy G. Cell cycle: regulatory events in G1-S transition of mammalian cells. *J Cell Biochem* 1994;54:379–386
2. Arellano M, Moreno S. Regulation of CDK/cyclin complexes during the cell cycle. *Int J Biochem Cell Biol* 1997;29:559–573
- 3a. Powell EA, Wells AD. The role of cyclin dependent kinases in T cell development, proliferation and function. *Crit Rev Immunol* 2006;26(3):189–272
- 3b. Hochegger H, Takeda S, Hunt T. Cyclin-dependent kinases and cell-cycle transitions. *Nat Revs Mol Cell Biol* 2008;9:910–915
4. De Virgilio C, Loewith R. The Tor signalling network from yeast to man. *Int J Biochem Cell Biol* 2006;38:1476–1481
5. Ekholm SV, Reed SI. Regulation of G1 cyclin dependent kinases in the mammalian cell cycle. *Curr Opin Cell Biol* 2000;12:676–684

6. Roovers K, Assoian RK. Integrating the MAP kinase signal into the G1 phase cell cycle machinery. *Bioessays* 2000;22:818–826
7. Johnson DG, DeGregori J. Putting the oncogenic and tumor suppressive activities of E2F into Context. *Curr Mol Med* 2006;6:731–738
- 8a. Kaelin WG. Function of the retinoblastoma protein. *Bioessays* 1999;21:950–958
- 8b. Polager S, Ginsberg D. E2F at the crossroads of life and death. *Trend Cell Biol* 2008;18:528–535
9. Liu F, Rothblum-Oviatt C, Ryan CE et al. Over production of human Myt1 kinase induces a G2 cell cycle delay by interfering with intracellular trafficking of cdc2-cyclin B1 complexes. *Mol Cell Biol* 1999;19:5113–5123
10. Nilsson I, Hoffmann I. Cell cycle regulation by the cdc25 phosphatase family. *Progr Cell Cycle Res* 2004;4:107–114
11. Roussel MF. The effectors of signal transduction and G1 progression. *Adv Cancer Res* 1998;72:1–15
- 12a. Cox CS, Lane DP. Tumor suppressors, kinases and clamps: how p53 regulates cell cycle in response to DNA damage. *Bioessays* 1995;17:501–507
- 12b. Yuan K, Chung L, Siegal GP, Zasyzaform M. CaM kinase II controls the growth of human osteosarcoma by regulating cell cycle progression. *Lab Invest* 2007;87:938–950
13. Jiang Y, Proper F, Verfaillie CM. Opposite effects of engagement of integrins and stimulation of cytokine receptors on cell cycle progression of normal human hematopoietic progenitors. *Blood* 2000;95:846–854
14. Alexandrov M, Moses H. TGFβ and cell cycle regulation. *Cancer Res* 1995;55:1452–1457
15. Hcevar BA, Howe PA. Mechanisms of TGFβ induced cell cycle arrest. *Miner Electrolyte Metab* 1998;24:131–135
16. Wang J, Sengina N, Ko TC, Gang J, Brattain MG. Autocrine and exogenous TGFβ control cell cycle inhibition through pathways with different sensitivity. *J Biol Chem* 2004;279:40237–40244
17. Samento LM, Huang H, Limon A et al. Notch1 modulates tuning of G1-S progression by inducing SKP2 transcription. *J Exp Med* 2005;202:157–168
18. von Boehmer H. Notch in lymphopoiesis and T cell polarization. *Nat Immunol* 2005;7:641–642
19. Pugacheua EN, Roegiers F, Golemis EA. Interdependence of cell attachment and cell cycle signaling. *Curr Opin Cell Biol* 2006;18:507–515
20. Mammoto A, Huang S, Moore K et al. Role of RhoA, mDia, and Rock in cell shape-dependent control of the Skp2-p27kip1 pathway and the G1/S transition. *J Biol Chem* 2004;279:26323–26330
21. Olson MF, Ashworth A, Hall A. An essential role for Rho, Rac and cdc42 GTPases in cell cycle progression through G1. *Science* 1995;269:1270–1272
- 22a. Adhikary S, Eilers M. Transcriptional regulation and transformation by Myc proteins. *Nat Rev Mol Cell Biol* 2005;6:635–645
- 22b. Cove-Smith A, Hendry BM. The regulation of mesangial cell proliferation. *Nephron Exp Nephrol* 2008;108:e74–e79
23. Gupta M, Gupta SK, Hoffman B, Liebermann DA. Gadd45a/45b protect hematopoietic cells from UV-induced apoptosis via distinct signal pathways. *J Biol Chem* 2006;281:17552–17558
24. Laiho M, Latonen L. Cell cycle control, DNA damage checkpoints and cancer. *Ann Med* 2003;35:391–397
25. Hermeking H, Benzinger A. 14–3–3 proteins in cell cycle regulation. *Semin Cancer Biol* 2006;16:183–192
26. Margolis SS, Perry JA, Forester CM et al. Role for PP2A phosphatase in regulation of 14-3-3 release from cdc25 to control mitosis. *Cell* 2006;127:759–774
27. Karlsson-Rosenthal C, Miller JBA. cdc25: mechanisms of check point inhibition and recovery. *Trends Cell Biol* 2006;16:285–292

28. Yamamoto M, Yoshida M, Ono K, Fujita T et al. Effect of tumor suppressor on cell cycle regulatory genes. *Exp Cell Res* 1994;210:94–100
29. Joerger AC, Fersht AR. Structural biology of the tumor suppressor p53. *Ann Rev Biochem* 2008;77:557–582
30. Ashcroft M, Vousden KH. Regulation of p53 stability. *Oncogene* 1999;18:7637–7642
- 31a. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature* 2000;408:307–311
- 31b. Sitko JC, Yeh B, Kim M et al. SOCS3 regulates p21 expression and cell cycle arrest in response to DNA damage. *Cell Signal* 2008;20:2221–2230
32. Wu L, Timmers C, Malti B. The E2F 1–3 transcription factors are essential for cellular proliferation. *Nature* 2001;414:457–462
33. Taylor WR, Stark GR. Regulation of the G2/M transition by p53. *Oncogene* 2001;20:1803–1815
34. Roche KC, Wiechens N, Owen-Hughes T, Perkins ND. The FHA domain protein SNIP1 is a regulator of the cell cycle and cyclin D1 expression. *Oncogene* 2004;33:8185–8195
35. Cui X, Zhang J, Ma P, Myers DE et al. cGMP-independent NO signaling and regulation of the cell cycle. *BMC Genomics* 2005 Nov3;6:151
36. Li G, Doeminoco J, Lucas JJ, Gelfand EW. Identification of multiple cell cycle regulatory functions of p57kip2 in human T lymphocytes. *J Immunol* 2004;173:2382–2391
37. Rowell EA, Walsh MC, Wells AD. Opposing roles for the cyclin-dependent kinase inhibitor p27^{kip1} in the control of CD4 T cell proliferation and effector function. *J Immunol* 2005;174:3359–3368
38. Charvet C, Canonigo AJ, Becart S et al. Vav1 promotes T cell cycle progression by linking TCR/CD28 costimulation to Foxo1 and p27 expression. *J Immunol* 2006;177:5024–5031
39. Modiano JF, Domenico J, Szepesi A, Lucas JJ, Gelfand EW. Differential requirements for interleukin 2 distinguish the expression and activity of the cyclin dependent kinases cdk4 and cdk2 in human T cells. *J Biol Chem* 1994;269:32972–32978
40. Song J, Salek-Ardakani S, So T, Croft M. The kinases aurora B and mTOR regulate the G1-S cell cycle progression of T lymphocytes. *Nat Immunol* 2007;8:64–73
41. Rodriguez PC, Quiceno DG, Ochoa AC. L-Arginine availability regulates T lymphocyte cell cycle progression. *Blood* 2007;109:1568–1573
42. Kothapalli D, Flores-Stewart SA, Assoian RK. Anti-mitogenic effects of prostacyclin on the G1 phase cyclin kinases. *Prostaglandins Other Lipids Mediat* 2005;78:3–13
- 43a. Buck M, Chojlier M. Signal transduction in the liver: C/EBPbeta modulates cell proliferation and survival. *Hepatology* 2003;37:731–738
- 43b. Papa S, Zazzummi F, Fu X-Y et al. GADD45beta promotes hepatocyte survival during liver regeneration in mice. *J Clin Invest* 2008;118:1911–23
44. Costa RH, Kalinichenko VV, Holterman A-XK, Wang X. Transcription factors in liver development, differentiation and regeneration. *Hepatology* 2003;38:1331–1347
45. Liu CWY, Wang R-H, Berndt N. Protein phosphatase 1 α activity prevents onco-genic transformation. *Mol Carcinog* 2006;45:648–656
46. Bartek J, Lukas J. Mammalian G1 and S-phase checkpoints in response to DNA damage. *Curr Opin Cell Biol* 2001;13:738–747
47. Macdonald F, Ford CHJ, Casson AG. *Molecular Biology of Cancer*. Chap. 3, Tumor suppressor genes. Taylor & Francis, Abingdon, U.K., 2004.
48. Urano T, Saito T, Tsukui T et al. Efp targets 14-3-3 for proteolysis and promotes breast tumour growth. *Nature* 2002;417,871–875
49. Srinivasan D, Sims JT, Plattner R. Aggressive breast cancer cells are dependent on activated Abl kinases for proliferation and survival. *Oncogene* 2008;27:1095–1105
50. Kaibuchi K, Kuroda S, Fukata M, Nakagawa M. Regulation of cadherin mediated cell-cell adhesion by Rho GTPases. *Curr Opin Cell Biol* 1999;11:591–596
51. Zhuge Y, Xu J. Rac.1 mediates type I collagen dependent MMP2 activation: role in cell invasion across collagen barrier. *J Biol Chem* 2001;276:16248–16246
52. Keely PJ. Rho GTPases as markers for tumour progression. *Lancet* 2001;358:1744–1745
53. Hunter KW. Ezrin in tumor metastasis. *Trends Mol Med* 2004;10:201–204

54. Beachy PA, Karhadkar SS, Berman DM. Tissue repair and stem cell renewal in carcinogenesis. *Nature* 2004;432:324–331
- 55a. Lum L, Beachy PA. The Hedgehog response network: sensors, switches and routers. *Science* 2004;304:1755–1759
- 55b. Yaucho RL, Gould SE, Scales SJ et al. A paracrine requirement for hedgehog signalling in cancer. *Nature* 2008;455:406–410
56. Lin X. Functions of heparin sulphate proteoglycans in cell signaling during development. *Development* 2004;131:6009–6021
57. Tas S, Avci O. Induction of the differentiation and apoptosis of tumour cells in vivo with efficiency and selectivity. *Eur J Dermatol* 2004;14:96–102
58. Bhardwaj G, Murdoch B, Wu D, Baker DP et al. Sonic hedgehog induces the proliferation of primitive human hematopoietic cells via BMP regulation. *Nat Immun* 2001;2:172–180
59. Bhatia M, Bonnet D, Wu D et al. BMPs regulate the developmental program of human hematopoietic stem cells. *J Exp Med* 1999;189:1139–1147
60. Duran-Scheel N, Weng L, Xin J, Du W. Hedgehog regulates cell growth and proliferation by inducing cyclins. *Nature* 2002;417:299–303
61. Watkins DN, Berman DM, Burkholder SG et al. Hedgehog signalling within airway epithelial progenitors and in small cell lung cancer. *Nature* 2003;422:313–322
62. Morton JP, Mongeau ME, Klimstra DS et al. Sonic hedgehog acts as multiple stages during pancreatic tumorigenesis. *Proc Natl Acad Sci U S A* 2007;104:5103–5108
63. Dierks C, Grbic J, Zirlik K et al. Essential role of stromally induced hedgehog signaling in B cell malignancies. *Nat Med* 2007;13:944–951
64. Rowbotto NJ, Hager-Theorides AL, Cebecauer M et al. Activation of Hedgehog signaling pathway in T lineage cells inhibits TCR repertoire selection in the thymus and peripheral T cell activation. *Blood* 2007;109:3757–3766
65. Liu H, Ferguson MM, Castilho R et al. Augmented Wnt signaling in a mammalian model of accelerated ageing. *Science* 2007;317:803–806
66. van Amerongen R, Berns A. Knock-out mouse models to study Wnt signal transduction. *Trends Genet* 2006;22:678–689
67. Baylin SB, Ohm JE. Epigenetic silencing in cancer. *Nat Rev Cancer* 2006;6:107–116
68. Wodarz A, Nusse R. Mechanisms of Wnt signaling in development. *Annu Rev Cell Dev Biol* 1998;14:59–88
69. Taipale J, Beachy PA. The Hedgehog and Wnt signalling pathways in cancer. *Nature* 2001;411:349–354
70. Labbe E, Lock L, Letamendia A et al. Transcriptional cooperation between the TGF-beta and Wnt pathways in mammary and intestinal tumorigenesis. *Cancer Res* 2007;67:75–84
71. Miles RH, van Es JH, Clevers H. Caught up in a Wnt storm: Wnt signaling in cancer. *Biochem Biophys Acta* 2003;1653:1–24
72. Ayyanan A, Civeni G, Ciarhoni L et al. Increased Wnt signaling triggers oncogenic conversion of human breast epithelial cells by a Notch dependent mechanism. *Proc Natl Acad Sci U S A* 2006;103:3799–3804
73. Bray SJ. Notch signalling. *Nat Rev Mol Cell Biol* 2006;7:678–689
74. Castellui J, Garcia A, de la Torre J et al. Ephrin B expression in ovarian neoplasms correlates with tumor differentiation and angiogenesis. *Human Pathol* 2006;37:883–889
75. Staal FJT, Luis TC, Tiemessen MM. WNT signalling in the immune system: WNT is spreading its wings. *Nat Rev Immunol* 2008;8:581–593
- 76a. Sharpe C, Lawrence N, Arias AM. Wnt signaling. *Bioessays* 2001;23:311–318
- 76b. Moran RT, Shoh K. Wnt and polarity. *Nature* 2002;417:239–240
77. Naito AT, Shiokima I, Kazawa H et al. Developmental stage-specific biphasic roles of Wnt/ β -catenin signaling in cardiomyogenesis and hematopoiesis. *Proc Natl Acad Sci U S A* 2006;103:19812–19817
78. Varas A, Hager-Theodorides AL, Sacedon R et al. The role of morphogens in T cell development. *Trends Immunol* 2003;24:197–206

79. Leong KG, Karson A. Recent insights into the role of Notch signaling in tumorigenesis. *Blood* 2006;107:2223–2231
80. George SJ. Wnt pathway; role in regulation of inflammation. *Arterioscler Thromb Vasc Biol* 2008;28:400–281
81. Gordon MD, Nusse R. Wnt signaling: multiple pathways, multiple receptors and multiple transcription factors. *J Biol Chem* 2006;281:22429–22433
82. Blumenthal A, Ehlers S, Lauber J et al. Wingless homolog Wnt5a and receptor Frizzled-5 regulate inflammatory responses of human mononuclear cells induced by microbial stimulation. *Blood* 108:965–973
83. Sen M, Ghosh G. Transcriptional outcome of Wnt-Frizzled signal transduction in inflammation: evolving concepts. *J Immunol* 2008;181:4441–4445
84. Green JL, Kuntz SG, Sternberg PW. ROR receptor tyrosine kinases: orphans no more. *Trends Cell Biol* 2008;18:536–544

Chapter 18

Cancer

Abstract This discussion of cancer considers the features of the cells and incitement by oncogenes. Cancer arises as a multistep process in which we now consider the role of transcription factors and cyclins and the implications of loss of cadherins or the activity of Cox-2 enzyme. In turn, breast cancer, prostate cancer, ovarian and renal cancers and finally colorectal tumours are scrutinised for clues regarding the operative mechanisms. Much current research is devoted to understanding how the cells spread (metastasis) and which genes determine such properties.

18.1 Cancer Cells

Cancer cells are characterised by their unrestrained growth. In theory, cancer might arise (a) as a result of autocrine growth factor production; (b) by mutation of growth factor receptors, rendering them hypersensitive; (c) because postreceptor intracellular signal pathways are enhanced; (d) on account of reduced sensitivity to cell proliferation inhibitors like transforming growth factor- β 1 (TGF β 1). Hanahan and Weinberg [1] summarised the six general features of cancer cells as (a) self-sufficiency in growth signals, (b) lack of sensitivity to antigrowth signals, (c) ability to avoid apoptosis, (d) limitless replicative potential, (e) sustained angiogenesis and (f) potential for tissue invasion and metastasis (spread).

The *oncogenes* are those genes that cause cell transformation in vitro. *Protooncogenes*, which are derived from retroviruses, code for vital proteins that determine cell growth. They have names like Myc for myelocytomatosis virus or Erb for erythroblastosis virus. Overexpression or mutation of a protooncogene turns it into a hyperactive oncogene (Table 18.1).

A *neoplastic* cell is one that has clonally expanded as a result of somatic mutations.

Cancer of a cell arises in a multistep process involving a series of alterations to the genome at initiation/promotion/progression (Fig. 18.1), which cause activation of oncogenes and inactivation of tumour suppressor genes at any step in the signal transduction processes that lead to cell cycle cyclin-dependent protein kinase

Table 18.1 Forms of oncogene

1. Genes coding for growth factors: sis gene for platelet-derived growth factor (<i>PDGF</i>), Int2 for fibroblast growth factor (<i>FGF</i>)
2. Genes coding for growth factors receptors: Erythroblastosis virus B (<i>erbB</i>) for a truncated epidermal growth factor (<i>EGF</i>) receptor neu (Erb2) for an EGF-like receptor trk for nerve growth factor receptor
3. Genes for guanosine triphosphatases (<i>GTPases</i>): Ha-ras, N-ras, Ki-ras Genes for nonreceptor tyrosine kinases: src, lck, abl, etc. Genes for serine-threonine kinases: raf, mos
4. Genes for nuclear proteins and transcription factors: jun, fos, myc

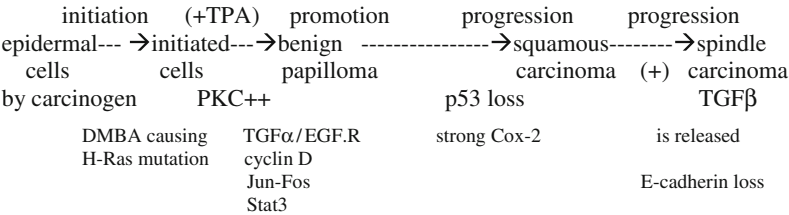


Fig. 18.1 Carcinogenesis in mouse skin

(CDK)-cyclin complexes. Each step might be implicated in induction of dysregulated cell growth and later in preparation for metastasis. There are about 150 cancer gene products, and they are proteins in the signalling pathways [2]. Accumulation of defective genes in cells is enhanced in a subset of tumours that have defects in DNA repair enzymes. So, as an extra feature, the hereditary predisposition to breast and colon cancers results from mutation of caretaker genes. These include mismatch repair (MMR), nucleotide excision repair (NER) and base-excision repair (BER) genes that correct the subtle double-stranded DNA faults that arise during DNA replication or from exposure to mutagens. We are all aware that cancer is more common in older persons. The activity of ATM kinase (**Fig. 8.4**) declines with age and is accompanied by decline in p53 responses to internal cell stress [3].

Methylation of genes was mentioned in Chapter 4 An important aspect of methylation is that it will inactivate the tumour suppressor genes [4]. Detection of hypermethylated CpG islands is developing. The problem is that it is critical to know the position of promoter methylation for every candidate gene in the context of progression of a given tumour type [5].

Ras GTPase is shown in **Fig. 5.1**. Activation of the Ras pathway is frequent in cancers even in the absence of ras mutations. There are Ras G proteins that have a defect in their intrinsic GTPase activity, so that once bound to GTP, they are unable to return to their inactive state, and the cell continues to receive an on signal. Mutant Ras genes occur in at least 30% of human tumours [6a, 6b]. Ras missense mutations with amino acid substitutions at positions 12, 13 or 61 impair the intrinsic

GTPase activity and confer resistance to Ras GTPase-activating proteins (GAPs). KRas mutations occur in pancreatic, colorectal, lung and cervical cancers. KRas or NRas occur in myeloid malignancies. There are mouse models of KRas initiated carcinogenesis [6c]. NRas predominates in melanoma and in bladder cancer. Mutant Ha-Ras creates an invasive phenotype that is accompanied by upregulation of EGF. Rs [7a]. Ras-like (Ral) proteins are effectors of Ras and they support malignancy [7b].

GAPs can also be involved. A GAP is mutated in basal cell carcinoma of the skin [8]. Basal cell cancer is more complicated than that. There is upregulation of the Forkhead protein FoxM1 and so upregulation of Gli-1 on the sonic hedgehog pathway (**Fig. 17.9**. In Chapter 4, reference was made to inactivation of genes critical for cancer by hypermethylation of CpG-rich promoter regions. Recently, 28 genes have been identified for Ras-mediated “epigenetic silencing” of the proapoptotic Fas gene. One of these is DNA methyltransferase 1 (DNMT1). Such cells will be freed of liability to apoptosis [9].

Discussion of oncogene activation is beyond the scope of this text. Often, it is created by viruses incorporated into the host cell DNA. Oncogenes can be destabilized by deletions or by point mutations, by chromosomal rearrangement (translocation) or by the process of gene amplification [10]. As has been emphasised, carcinogenesis is a multistep mutational process (**Fig. 18.1**). The minimum number of genetic events needed to produce a cancer cell has been examined [11]. There needs to be (a) maintenance of the length of telomeres by expression of the telomerase catalytic unit hTERT; (b) provision of a viral oncoprotein like SV40 T antigen, which inhibits the tumour suppressor genes p53 and Rb (retinoblastoma); and (c) an activated Ras gene. One can add that Myc cooperates with oncogenes that function in the Ras pathway to cause cellular transformation [12]. Ras itself activates p110 α PI3 kinase (PI3K) for oncogenesis [13]. Myc connects with cdc25A and cdc25B (cell division cycle 25) phosphatases (cf. **Fig. 17.2**. Both cdc25A and cdc25B are overexpressed in breast and colorectal cancers. Cdc25A is overexpressed in melanoma and cdc25B in prostate cancers and gliomas.

The normal ageing process leads to a reduction of the length of telomeres [14]. Telomeres at the ends of chromosomes consist of multiple tandem repeats of six bases (TTAGGG). At each replication cycle, several of the telomere repeats are lost. When tumour cells lose growth control and lose “density-dependent inhibition of growth”, proliferation is enhanced, and telomeres shorten. Yet, at a critical length, telomerase is reactivated [15], stabilising the telomeric repeats, and the cells become immortal [16, 17]. The activity of telomerase in tumours has diagnostic and prognostic value. If normal cells are engineered to overexpress Ras (or Mos or Cdc6), paradoxically they become senescent [18]. Simultaneously, there is a DNA damage response (DDR), which we know causes cell cycle arrest via ATM/chk2/p53 (**Fig. 8.4**). Obviously, the senescence response helps to prevent tumours, but it also causes tissue ageing. High-level Ras activation causes senescence because it leads via p53 to the inhibitor of cdk4a (INK4a)-ARF checkpoint [19]. Yet, cells with Ras activation can avoid that if protein kinase C δ (PKC δ) is activated concurrently [20].

Chronic inflammation is associated with emergence of malignancy (R. Virchow 1863). Many cancers are associated with activation of nuclear factor kappa B (NF- κ B) [21]. Inflammation seems to be a tumour promoter: the proinflammatory cytokines such as interleukin 1 (IL-1), tumour necrosis factor- α (TNF α) and IL-6 that are produced by cells in a tumour stroma help cancer cell growth [22]. Deletion of NF- κ B from hepatocytes prevents their transformation. NF- κ B will help carcinogenesis by preventing death of cells [23]. Yet, chronic inflammation is also associated with the generation of local reactive oxygen species (ROS). In the context of mitogenic signalling, ROS (determined by p53 [24]) often lead to senescence and thus loss of cells [25]. This is adjunctive to the usual metabolism of malignant cells, which relies on anaerobic glycolysis rather than oxidative phosphorylation [26, 27]. Macrophage inhibitory factor (MIF) is a proinflammatory cytokine of chronic inflammation that induces hypoxia-inducing factor 1 α (HIF-1 α), which delays premature senescence. In addition, MIF bypasses p53 and regulates tumour suppressor genes.

The cell cycle and its controls were considered in Chapter 17. Deregulation of the cyclin D1-cdk-CDK inhibitor (CKI)-Rb system is involved in tumourigenesis. The suppressors of the D cyclins like p53 and pRb Section (Fig. 17.1) are usually mutated. Thus, phenotypic alterations in the Rb path were recorded in oral cancers [28]. In Section **Fig. 8.4**, one is reminded that the normal p53 suppressor stimulates Bax to cause apoptosis of cells that have altered DNA, and it stimulates p21^{waf} so that the cell cycle is arrested. Among the CKIs, the p16 stands out as an important suppressor Section (Table 17.1) Cdk4 mutations that stop interaction with p16 are found in cancers like melanoma. Cdk4 is overexpressed in gliomas and sarcomas. Cyclin E is highly expressed in various cancers [29]. Overexpression of cyclin E with A results in anchorage-independent growth of cells in agar suspension. There is an oncogene PRAD1 for parathyroid adenomatosis. PRAD1 product turns out to be cyclin D1.

We realize now that the p53 network ((**Fig. 8.4**), which acts as a sensor for many cancer-associated stress signals, engenders microRNA (miRNA) miR-34, and this causes posttranscriptional gene silencing, so that Bcl2 is inhibited and there is cellular apoptosis. It inhibits cell cdk4/cdk6, encouraging cellular senescence [30a]. If cell senescence is thwarted, high grade malignancy can result [30b].

In growing cells PI3K stimulates Akt, which accounts for antiapoptosis. In cancers, enhanced glycolysis (Warburg effect) and low mitochondrial respiration enhance PI3K/Akt activation. Akt expression is demonstrable in breast, ovarian and pancreatic cancers. Akt sustains growth through mTOR (**Fig. 11.6**) [31]. The regulatory unit of PI3K is able to interact directly with oncogenes. In cancers, there are activating mutations in specific locations in the p110 α catalytic unit of PI3K [32] and additional Akt mutations. Thus, in glioblastomas, receptor tyrosine kinases of PDGFR and EGFR are amplified so that they activate PI3K. PI3K should work as an antiapoptotic effector in the proliferative response of epithelial cells to mutant Ras [33]. Increased PI3K activity is implicated in the generation of lymphoma [34]. Hence, PI3K antagonists are being used to combat various tumours [35].

In normal cells, PTEN, which is PI(3,4,5)P3 phosphatase ((**Fig. 2.6**), down-regulates PI3K signalling. PTEN supports the p27^{kip1} cell cycle inhibitor. However,

PTEN suppressor gene at chromosome 10q23 is often deleted in cancers [36]. Epithelial cell malignancy stems from loss of polarity, uncontrolled cell proliferation and resistance to apoptosis. There is an inhibitor of apoptosis (IAP) called *survivin* that is determined by a gene on chromosome 17q25 [37]. When it is promoted by insulin-like growth factor 1 (IGF1)-mTOR, survivin inhibits the activity of caspase 9. Survivin is not found in normal tissues. It appears at an early stage of cancer [38]. Cells of the lymphomas are protected by antiapoptotic oncogene Bcl-2 [39]. The glycoprotein *clusterin* in cancers inhibits apoptosis of the cells by interfering with Bax activation. Clusterin is overexpressed in breast, prostate and squamous cell cancers. PAK, p21-activated kinase, is upregulated in many cancers [40]. In normal cells, one recognises PAK to be associated with cytoskeletal dynamics and cell motility. In breast cancers, heregulin stimulates relocalisation of PAK to the leading edge of motile cells.

Malignant cells can hijack the functioning of G protein-coupled receptors (GPCRs) [41] to proliferate autonomously, invade tissues and disseminate and increase their nutrient and oxygen supply, often by activating HIF to switch to lactic acid formation [27]. Aberrant overexpression of GPCRs and their autocrine and paracrine activation by mediators released by tumour or stromal cells is a strategy at the head of various signal networks used by tumour cells. Since GPCRs are the target of inflammatory mediators, this suggests a link between chronic inflammation and cancers [22].

18.2 Multistage Carcinogenesis in Skin

The phases of (squamous cell) tumourigenesis are initiation, promotion and progression. At *initiation*, the DNA of cells is mutated by chemical or physical carcinogens, leading to activation of oncogenes or inactivation of tumour suppressor genes. Then, at tumour *promotion* there is clonal expansion of the initiated cells and reduced cell death (Fig. 18.1). There is strong cyclooxygenase 2 expression in keratinocytes and in infiltrating inflammatory cells. *Progression* involves invasion and metastasis. Additional mutations occur at this stage. In the meantime, Cox-2 supplies prostaglandin E2 (PGE2), and by acting on EP2 prostanoid receptors of squamous cancer, there is EGFR transactivation that helps the growth momentum [42]. Intracellular NO also helps [42].

In mice, the sequential application of a skin carcinogen like 7,12-dimethylbenzanthracene (DMBA) followed by repeated application of the promoter TPA (phorbol ester, 12-O-tetra-decanoyl-phorbol-13-acetate) leads to formation of a benign papilloma that will probably progress to cancer. The DMBA causes damage to DNA, resulting in mutation of H-ras oncogene. The promotion elevates PKC activity and increases expression of TGF α and of c-jun and c-fos.

TGF α acts on the EGFR. Progression occurs because of a high level of genetic instability, arising from chromosome abnormalities. ie p53 suppressor is lost, and gene expression of H-ras and cyclin D1 is elevated. At a later stage, the squamous cell cancer may change to a spindle cell cancer, revealed as altered epithelial

Table 18.2 Transition of a naevus to a melanoma

Molecular biology	BRaf mutation	PTEN loss	Increased	Loss of cadherins	Melastin 1 gene
		CDKN2A loss	Cyclin D Cdk4	α V β 3- integrin MMP2 Survivin	
Benign naevus	+	0	0	0	0
Dysplastic naevus	+	+	0	0	0
Radial growth	+	+	+	0	0
Melanoma	+	+	+	+	0
Metastatic melanoma	+	+	+	+	+ $\rightarrow$ 0

MMP matrix metalloproteinase, *PTEN* phosphatase

differentiation markers like E-cadherin. This ultimate change is explained by the action of TGF β . Skin cancers overexpress TGF β 1, and the result is epithelial-to-mesenchymal transition (EMT), explaining formation of spindle cells (Fig. 18.1).

The dreaded melanoma comes in various forms and, when on mucosal surfaces, does not arise from UV light exposure. In those cases without sun-induced damage, there are N-Ras mutations enhancing c-Myc or mutations of B-Raf on the path to MAPK activation [6b, 6c]. The B-Raf $\rightarrow$ MAPK signal pathway is essential for cancer immune evasion [43a]. Accompanying loss of PTEN is supportive the more so when there is active Notch signalling [43b]. Such cases can respond to the Raf kinase inhibitor sorafenib. In most melanomas, there is enhanced expression of downstream cyclin D1 or of CDK4 on the cell proliferation pathway [44]. Some 25–40% of members of melanoma-prone families have mutations in the cdk inhibitor CDKN2A [45]. The features of the development of a naevus into a melanoma, akin to the sequence in Fig. 18.1, are summarised in Table 18.2.

18.3 Breast Cancer

To illustrate aspects of carcinogenesis, consideration is now given to breast, prostate, ovarian, renal and then colon cancers. In breast and colon cancers, there is a strong genetic influence. There are different forms of breast cancer, and their aetiologies differ (Fig. 18.2). For example, lobular breast carcinoma is associated with cell proliferation driven by fibroblast growth factor receptor (FGFR). Estrogen-driven proliferation of the breast duct epithelium begins at puberty and continues for much of adult life. Unfortunately, estrogen and its metabolites are genotoxic [46]. Other hormones like progesterone, IGF-1, growth hormone and leptin also contribute to ductal elongation at puberty, and prolactin determines side-branching and alveologenesis in pregnancy. The wild-type breast cancer gene BCRA1 suppresses estrogen receptor (ER)-induced transcription of epithelial proliferation in the breast [47], but it aids progesterone-induced breast development [48]. Progestins act directly on the

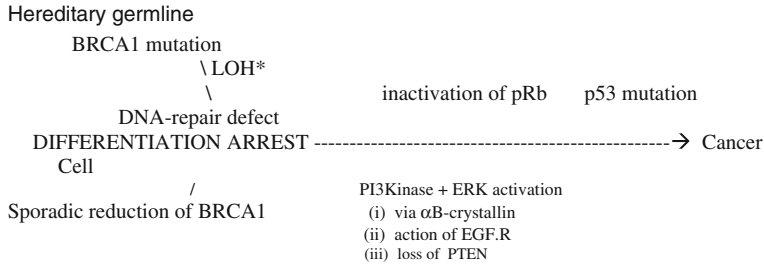


Fig. 18.2 Possible pathogenesis of basal cell breast cancer. Breast cancer gene BRCA1 is inactivated by loss of heterozygosity (*LOH*) of the BRCA1 locus. Molecular chaperone α B-crystallin induces PI3 kinase (*PI3K*) $\rightarrow$ Akt and MEK $\rightarrow$ *ERK* activation

mammary gland to induce ductal side-branching and alveologensis. Progestins increase breast cancer in postmenopausal women. Progesterone receptor expression corresponds to that of fatty acid synthase [49].

BRCA1 is normally required for the response to DNA damage (**Fig. 8.4**). Double-stranded DNA (dsDNA) damage activates BRCA1, so that there is expression of growth arrest and GADD45 and $p21^{cip1}$, which mediate cell cycle arrest and apoptosis. BRCA1 also plays a role in mitotic spindle assembly [50], and it is involved in gene transcription and mammary gland development [51]. Of overall risk of breast cancer, 15–20% is attributable to mutations of the caretaker genes BRCA1 and BRCA2, but they are rare in the general population [52, 53]. Inheritance of one mutated BRCA1 in the germline will be followed by inactivation of the second allele within the somatic cells of the breast. A breast stem cell that has lost both alleles will produce malignant progeny when there are additional gene alterations [54, 55]. Actually, BRCA1/2 cancers differ in morphology and have different gene expression profiles [56].

There are five breast cancer subtypes: (a) basal-like, which are BRCA1 related, (b) ERB-B2 (i.e. heregulin positive (Her2⁺)/ER⁻) ductal cancers, (c) normal breast-like, (d) luminal A subtype and (e) luminal B subtype. In both mice and humans, inactivation of the tumour suppressor gene PTEN leads to basal-like mammary tumours [57]. Basal-like tumours have the worst prognosis, as in African women, and luminal A type the best prognosis. Often, there are also p53 gene mutations, or compromised HoxA5 can limit expression of the wild-type p53. Curiously, TP53 mutations tend to occur in stromal cells, and they are associated with lymph node metastases in sporadic (nonhereditary) breast cancer [58]. Overexpression of Cox-2 in mammary glands under the control of murine mammary tumour virus promoter will create gland hyperplasia, dysplasia and ultimately metastatic cancer [59].

The mammary epithelial stem cells are located in the ducts. In culture, one can find bipotential progenitor cells that form luminal epithelial and myoepithelial cells [60]. Those breast cancers that arise from basal myoepithelial cells are ER⁻, PR⁻ and HER2⁻, and they are similar to hereditary BRCA1 tumours [61]. The progenitor

cells express high levels of IL-6 [62]. Of course, 80% of newly diagnosed breast cancers are ER and PR (progesterone receptor) positive. Yet, actually in the normal mammary gland only 7% of the epithelial cell population are receptor positive. This is because R⁺ cells are held in a nonproliferative state by strongly inhibitory molecules like TGF β or the p27 CKI.

Maintenance of luminal epithelial cell differentiation in normal breast requires the zinc finger TF called GATA3. Methylation of FoxA1 promoter is a feature of breast cancer, and it may compromise normal cell proliferation. Deregulation of Fox proteins is associated with many malignancies, such as deregulation of Foxo3A (Forkhead TF 3A), which generally confers resistance to oxidative stress and protects cells from apoptosis [63]. Those tumours that arise from luminal epithelial cells bear ERs: They are ER⁺ and PR⁺, and they express luminal cytokeratin 8. ER⁻ tumours are poorly differentiated. Estrogens shift dormant epithelial cells from Go into the G1/S phase of the cell cycle. Estrogen controls several key G1-phase cell cycle regulators like cyclin D1, c-Myc, cdk2 and 4, CDKIs and cdc25A. Estrogen metabolites like 16-hydroxyoestrone are mutagens that cause aberrant gene expression and chromosome anomalies. In vitro, 17 β -estradiol has been shown to transform the cell line MCF-10F to anchorage-independent growth and invasiveness, accompanied by chromosomal deletions [64]. Estrogen metabolites generate free radicals capable of damaging DNA [65]. They can also bind to DNA and cause depurination. There are mechanisms to repair such damage, but they are error prone. Incidentally, Muc-1 oncoprotein has been shown to stabilize and activate the ER α , and it protects against apoptosis by offsetting cell oxidative stress [66].

The PR has 14 phosphorylation sites and is susceptible to subphysiological levels of progestins. Thus, PR ser294 is a site for regulation by multiple kinases. Cytoplasmic and nuclear PRs co-opt EGFR signalling to induce breast cancer cell proliferation [67].

In early breast cancer, TGF α , akin to EGF, is relevant, as it is in skin cancers. Such growth factors activate telomerase, preserving the length of the telomeres [68]. TGF α acts on EGFRs induced by oncogene c-erb. Prolactin potentiates TGF α induction of mammary neoplasia in mice [69]. Also, H-Ras augments EGFRs on mammary epithelium, and it causes sustained activation of MAPK [70]. There is also a mammary gland-specific EGFR transgene whose activity leads to loss of differentiation of breast tissue [71]. Furthermore, estrogen or progesterone promote the production of TGF α , and they inhibit the TGF β that might suppress cell proliferation. TGF β restrains proliferation of ER⁺ mammary epithelial cells. The actual situation is that TGF β is a growth inhibitor in early-stage breast cancer, but at a later stage it is prooncogenic, and it certainly encourages metastases. There is a strong correlation between elevated plasma TGF β 1 and progression of breast cancers.

In TGF α transgenic mice that will develop mammary cancer, the TGF α works in conjunction with Wnt growth factors [72]. Canonical Wnt signalling (Fig. 17.10) is often disrupted in cancers. Increased Wnt signalling carries the liability to oncogenic transformation by a Notch-dependent mechanism [73]. Then, EMT will arise because the action of β -catenin induces Axin 2, which incites Snail 1 zinc finger TF, which is a potent inducer of EMT [74]. One must be reminded also of the role of factors from

the breast stroma that regulate ductal growth [75]. Notch has been mentioned. Then also, estrogen acting on ER⁺ receptors promotes the release of TGF α , which induces vascular endothelial growth factor (VEGF), the angiogenic factor. C-myc also synergises with TGF α [76]. Estrogen receptor/MAPK cross talk enhances estrogen-mediated signalling and tumour growth [77]. It has been shown that progesterone pretreatment potentiates EGFR signalling in the breast cancer cell line ZR-75 [78]. Clearly, such networks induce *epithelial-mesenchymal transition* (EMT), which is the basic cancer process. This fact creates complicated discussions [30b]. TGF β causes VEGF to be released by cancer cells, as does local hypoxia acting through HIF1 (Chapter 4) and surface integrins that promote PI3K $\rightarrow$ Akt [31], which is central to EMT. VEGF will bind to cancer cell receptors like neuropilin and Flt-1 [79]. The contribution of the stroma to carcinogenesis merits serious consideration [80].

In human breast cancer tissue, there can be amplification of oncogenes erbB, erbB2 and erbB3 and amplification with overexpression of H-Ras and c-Myc. These are the HER2/ErbB2 tumours that are ER⁻. ErbB2-B3 heterodimers induce potent biological responses [81]. ErbB2 is a ligand orphan receptor that amplifies these other receptors by dimerizing with them [81]. Oncogenic ErbB2 helps to promote c-myc-induced cell proliferation [82]. The breast oncogene heregulin (Her2/neu), which acts on ErbB3/4, regulates epithelial cell proliferation and differentiation [83]. In contrast to EGF, heregulin induces more intense and longer activation of PI3K, MAPK and the p38 pathway. HRG acts via Pak1 and PI3K to cause cytoskeletal reorganization and thus the potential for metastasis [84]. By upregulating VEGF-C, HRG sustains tumour development. In fact, there is now a dual ErbB2/VEGF receptor inhibitor (AEE788) with potent anti-tumour activity.

Either estrogen or IGF-1 stimulate Akt to help cell proliferation. In breast cancer, the IGF-1 receptor undergoes autophosphorylation. Furthermore, caveolins can stimulate Akt. PI3K/Akt stimulate NF- κ B. High levels of NF- κ B are associated with progression of breast cancer cells to independent growth. Hepatocyte growth factor (HGF) is strongly expressed along with its Met receptors [85]. High serum HGF indicates short survival for the patient. HGF enhances CXCR4 expression that favours breast cancer cell invasiveness [86a]. CXCR4 and CCR7 are highly expressed in breast cancer cells. CXCR4 is under the control of HIF1 α . Furthermore, ErbB2, occurring in 30% of breast cancers, limits the degradation of CXCR4 [86b].

Stat (signal transducer and activator of transcription) proteins promote cell growth. Stat proteins are activated by oncogenes and are activated in tumours [87]. In breast cancer-activated Stats 1, 3 and 5 are noted [88]. There are cell surface ligand-receptor complexes relevant to breast cancer like EGF, PDGF, HGF and IGF-1 that activate Stat3, and EGF, PDGF, prolactin and growth hormone that activate Stat5 [88]. Stat3 and 5 promote cell proliferation, and they prevent apoptosis [87]. A dominant-negative inhibitor of Stat3 will block the survival of breast cancer cells. Stat3 supports tumour cells by upregulating cell cycle activation via Myc and cyclin D1. It induces antiapoptotic Bcl-XL. Furthermore, Stat3 inhibits the expression of mediators necessary for activation of immune defence cells against tumour cells. Stat3 aids angiogenesis by induction of VEGF, HIF1 α and matrix metalloproteinase 2 (MMP-2) [89].

Cyclin D1 is overexpressed in breast cancer since it is stimulated by PI3K, MAP kinases, c-Myc and the Ras oncogenes. Often, breast tumours have hyperactive Neu or Ras and thus abundant cyclin D1, which explains proliferation of breast epithelial cells. There is overexpression of the phosphatase cdc25A, and that titrates the p21 inhibitor off the cyclin E/cdk2 complex, promoting cell activation. Cyclin D1 competes with and stops the restraining action of BRCA1 on ER α -receptors. Cdc2 and cyclin B are also overexpressed. Using MCF-7 breast cancer cells, it has been shown that blockade of activator protein 1 (AP-1) complexes, which are normally activated by peptide and steroid growth factors, will decrease the expression of D and E cyclins, reduce cdk2 and cdk4 and increase the CDK inhibitor p27 (Figs. 17.1, Figs. 17.2) [90]. When cells are about to metastasize, E-cadherins are lost, so that intercellular junctions are weakened. Actually, loss or downregulation of E-cadherin (Sect 18.5) predicts invasion, defining E-cadherin as an invasion suppressor gene.

The stromal hexameric glycoprotein tenascin-C, which is suppressed in normal breast tissue, appears periductally in tumours, which portends invasion. Tenascin-C is influenced by FGFs. It may control epithelial cell growth by affecting EGFRs. The c-erb B2 receptor, akin to the EGFR, accounts for the ability of breast cancer cells to invade extracellular matrix. ErbB2 connects with actin fibre controls. Of course, matrix metalloproteinases aid tumour invasion [91]. There are hypoxic areas within tumours where adhesion molecules like integrin $\alpha 6 \beta 4$ are induced more on the cancer cells, aiding spread [92]. One may yet discover exotic reasons for anchorage-independent growth, like the fact that chromosomal translocation affecting high-mobility group A2 disrupts its link to let-7 [93].

One can expect tumours to be attacked by cytotoxic CD8⁺ T cells. However, tumours escape from immune surveillance in various ways, in particular by producing immunosuppressive cytokine TGF β . TGF β inhibits cytolytic gene products in cytotoxic T lymphocytes (CTLs) [94]. Also, tumours induce myeloid stem cells to produce monocytes that are immunosuppressive for CD8⁺ T cells [95a], and myeloid suppressor cells responding to complement inhibit CTLs [95b].

EGFR, activated PI3K and MEK all help to upregulate VEGF production, and that supports angiogenesis, which aids tumour spread. Small GTPases like RhoA and RhoC, which are stimulated by lysophosphatidic acid (LPA) ((**Fig. 7.5**), are implicated in cancer progression. These small GTPases aid cell motility (Chap. 9). In addition, Snail downregulates E-cadherins, and Snail that is phosphorylated by glycogen synthase kinase 3 β (GSK3 β) enhances the motility and invasiveness of cancer cells [96]. Protease-activated receptors (PARs) respond to proteases in a tumour microenvironment [97]. PAR1 expression correlates with the invasiveness of breast cancers. All these could be targets in breast cancer therapy. To date, trastuzumab (Herceptin), which targets metastatic tumours expressing EGFR2 (Her2), has been accorded success. It contributes to apoptosis, downregulation of surface HER2 and suppression of VEGF production [98], but there can be resistance. Tumour Cox-2 expression helps progression and correlates with MMP2 activity. Cox-2 inhibition is discussed more in later sections.

18.4 Prostate Cancer

The cell type that gives rise to prostate cancer is still unknown, but those cells will have phenotypic attributes of luminal epithelial and basal cells [99]. The cell surface marker Sca-1 certainly defines a cell population that can beget cancer [100]. There will be initiation, followed by promotion, and then a series of genetic changes. Androgen action on stromal cells leads to secretion of *andromedins*, which regulate proliferation of prostatic epithelial cells. Both fibronectin and tenascin in the stroma help confer tumour-inducing activity. Stromal cells also express basic FGF and TGF α , and the latter acts on EGFRs of androgen-dependent cells. The action of testosterone is mediated mainly through the stroma, where there are positive androgen receptors (AR⁺). Stromally expressed c-jun of fibroblasts regulates the proliferation of prostate epithelial cells. Via its induction of IGF-1, c-jun promotes prostatic hyperplasia. Thus, it promotes epithelial cell MAPK and downregulates the cell cycle inhibitor p27 [101]. There are various indications that the stromal AR plays a vital role in the homeostasis of the prostate gland [102]. *Prohibitin* is a molecule that regulates the cell cycle, and it is targeted by androgens to cause cell proliferation [103]. Estrogen inhibits rat adenocarcinoma by increasing expression of TGF β and its receptors [104]. As has been emphasized repeatedly, TGF β is antiproliferative since it boosts p21 and p15 cell cycle inhibitors, and it downregulates c-Myc and Id1/2. However, with time there is loss of inhibition of growth by TGF β [105]. Elevated TGF β 1 is found consistently, but cancer cells show loss of T β RI and II [106a]. In fact downregulation of TGF β can restore immunogenicity [106b].

At the stage of intraepithelial proliferation, there is overexpression of cdc25B and an AR coactivator [107], and the expression of p27^{kip1} is reduced [108]. The AR coactivator CARM1 influences Grip1 (glucocorticoid receptor interacting protein 1), which is attached to the AR and allows it to function at lower androgen levels [109]. Proliferative advantage is conferred by the TF E2F3 [110]. 15-Lox-1 is pro-tumourigenic in rodent prostate cells. 15-Lox-2 is a functional tumour suppressor that regulates prostate epithelial cell differentiation, growth and senescence [111]. The level of 12-Lox correlates with advanced stage and poor differentiation of human prostate cancer [112]. All these features are slowly being assembled [113]. The tumour suppressor gene Kruppel-like factor 6 (KLF6) is mutated in prostate cancer. The wild-type KLF6 upregulates p21 and thereby reduces cell proliferation. KLF6 influences cell growth and differentiation by its action on TGF β receptors and the urokinase-type plasminogen activator [114]. PTOU-1 is a novel protein expressed in prostate cancer, and it drives cell proliferation [115]. Two Ets TFs, ERG and ETV1, turn out to be protooncogenes that are overexpressed, and they engage in gene fusion [116]. Furthermore, androgen-responsive promoter elements of TMPRSS2, a prostate-specific androgen-regulated gene, cause overexpression of Ets family members [117]. The TMPRSS2-ERG-ETV1 fusion product is an androgen-responsive oncoprotein. Pim-1 kinase is implicated in the development of prostate cancer. Indeed, measurement of Pim1 messenger RNA (mRNA) by quantitative polymerase chain reaction (PCR) could turn out to be helpful in diagnosis [118].

Within this array of facts, you will be gratified to find some easily recognisable features. Inhibition of Ras by S-trans-farnesylthiosalicylic acid shows its involvement in both androgen-dependent and androgen-independent cancer cells [119]. These cells maintain an elevated Akt, together with loss of PTEN [120]. The Akt synergises with androgen receptor signalling. Also, there is upregulation of mTOR, starting with prostate intraepithelial neoplasia [121]. Foxo2 could be involved in the progression to androgen independence [122]. Perlecan, which binds to sonic hedgehog, has been shown to enhance signalling in aggressive tumours [123].

The AR continues to be essential at the stage of androgen-independent growth [124], that is, because prostate cancer cells can utilise low levels of androgen for survival by increased sensitivity of their ARs to testosterone, by increased local conversion of testosterone to dihydrotestosterone by 5 α -reductase and by display of increased numbers of ARs [125]. This also depends on the PI3K-Akt-mTOR pathway [106a], and is sustained by neuroendocrine factors [126, 127]. Cross talk with other signalling pathways will allow the ARs to function in the presence of very low levels of androgens. Nonandrogenic steroid molecules can bind and activate the ARs. Furthermore, at that hormone-independent stage, granulocyte-macrophage colony-stimulating factor (GM-CSF) has been shown to promote MAPKs and elevation of Ki-67. There is overexpression of transcriptional coactivators, and the ARs are themselves overexpressed as a consequence of gene amplification. A bypass pathway upregulates Bcl-2, so that cancer cells are protected from apoptosis. There is dysregulation of annexins [128], which will portend spread of cells. Cytokine stimulation of prostatic cells also leads to recruitment of β -catenin by wild-type or mutant ARs [129]. When the AR connects with β -catenin signalling (Sect. 18.5), control of E-cadherins is lost, similar to the situation in colon cancer [130]. Then, elevation of Cox-2 enzyme corresponds to the reduction of E-cadherin expression [131]. Cellular oxidative stress must also be considered for there are extensive mitochondrial DNA mutations in prostate neoplasia [132].

Ras is a convergence point for diverse signalling pathways, and the Ras-MAPK pathway must participate in progression. Yet, curiously Ras mutations are infrequent. Alternatively, Rho GTPases have a central role in promoting prostate cancer cell growth [41]. Thrombin, angiotensin, bradykinin, endothelin and LPA receptors are all overexpressed in prostate cancers, and through GPCRs like G α_q and G α_{13} , they stimulate RhoA. Growth of androgen-independent tumour cells is inhibited by use of antagonists for bradykinin, AT1, ET-A and gastrin-releasing peptide. It appears that aberrant activity of GPCRs might determine progression from the hormone-dependent to hormone-independent stage [133].

When prostate cancer is found early at the clinic, it is dependent on androgen for growth and so is responsive to pharmacological or surgical ablation of circulating androgen. However, the later stages, when there are metastases, are marked by apparent androgen independence. Since the ARs are retained [134], the cells at this stage are probably still responsive to low levels of androgen. Hence, we need to know much more about the controls affecting these receptors. Loss of the tumour suppressor Foxo1A through chromosomal deletion 13q14 is observed in many prostate cancers and will help progression to androgen independence. An androgen-induced gene FKBP51 has been shown to increase AR transcriptional activity

[135]. Furthermore, the ErbB2/ErbB3 path is critical in hormone-refractory cancer since it stabilises the AR and enhances AR binding to the promoter of AR-responsive genes [136]. It has also been noted that there is a reduction of the EGFRs in the stroma but overexpression of active variant EGFR (termed EGFRvIII) on the epithelium [137]. In PTEN-mutant mice, there is androgen independence, but nevertheless the cells show AR-dependent growth [138].

At this later stage [139], the cells show increased production of various growth factors (TGF α , EGF, KGF, bFGF, IGF-1, IGF-2), for which they have receptors. These growth factors will act via Ras-Raf-MEK-ERK to drive androgen response element (ARE) promoters [140]. Actually, androgen-refractory tumours often have AR mutations, so that a receptor that is usually activated by DHT will be activated by other steroids and even antiandrogens [134]. One must take account of the neuroendocrine cells in the stroma for they secrete serotonin, bombesin, calcitonin and PTHrP, which help prostate cells to proliferate since they signal through protein kinase A to stimulate Raf-MAPK [134].

There will be expression of antiapoptotic genes like Bcl-2. Loss of PTEN (Fig. 2.6) increases Akt activity and blocks apoptosis. In aggressive cancers, IL-6 acts likewise [141]. Prostate cancer is characterised by epigenetic silencing of expression of 14-3-3 protein since there is CpG methylation [142]. This means that those cells escape from DNA damage-induced arrest. We discuss metastases at the end of this chapter. Overexpression of HIF-1 α stimulates the invasive potency of prostate cancer cells [143]. Expression of heterotrimeric G proteins G α 12/13 in cell lines has been shown to induce invasion [144]. It was shown that inhibition of macrophage MIF or its receptor will stop the growth and invasion of DU-145 prostate cancer cells [145]. So will genistein of soy protein, which can block activation of metalloproteinase 2 [146].

In addition to androgen deprivation, combination therapies might be most useful for prostate cancer. Inhibition of Akt, Cox-2 and MMP9 activation should stop the activation of the ARs that still occurs in the androgen-independent stage of prostate cancer [147].

18.5 Cadherins and Catenins

Before dealing with other cancers, consideration of cadherins and catenins is required. Cadherins are calcium-dependent glycoproteins that mediate cell–cell adhesion at adherens junctions; thus, they organise epithelia. Recall that cAMP induces EPAC (exchange protein directly activated by cAMP), so cell–cell adhesions and integrity of epithelium is ensured. The intracellular anchorage of the cadherins is determined by the catenins. E-cadherin joins with either α -catenin or β -catenin/plakoglobin. In turn, they bind to γ -catenin, which links them to actin microfilaments in the cell (Fig. 18.3).

Plakoglobin (γ -catenin) has the potential to be a tumour suppressor. β -Catenin has been shown to induce antiapoptotic Bcl-2. That potential is modulated by plakoglobin [148].

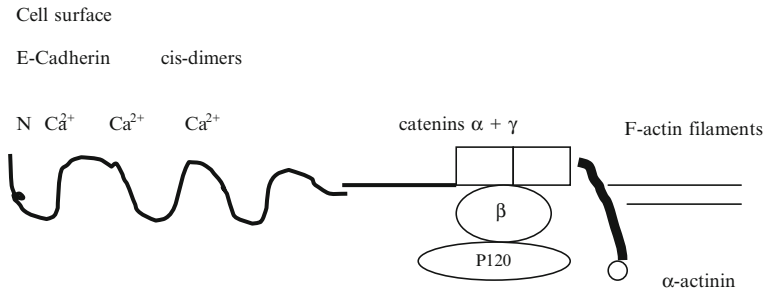


Fig. 18.3 E-Cadherin connecting with catenins. β -Catenin with α -catenin mediate cell adhesion. β -Catenin is a signalling molecule. α -Catenin inhibits Arp2/3-induced actin branching but aids actin bundling. The p120 catenin connects with Rac and Rho GTPases. Wnt or other g-factor can stimulate β -catenin, which connects $\rightarrow$ Myc $\rightarrow$ cyclin D $\rightarrow$ pRb $\rightarrow$ cell cycle

More than 90% of cancers arise in epithelia. In cancers, the E-cadherin of epithelial cells may be increased, but more often it is decreased as a prelude to invasion, and β -catenin is decreased. So, the desmosome complexes are defective. When E-cadherins are downgraded, that helps metastasis [149]. The Snail family TFs Snail (SNA1) and Slug (SNA2) are recognised to downregulate E-cadherins. Ectopic SNA1 expression induces epithelial and mesenchymal mouse tumours. The prototypic cadherin CDH1 is highly expressed in normal thyroid. SNA1/2 are not expressed in normal thyroid tissue, but they are highly expressed in thyroid cancers [150]. In embryogenesis, there is utilisation of the Wnt/Wingless pathway for signalling (Fig. 17.10). β -Catenin acts independently of cadherin, and it transduces signals from the cytoplasm into the nucleus by activating DNA binding proteins of the LEF-1/Tcf family [151]. Wnt and hedgehog signalling paths are important in embryonic patterning [152] (Fig. 17.9), (Fig. 17.10) since they regulate stem cell numbers in epithelia like the skin and intestine.

Formation of transdimers of E-cadherin causes activation of c-Src PTK. C-Src via Crk and C3G (a guanine-nucleotide exchange factor [GEF]) activates Rap1 GTPase. Then, Rap1 activates Rac1 through PI3K and Vav2 (the GEF for Rac1), so there is reorganisation of the actin cytoskeleton.

18.6 Ovarian Cancers

Ovarian cancers show considerable heterogeneity, and there are few good tumour markers. Abnormalities of chromosome 17 play a role. The breast/ovarian cancer gene BRCA1 is at 17q21, and BRCA2 is on chromosome 13. The p53 locus is on the short arm of chromosome 17. Site-specific ovarian cancer with early age of onset is an autosomal dominant trait. The breast-ovarian cancer syndrome is typified by early onset of breast or ovarian cancer. In high-grade serous and endometrial

cancers, there is chromosomal instability due to BRCA1/2 and TP53 mutations. PI3K is amplified in 80% of ovarian cancers. Mutation of the PIK3CA gene, or its amplification is found in 30% of ovarian cancers [153]. Loss of PTEN is common.

The incessant ovulation hypothesis postulates that repetitive wounding and cell proliferation in postovulatory repair of the ovarian surface epithelium results in a stepwise accumulation of genomic abnormalities [154]. Thirty percent of ovarian cancers carry amplified ERB-B2. The EGFR/HER2/PI3K path is implicated in ovarian cancer [154]. Introduction of K-Ras can transform the ovarian epithelium into tumours, as is the case for the uterine endometrium. Expression of K-Ras or B-raf point mutations or PTEN deletion within the ovarian surface epithelium gives rise to preneoplastic ovarian lesions [155]. K-Ras mutations occur in 45% of mucinous forms and in 15% of serous cancers. Aberrant embryonic homeobox gene reexpression (HOXA7/A9/10/11) is usual [154, 156], and Muc1 splice variants A, D, X, Y, Z and REP are associated [157]. Hypermethylation of gene ZAC is an early event [158].

The E2F family of TFs (Fig. 17.2) (i.e. E2F1, E2F2 and E2F-3a) are promoters of cell proliferation [159]. These TFs form heterodimeric complexes with DP proteins (also in Fig. 17.2). Overexpression of E2F2 will probably overwhelm the proapoptotic effects of E2F1 [160]. LPA is a potent mitogen secreted into ascites fluid by ovarian cancer cells. It promotes growth, survival and resistance to chemotherapy by stimulation of LPA-sensitive GPCRs on ovarian tumour cells. LPA stimulates further LPA release, establishing an autocrine loop that drives uncontrolled growth [161]. Expression of the tyrosine kinase receptor EphB4 correlates with advanced stage and the presence of ascites [162]. One would also suspect an important role for cadherins. Mucinous tumours strongly express E-cadherin, serious cancers are N-cadherin and P-cadherin positive, and endometrial tumours express nucleocytoplasmic β -catenin [163]. In serous neoplasms, high-grade tumours have reduced expression of E-cadherin and nuclear staining for γ -catenin, and there is overexpression of CD44s and CD56 [164]. Normal ovarian samples do not express E-cadherin, but E-cadherin is uniquely elevated in over 85% of benign, borderline and malignant ovarian cancers [165]. Yet, in serous and endometrial histological types, there is reduced expression of E-cadherin, β -catenin and CD44, the hyaluronan-binding protein [166]. CD44 isoforms are formed by the splicing of ten variant exons [167], and variants of CD44 often accompany an aggressive phenotype in cancers. KLF6 of the Kruppel-like family of TFs is a tumour suppressor gene; when it is reduced, it reflects low E-cadherin expression [168]. In the ovarian cancer field, there does seem to be scope for surprises. The final answers still seem distant. Programmed cell death ligand (PD-L1) is expressed on ovarian cancer cells, especially those with a poor prognosis; naturally, this means that tumour-infiltrating CD8 CTLs are suppressed [169].

It was shown that clofibrate acid as ligand for peroxisome proliferator-activated receptors (PPAR α s) and pioglitazone ligand for PPAR γ receptors together suppress VEGF expression by human ovarian cancer cells; reduce AP-1, the transcriptional regulator of Cox-2; and cause apoptosis and necrosis of cells [170]. Inhibition of Cox-2 is relevant to other cancers.

18.7 Renal Cancer

There are several types of renal cancer [171], but the type that is found more often is the clear cell form [172]. In many of these tumours, the von Hippel-Lindau (VHL) tumor-suppressor gene is inactivated (**Fig. 4.19**), and there is aberrant localisation of E-cadherin. When cells are deficient in VHL, there is inappropriate accumulation of HIF-1 α under conditions of normal oxygen tension. This means that there is increased expression of the HIF-regulated genes, including those for angiogenic factors. So, VEGF is expressed, as are the growth factors PDGF β and TGF α . Keep in mind that mTOR simulates HIF, which in turn means that mutation of the tuberose sclerosis complex TSC1/2 (**Fig. 11.6**) could be relevant to this cancer. Poignantly, the agents sorafenib or sunitinib, which inhibit both PDGFR β and VEGFR2, can be used in treatment, as can the anti-VEGF antibody bevacizumab whenever there are metastases.

As is the case in breast cancer, there is cross talk in clear cell renal carcinoma between the IGF-1 $\rightarrow$ IGFBP-3 system and TGF β signalling [173]. Thus, TGF β regulates endogenous levels of IGFBP-3 in these cells. In turn, IGFBP-3 potentiates TGF β 1 effects by enhancing the phosphorylation of Smad 2 and Smad 3. Also, Notch1 and its ligand Jagged-1 are highly expressed in clear cell renal cancer, the implication being that Notch blockade could be useful [173b].

18.8 Colon Cancer

Colon cancer arises from erroneous renewal of crypt cells in the lower gut epithelium. Mutations in stem cells in the mucosa or other proliferative progenitors lead to the accumulation of mitotic cells that invade the stroma. One will suspect that there must be an altered role of E-cadherin and with that there will be epithelial-mesenchymal transformation. Local papillomas or adenomas are quite common, and they should be considered first (Table 18.3).

APC (Fig. 18.4) is a tumour suppressor gene that stops Wnt signalling and so cell proliferation in intestine. APC acts as a partner molecule for γ -catenin and for β -catenin (plakoglobin) [174]. Normally, the catenins are degraded and inactivated by binding to APC, functioning as a negative regulator of the Wnt signal transduction cascade [174, 175]. However, when APC is truncated by mutation,

Table 18.3 Genetic changes in colon cancer development

Hyperproliferative colonic mucosa	5q loss (adenomatous polyposis coli gene)
Early adenoma	DNA hypomethylation
Intermediate adenoma	K-Ras or B-Raf activation
Late adenoma	18q loss affecting DCC suppressor
Colon cancer	17q loss affecting p53 suppressor

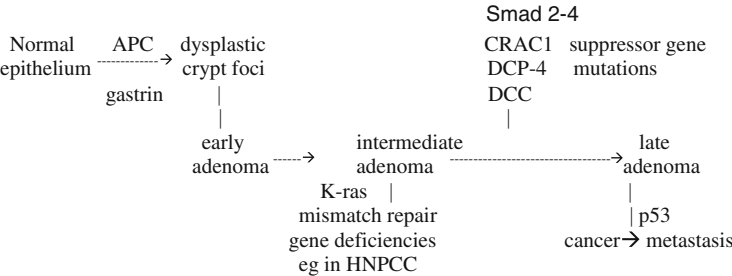


Fig. 18.4 Genesis of colorectal tumours. APC = adenomatous polyposis coli

it cannot control β -catenin, which thus accumulates, and acts as a cotranscription factor for Tcf-4, Myc Fig. 17.10 [176, 177]. Plakoglobin is similar. There is then expression of TCF/ β -catenin and TCF/plakoglobin target genes like c-Myc that can lead to cancer on APC mutation the phosphatase PTEN exerts a vital role in preventing adenoma formation, which could be consequent upon increased Akt activation [177b].

There is a relationship between consumption of red meat or fat and the prevalence of breast, colon and prostate cancers. A high saturated fat intake alters the bowel flora so that carcinogenic bile acids are produced. Bile acid activation of PKC stimulates the proliferation of colonic epithelial cells [178, 179]. A normal content of APC protein regulates proliferation, migration, differentiation and apoptosis of the epithelial cells. A half amount of APC proteins renders epithelium susceptible to development of cells with unrestrained proliferation, so that adenomas are formed [180]. Whenever β -catenin is unrestrained, the cyclin D promoter is activated [181]. Unrestricted plakoglobin leads to the activation of c-Myc [182]. Truncated, mutated APC proteins induce the proliferation of intestinal crypt cells so that polyps form. Gastrin supports this [183]. Growth of adenomas is accompanied by activation of cyclin D, or c-Myc, and expression of metalloproteinase.

In 1990, Fearon and Vogelstein [184] proposed that there is multistage colon carcinogenesis as a result of successive mutations of key genes [185]. They can be acquired, but often they are inherited (Table 18.2). Initially, there will be mutation of tumor suppressor gene APC [186], then K-Ras mutations and thereafter change in the tumour suppressors like Smads 2–4 and p53 [187]. In sum, at least four genetic changes are required for colon cancer: change in K-Ras and in suppressors APC, Smad 4 and TP53. Lesions of the DCC gene are actually rare. DCC can cause cell apoptosis by a pathway that is inhibited by netrin-1. More commonly, netrin mutations account for epithelial hyperplasia and neoplasia [188]. In keeping with the emphasis on the oxidative process in signal transduction and as a means of mutagenesis, note that nicotine activates the phosphorylated receptor of EGFR on colon cancer cells, and that the EGFR then activates Src kinases and 5-lipoxygenase [189]. The role of oxidative stress in carcinogenesis is a separate topic that has been reviewed by Klaunig and Kamendulis [190].

In HNPCC (hereditary nonpolyposis colorectal cancer; Lynch syndrome), there is a defect of MMR genes such as MSH2, MSH6 and MLH1 that can be detected by immunohistochemistry. Microsatellite instability is detectable [191, 192]. Normal DCC induces apoptosis of such cells [193, 194], but that protection is lost. Another colorectal adenoma and cancer gene CRARC1 is found on 15q14-q22. Smad 4 of the TGF β signal pathway (Fig. 14.1) is a tumour suppressor. Smad 4 is missing in colorectal and pancreatic cancers. Alternatively progression of adenocarcinoma is often heralded by mutation of the gene for the TGF β RII receptor [195]. Furthermore, tumour-induced PKC β II represses the expression of T β RIIs. The molecular diagnosis of colorectal adenomas or cancers can now be made from the expression profiles of some 50 genes by multiplex PCR assay [196].

Various cell surface molecules aid the spread of colon cancer [197]. Loss of E-cadherins and of β -catenin help since this predisposes to EMT (see Sect. 18.9). So does the expression of CD44, hyaluronan-binding protein, and its variants [198]. Transdifferentiation of colon cancer epithelium into invasive cells has drawn attention to the ability of nuclear β -catenin/Tcf (Fig. 17.10) to induce CD44, matrilysin and uPAR and so to promote invasiveness. Conversely, the inhibition of β -catenin can stop metastasis [199].

Cytoplasmic β -catenin associates with normal APC protein, resulting in APC phosphorylation and so recruitment of ubiquitin-ligase components, which will lead to APC degradation. The phosphorylation then needed for degradation of β -catenin is effected first by casein kinase I and then by GSK3. However, when the Wnt pathway is being stimulated because there is an APC mutation, GSK3 is inhibited [200]. Hence, β -catenin is stabilised, in part by the action of PGE₂, and so it moves into nuclei, where it drives T cell factors to activate genes (Fig. 17.10) [201]. β -Catenin then induces genes that stimulate cell proliferation, such as cyclin D1 and Myc, whose promoters contain LEF/Tcf binding sites, and genes for cancer progression like matrilysin, metalloproteinase, fibronectin and the multidrug resistance protein Mdr-1. Should PTEN be expressed, GSK3 is thereby activated, there is degradation of β -catenin, and cyclin D1 falls. However, mutations of the PTEN suppressor gene are common in cancers, so GSK3 β is not activated.

A germline APC mutation is the genetic cause of familial adenomatous polyposis (FAP). FAP patients inherit one defective APC allele and so develop large numbers of colon adenomas or polyps. Some will become malignant adenocarcinoma. Several EphB genes are initially upregulated as Wnt/Tcf4 target genes in early adenomas.

Following APC mutation, amongst the targets for Tcf4 is the CD44 gene [201], and thus there is enhanced CD44 expression. Normal CD44 binds to hyaluronan of the extracellular matrix. Variant CD44 imparts enhanced cell motility [202]. Furthermore, HGF (scatter factor) signals into the cell via c-Met receptors and by the tails of CD44 molecules so that cell proliferation is promoted, and there is enhanced motility and decreased apoptosis of cells [202]. MMPs like collagenase 1 and stromelysin 1 enable actin rearrangements that help movement of cells. Indeed, autocrine TGF β can mediate Smad-independent motility in cancer cells [203]. In addition, drive-by Rho GTPases like Rho, Rac and cdc42 explain motility and metastasis [204]. The Rho GTPases are required for functional cell–cell contacts

and polarity of epithelial cells. Their mutants permit disassembly. On occasion, mutation of ROK, Rho-activated kinase, confers invasive potential. When it was found that angiotensin-converting enzyme (ACE) inhibitors and angiotensin II (Ang II) receptor blockers could benefit cancer [205], it was also realised that Ang II receptors (**Fig. 5.10**), by their ability to cause EGFR transactivation, must help progression and spread of cancer.

Malignancy stems from the accumulation of multiple genetic alterations. Receptor tyrosine kinase RON (recepteur d'origine nantais) belongs to the Met protooncogenes. It shows increased expression in colon cancers [206], correlating with enhanced cell motility and altered β -catenin (**Fig. 17.10**).

The characteristics of colon cancer cells are increased basal PI3K activity, TCF/ β -catenin signalling, and increased Cox-2 and thereby increased PGE2 biosynthesis. PGE2 that interacts with the PI3K path further activates TCF/ β -catenin signalling, so that there is positive feedback. One can reduce the growth of colonic polyps by ingestion of a Cox inhibitor like celecoxib [207], aspirin [208, 209], or sulindac. Aspirin actually induces apoptosis of cells by inhibition of the proteasome [210]. The Cox-2 product PGE2 promotes cell growth, cell adhesion and enhanced cell motility, and apoptosis is inhibited [211]. In fact, EP2 and EP4 are coupled to $G_{\alpha s}$, and they stimulate cAMP accumulation [212]. Moreover, PGE2 stimulates growth of colon cancer cells by induction of amphiregulin [213]. PGE2 acting on its EP receptors also elevates the expression of Bcl-2 [214]. It also looks as though PGE2, via receptor EP2, stimulates the β -catenin pathway [215]. Cox-2 upregulation is a feature that facilitates mitogenic growth factor signalling and angiogenesis and so carcinogenesis and metastasis [215]. PGE2 is known to transactivate the receptors for EGF [216]. Cox-2 is also formed in stromal cells in the intestinal wall from the early stage of colon cancer, and this PGE2 induces VEGF, which causes formation of new blood vessels within the tumour [211].

Of course, persistent inflammation in the colon is associated with elevated risk of colorectal cancer. Inflammatory cells produce cytokines, TNF α in particular, which has a critical role in promoting neoplastic transformation. There is NF- κ B activation in the epithelial cells [217].

Nonsteroidal anti-inflammatory drugs (NSAIDs) like aspirin and indomethacin attenuate β -catenin/Tcf4 signalling [218]. By inhibition of Cox-1 enzyme, NSAIDs cause an accumulation of arachidonic acid [219], which if converted to ceramide, will promote cell apoptosis, protecting against cancer. Furthermore, NSAIDs cause acetylation of polyamines, so that they are depleted [220]. Cox-2 inhibition promotes IFN γ -dependent enhancement of antitumour cell responses [221]. Genetic ablation of Cox-2 strongly reduces tumour formation induced by the two-stage carcinogenesis protocol in mouse skin [222] and likewise development of colonic polyps in the APC^{Min} mouse model [223]. One has to admit that there have been disappointments in the treatment of human colonic polyps. Nevertheless, the importance of Cox-2 for growth of cancer cells is emphasized by the fact that one third of colon cancers that are Cox-2⁺ are prevented by regular aspirin use [224], and the Cox-2 inhibitors protect against gastric and pancreatic cancer in humans [225]. There is recent evidence that Cox-2 inhibition also results

in inhibition of EGFR transactivation [226]. It could be appropriate to use a Cox-2 inhibitor together with an EGFR antagonist.

Since PPAR γ s are activated by eicosanoids, fatty acids and some drugs, Cox-2 inhibition to stop eicosanoid production is helpful. PPAR γ s are abundant on colonic epithelium [227], where they activate various genes in conjunction with retinoid X (**Fig. 4.14**). In colon polyps and cancers, the free β -catenin and plakoglobin that move into cell nuclei act via TF Tcf4 to induce PPAR γ [228].

Vitamin D analogues have been shown to inhibit the growth of cancer cells. Natural vitamin D, the 1 α ,25(OH) $_2$ D $_3$, regulates the differentiation, proliferation and apoptosis of cells. Mice with a dominant mutation in the APC gene that are fed a Western-style diet, low in calcium and vitamin D, develop more cancers and invasive tumours [229]. Their tumour load is reduced by 36% by vitamin D therapy. In fact, VDR knockout animals have colon cells showing high proliferating cell nuclear antigen (PCNA) and cyclin D1 expression, and there is mucosal hyperproliferation and oxidative stress. So, 1 α ,25(OH) $_2$ D $_3$ and its analogues inhibit proliferation of human cancer cells by upregulation of p21 and p27, and they induce differentiation such as E-cadherin expression and apoptosis that is associated with upregulation of proapoptotic Bak. Vitamin D represses the transcription of Tcf-target genes [230]. A new era of vitamin D therapy has started [231].

Patients with a familial risk form only 20% of cases of colorectal cancer. In sporadic cases, cytomegalovirus (CMV) may turn out to be implicated [232].

18.9 Epithelial-Mesenchymal Transition

Detachment of cancer cells and spread into the stroma [233] recapitulate the developmental process of (a) loss of cell–cell adhesion by E-cadherin downregulation, (b) upregulation of matrix-degrading proteases, and (c) reorganization of the actin cytoskeleton of cells by Rho GTPase activation of the cell motility machinery (**Fig. 9.7**). Cofilin is a downstream target of Rho GTPase that promotes F-actin recycling. In fact, cofilin is a regulator of cell protrusion that aids formation of lamellipodia [234a]. The TFs Snail and Twist are especially known to trigger EMT [234b]. It acts as a molecular organizer by downmodulating epithelial cell genes and upregulating mesenchymal genes. Snail is expressed at the invasive front of cancers. When TGF β is inactivated in colon cancer epithelium, there is an increase of chemokine CCL9, which promotes recruitment of MMP2- and -13-expressing stromal cells, which aid tumour invasion [235].

18.10 Genes for Metastasis

By now, we know of metastasis suppressor genes, which occur within every cell in positions where signals are being amplified [236]. There are also metastasis tumour antigens (MTAs) [237]. Nm23 gene (murine melanoma nonmetastatic clone 23)

forms a complex with scaffold protein KSR, kinase suppressor of Ras (**Fig. 4.3**). Melanocytes can metastasize aggressively. Their TF Slug is a mediator of high migratory activity. Conversely, VDUP (induced by vitamin D) is a thioredoxin inhibitor that also suppresses the MAPK pathway. KAI-1 is a tetraspanin molecule that desensitises the EGFR, so it suppresses formation of lamellipodia and signals for cell migration. Likewise, E-cadherin overexpression decreases tumour cell motility and invasion. Tissue inhibitors of metalloproteinase (TIMPs) inhibit the metalloproteinases, and RhoGDI2 are Rho-GDP (guanosine diphosphate) dissociation inhibitors that stabilise GDP in bound inactive form. Important developments might arise from this knowledge, although to date they have been slow. It is known that p38 MAPK activates MAPKAPK2, which in turn activates heat shock protein 27 (hsp27), and both regulate metalloproteinase MMP2 and cell invasion [238].

One will suspect rightly that alterations in tumour characteristics will increase the invasive potential. The transition to overt metastasis coincides with the expansion of clones with cytogenetic abnormalities. Tumours use induction of tolerance and immunoediting, by which there is loss of expression of neoantigens and major histocompatibility complex (MHC) molecules. Such cells upregulate invasive factors [239]. It has been shown that VEGFR1 bone marrow progenitors, which also express VLA4, facilitate the premetastatic niche. VEGFR1, CD133, CD34 and c-kit tend to be associated on tumour cells. Factors derived from the tumour upregulate fibronectin, which is the ligand for VLA4 [240]. Cancer cells often release S100A8/A9, which attach to myeloid or endothelial cells of the lungs and so form hot spots for the adhesion of metastatic cells [241]. The Met protooncogene is a key regulator of invasive growth that is expressed not only in the rare hereditary kidney cancer, but also by melanomas, breast, lung and head and neck squamous cancers [242, 243]. Met works in conjunction with other cell surface receptors like integrin $\alpha 6 \beta 4$, semaphorins and CD44, EGFR and RON, and it elicits a prolonged phosphorylation of Gab1 (Grb-associated binder) (cf. (**Fig. 4.2**), which functions as a multiprotein assembly centre. Met leads to transcriptional activation of a set of genes that control blood coagulation [242], so it facilitates spread of tumour cells. Furthermore, in hypoxic regions of tumours, hypoxia induces Met expression since HIF-1 acts on the Met promoter [243]. Of course, angiogenic switch is integral to tumour spread. Therefore, one notes that p53 protein inhibits neovascularisation, and it represses VEGF. p53 stops the action of metalloproteinases [244], and it promotes formation of PAI-1, which inhibits plasminogen activator and therefore plasmin. Of course, plasminogen activator and plasmin do play major roles in the spread of cancer cells [245, 246]. Coopting blood platelets, and using them as shields, helps circulating tumour cells. VEGF production by metastatic cells, and so Src kinase activation in endothelial cells, results in dissolution of endothelial cell junctions, and it helps the spread of neoplastic cells. In response to VEGF stimulation, endothelial cells release chemokine IL-8. Many cancer cells use IL-8, Gro α and ENA78 (CXCL5) to promote angiogenesis [247]. Of course, released growth factors act on stromal cells, and fibroblasts are activated. The formation of chemokine CXCL12 by breast cancer-associated fibroblasts augments movement of CXCR4-expressing breast cancer cells. Release of TGF β from dissolved bone matrix drives further expression of some metastasis mediators.

References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70
2. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control: Nat Milestones Cancer 2006;April supplement:S33–S43
3. Feng Z, Hu W, Teresky Y et al. Declining p53 function in the ageing process: tumor incidence in older populations. *Proc Natl Acad Sci U S A* 2007;104:16633–16638
4. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med* 2003;349:2042–2054
5. Baylin SB, Ohm JE. Epigenetic gene silencing in cancer. *Nat Rev Cancer* 2006;6:107–115
- 6a. Li W, Zhu T, Guan K-L et al. Transformation potential of Ras isoforms correlates with activation of PI-3kinase but not ERK. *J Biol Chem* 2004;279:37398–37406
- 6b. Karnoub AE, Weinberg RA. Ras oncogenes. *Nat Rev Mol Cell Biol* 2008;9:517–531
- 6c. Janssen K-P, Abala M, El Marjou F, et al. Mouse models of K-Ras-initiated carcinogenesis. *Biochim Biophys Acta* 2005;1756:145–154
- 7a. Theodorescu D, Cornil I, Sheehan C et al. Has-Ras induction of invasive phenotype results in upregulation of EGF receptors. *Cancer Res* 1991;51:4486–4491
- 7b. Bodemann BO, White MA. Ral GTPases and cancer. *Nat Rev Cancer* 2008;8:133–139
8. Friedman E. Role of Ras-GTPase activating protein in human tumorigenesis. *Pathobiology* 1995;63:348–350
9. Gazin C, Wajapeyee N, Gobeil S et al. An elaborate pathway for Ras mediated epigenetic silencing. *Nature* 2007;449:1073–1077
10. Coleman W B, Tsongalis GJ. Molecular mutations in human neoplastic diseases. Chap. 14, pp. 293–316 in *Molecular Diagnostics*, B Coleman, GJ Tsongalis (Eds), Humana Press, Totowa, NJ, 1997
11. Weitzman JB, Yaniv M. Rebuilding the road to cancer: *Nature* 1999;400:401–402
12. Land H, Parada LF, Weinberg RA. Tumorigenic conversion of primary embryo fibroblasts requires at least 2 cooperating oncogenes. *Nature* 1983;304:596–602
13. Gupta S, Ramjaun AR, Haiko P et al. Binding of Ras to PI3kinase p110 α is required for Ras driven tumorigenesis in mice. *Cell* 2007;129:957–968
14. von Zglinicki T. Oxidative stress shortens telomeres. *Trends Biochem Sci* 2002;27:339–343
15. Wang J, Harman GJ, Beach DH. Risky immortalization by telomerase. *Nature* 2000;405:755–756
16. Kim NW, Piatyszek MA, Prowse KR et al. Specific association of human telomerase activity with immortal cells and cancer. *Science* 1995;266:2011–2015
17. Buys CH. Telomeres, telomerase and cancer. *N Engl J Med* 2000;342:1282
18. Cao L, Finkel T. Cancer gets the Chk'ered flag. *Nat Med* 2006;12(12):1354–1356
19. Sarkisyon CJ, Keister BA, Stairs DB et al. Dose dependent oncogene induced senescence and its evasion during tumorigenesis. *Nat Cell Biol* 2007;9(5):493–505
20. Xia S, Forman LW, Faller DV. Protein kinase C δ is required for survival of cells expressing activated p21Ras. *J Biol Chem* 2007;282:13199–13210
21. Shishodia S, Aggarwal BB. Nuclear factor-kB: a friend or foe in cancer. *Biochem Pharmacol* 2004;68:1071–1080
22. Karin M, Greten FR. NF-kB: lining inflammation and immunity to cancer development and progression. *Nat Rev Immunol* 2005;5:749–759
23. Pikorsky E, Porat RM, Stein I et al. NFkB functions as a tumor promoter in inflammation associated cancer. *Nature* 2004;431:461–466
24. Bensaad K, Vousden KH. p53: new roles in metabolism. *Trends Cell Biol* 2007;17:286–291
25. Ramsey MR, Sharpless NE. ROS as a tumour suppressor? *Nat Cell Biol* 2006;8:1213–1215
26. Shaw RJ. Glucose metabolism and cancer. *Curr Opin Cell Biol* 2006;18:598–608

27. Brahimi-Horn MC, Chiche J, Pouyssegur J. Hypoxia signalling controls metabolic demand. *Curr Opin Cell Biol* 2007;19:223–229
28. Jayasurya R, Sathyan KM, Lakshminarayanan K et al. Phenotypic alterations in Rb pathway have more prognostic influence than p53 pathway proteins in oral cancer. *Mol Pathol* 2005;18:1056–1066
29. Marx J. How cells cycle towards cancer. *Science* 1994;263:319–321
- 30a. He L, He X, Lowe S, Hannon GJ. Micro-RNAs join the p53 network. *Nat Rev Cancer* 2007;7:819–822
- 30b. Weinberg RA. Twisted epithelial-mesenchymal transition blocks senescence. *Nat Cell Biol* 2008;10:1021–1023
31. Coloff JL, Rathmell JC. Metabolic regulation of Akt. *J Cell Biol* 2006;175:845–846
32. Vogt PK, Kang S, Elsliger M-A, Gymnopoulos M. Cancer specific mutations in phosphatidylinositol 3-kinase. *Trends Biochem Sci* 2007;32:342–348
33. Gire V, Marshall C, Wynford-Thomas D. PI 3-kinase is an anti-apoptotic effector in the proliferative response of primary human epithelial cells to mutant Ras. *Oncogene* 2000;19:2269–2276
34. Borlaco L, Redondo C, Alvarez B et al. Increased PI3kinase activity induces a lymphoproliferative disorder and contributes to tumor generation in vivo. *FASEB J* 2000;14:895–903
35. Razzini G, Berrie CP, Vignati S, Broggin M. Novel functional PI3kinase antagonists inhibit cell growth and tumorigenicity in human cancer. *FASEB J* 2000;14:1179–1187
36. Bruni P, Boccia A, Baldassarre G et al. PTEN expression is reduced in a subset of sporadic thyroid cancers. *Oncogene* 2000;19:3146–3155
37. Eichhorst ST, Krammer PH. Derangement of apoptosis in cancer. *Lancet* 2001;358:345–346
38. Altieri DC, Marchisio C. Survivin apoptosis. *Lab Invest* 1999;79:132–136
39. Kramer MH, Hermans J, Wijburg E et al. Clinical significance of bcl-2, bcl-6 and Myc rearrangements in diffuse large B cell lymphoma. *Blood* 1998;92:3152–3162
40. Kumar R, Gururaj AE, Barnes CJ. p21 activated kinases in cancer. *Nat Rev Cancer* 2006;6:459–471
41. Dorsam RT, Gutkind JS. G-protein coupled receptors and cancer. *Nat Rev Cancer* 2007;7:79–95
42. Donnini S, Finetti F, Solito R et al. EP2 prostanoid receptor promotes squamous cell cancer growth through EGF-receptor transactivation. *FASEB J* 2007;21:2418–2430
- 43a. Sumimoto H, Imabayashi F. The BRAf-MAPK signal pathway is essential for cancer-immune evasion in human melanoma cells. *J Exp Med* 2006;203:1651–1656
- 43b. Bedogni B, Warneke JA, Nickoloff BJ et al. Notch1 is an effector of Akt and hypoxia in melanoma development. *J Clin Invest* 2008;118:3660–3670
44. Curtin JA, Fridlyand J, Kageshita T et al. Distinct sets of genetic alterations in melanoma. *N Engl J Med* 2005;353:2135–2147
45. Miller AJ, Mihm MC. Melanoma. *N Engl J Med* 2006;355:51–65
46. Yager JD, Liehr JG. Molecular mechanisms of estrogen carcinogenesis. *Annu Rev Pharmacol Toxicol* 1996;36:203–232
47. Fan S, Ma Y-Y, Wang C, Yuan R et al. Role of direct interaction in BRCA1 inhibition of estrogen receptor activity. *Oncogene* 2001;20:77–87
48. Poole AJ. Prevention of BRCA1 mammary tumorigenesis in mice by a progesterone antagonist. *Science* 2006;314:1467–1470
49. Esslimani-Sahla M, Thezenas S, Simony-Lafontaine J et al. Increased expression of fatty acid synthase in early steps of human mammary carcinogenesis. *Int J Cancer* 2006;120:224–229
50. Clarke PR, Sanderson HS. A mitotic role for BRCA1/BARD1 in tumor suppression. *Cell* 2006;127:453–455
51. Xu X, Wagner KU, Larson D. Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumor formation. *Nat Genet* 1999;22:37–43

52. Deng C-X, Scott F. Role of tumor suppressor gene BRCA-1 in genetic stability and mammary gland tumor formation. *Oncogene* 2000;19:1059–1064
53. Deng C-X, Brodie SG. Roles of BRCA-1 and its interacting proteins. *Bioessays* 2000;22:728–737
54. Li S, Ting NSY, Zheng L et al. Functional link of BRCA1 and ataxia telangiectasia gene product in DNA damage response. *Nature* 2000;406:210–215
55. Haber D. BRCA1: an emerging role in the cellular response to DNA damage. *Lancet* 2000;355:2090–2091
56. Hedenfalk I, Duggan D, Cen YD et al. Gene expression profiles in hereditary breast cancer. *N Engl J Med* 2001;344:539–544
57. Saal L, Gruvberger-Saal SK, Persson C et al. Recurrent gross mutations of the PTEN tumor suppressor gene in breast cancers with deficient DSB repair. *Nat Genet* 2007;40:102–107
58. Patocs A, Zhang L, Xu Y et al. Breast cancer stromal cells with TP53 mutations and nodal metastases. *N Engl J Med* 2007;357:2543–2551
59. Liu CH, Chang SH, Narko K, Trifan OC et al. Overexpression of Cox2 is sufficient to induce tumorigenesis in transgenic mice. *J Biol Chem* 2001;276:18563–18569
60. Polyak K. Breast cancer: origins and evolution. *J Clin Invest* 2007;117:3155–3163
61. Yehiely F, Moyano JV, Evans JR et al. Deconstructing the molecular portrait of basal-like breast cancer. *Trends Mol Med* 2006;12:537–544
62. Sansone P, Storci G, Tavolari S et al. Il-6 triggers malignant features in mammospheres from human ductal breast carcinoma. *J Clin Invest* 2007;117:3988–4002
63. Myatt SS, Lam EW-F. The emerging roles of forkhead box(Fox) proteins in cancer. *Nat Rev Cancer* 2007;7:847–860
64. Russo J, Fernandez SV, Russo PA et al. 17 β -estradiol induces transformation and tumorigenesis in human breast epithelial cells. *FASEB J* 2006;20:1622–1634
65. Yager JD, Davidson NE. Estrogen carcinogenesis in breast cancer. *N Engl J Med* 2006;354:270–282
66. Wei X, Xu H, Kufe D. Muc1 oncoprotein stabilizes and activates estrogen receptor- α . *Mol Cell* 2006;21:295–305
67. Lange CA, Gioeli D, Hammes SR, Marker PC. Integration of rapid signaling events with steroid hormone receptor action in breast and prostate cancer. *Annu Rev Phys* 2007;69:171–199
68. Maida Y, Kyo S, Kanaya T et al. Direction activation of telomerase by EGF through Ets mediated transactivation of TERT via MAP kinase signaling pathway. *Oncogene* 2002;21:4071–4079
69. Arendt LM, Rose-Hellekant TA, Sondaren EP, Schuler LA. Prolactin provides TGF α induction of mammary neoplasia in transgenic mice. *Am J Pathol* 2006;168:1365–1374
70. Martinez-Lacoci I, Karnan S, DeSantis M et al. Ras transformation causes activation of EGF receptors and elevation of MAP kinase in human mammary epithelial cells. *Int J Cancer* 2000;88:44–52
71. Brandt R, Eisenbrandt H, Leenders F et al. Mammary gland specific hEGF-receptor transgene expression induces neoplasia. *Oncogene* 2000;19:2129–2134
72. Schroeder JA, Troyer KL, Lee DC. Cooperative induction of mammary tumorigenesis by TGF α and Wnt. *Oncogene* 2000;19:3193–3199
73. Ayyanan A, Civinni L, Cialani L et al. Increased Wnt signaling triggers oncoconversion of human breast epithelial cells by a Notch dependent mechanism. *Proc Natl Acad Sci U S A* 2006;103:3799–3804
74. Yook JI, Li X-Y, Ota I et al. A Wnt-Axin2-GSK3 β cascade regulates Snail1 activity in breast cancer cells. *Nat Cell Biol* 2006;8(12):1398–1406
75. Wiseman BS, Werb Z. Stromal effects in mammary gland development and breast cancer. *Science* 2002;296:1046–1049
76. Rose-Hellekant TA, Sandgren EP. TGF α and c-myc induced mammary carcinogenesis in transgenic mice. *Oncogene* 2000;19:1092–1096
77. Atanaskova N, Keshamouni VG, Krueger JS et al. MAPkinase/estrogen receptor crosstalk enhances estrogen mediated signaling and tumor growth. *Oncogene* 2002;21:3000–3008

78. Carvajal A, Espinoza N, Kato S et al. Progesterone pre-treatment potentiates EGF pathway signaling. *Breast Cancer Res Treat* 2005;94(2):171–183
79. Mercurio AM, Backelder RE, Bates RC, Chung J. Autocrine signaling in carcinoma: VEGF and $\alpha 6 \beta 4$ integrin. *Semin Cancer Biol* 2004;14:115–122
80. Comoglio PM, Trusolino L. Cancer: the matrix is now in control. *Nat Med* 2005;11:1156–1159
81. Holbro T, Hynes NE. ErbB receptors: directing key signaling networks throughout life. *Annu Rev Pharmacol Toxicol* 2004;44:195–217
82. Menard S, Tagliabue E, Campiglio M, Pupa SM. Role of Her gene overexpression in breast cancer. *J Cell Physiol* 2000;182:150–162
83. Li L, Cleary S, Mandarano MA et al. Breast protooncogene HGR α regulates epithelial proliferation and lobuloalveolar development in mouse mammary gland. *Oncogene* 2002;21:4900–4907
84. Adam L, Vadlamudi RK, Kondapaka SB et al. Heregulin regulates cytoskeletal reorganization and cell migration through PAK.1 and PI3kinase. *J Biol Chem* 1998;273:28238–28246
85. Rosario M, Birchmeier W. How to make tubules: signaling by the Met receptor tyrosine kinase. *Trends Cell Biol* 2003;13:328–335
- 86a. Matteucci E, Locati M, Desiderio MA. Hepatocyte growth factor enhances CXCR4 expression favouring breast cancer cell invasiveness. *Exp Cell Res* 2005;310:176–185
- 86b. Li YM, Pan Y, Wei Y, Cheng X et al. Upregulation of CXCR4 is essential for Her2 mediated tumor metastasis. *Cancer Cell* 2004;6:459–469
87. Regis G, Pensa S, Boselli D et al. Ups and downs: the STAT1:STAT3 seesaw of Interferon and gp130 receptor signalling. *Semin Cell Dev Biol* 2008;19:351–359
88. Clevenger CV. Roles and regulation of Stat family transcription factors in human breast cancer. *Am J Pathol* 2004;165:1449–1458
89. Yu H, Kortylewski M, Pardoll D et al. Crosstalk between cancer and immune cells: role of Stat3 in the tumour microenvironment. *Nat Rev Immunol* 2007;7:41–51
90. Liu Y, Lu C, Shen Q, Muniz-Medelling D et al. AP-1 blockade in breast cancer cells causes cell cycle arrest by suppressing G1 cyclin expression and reducing cyclin-dependent kinase activity. *Oncogene* 2004;23:8238–8246
91. Arribas J. Matrix metalloproteases and tumor invasion. *N Engl J Med* 2005;352:2020–2021
92. Koike T, Kimura N, Miyazaki K et al. Hypoxia induces adhesion molecules on cancer cells. *Proc Natl Acad Sci U S A* 2004;101:8132–8137
93. Mayr C, Hermann MT, Bartel DP. Disrupting the pairing between let7 and Hmga2 enhances oncogenic transformation. *Science* 2007;16:1576–1579
94. Thomas DA, Massague J. TGF β targets cytotoxic T cell functions during tumor evasion of immune surveillance. *Cancer Cell* 2005;8:369–380
- 95a. Gallina G, Dolcetti L, Serafini P et al. Tumors induce a subset of inflammatory monocytes with immunosuppressive activity on CD8 T cells. *J Clin Invest* 2006;116:2777–2790
- 95b. Markiewski MM, DeAngelis RA, Benencia F et al. Modulation of the antitumor immune response by complement. *Nat Immunol* 2008;9:1225–1235
96. Schlessinger K, Hall A. GSK-3 β sets snail pace. *Nat Cell Biol*.2004;6:913
97. Coughlin SR. Thrombin signalling and protease activated receptors *Nature* 2000;407:258–264
98. Burstein HJ. The distinctive nature of HER2-positive breast cancers. *N Engl J Med* 2005;353:1652–4
99. De Marzo AM, Nelson WG, Meeker AK, Coffey DS. Stem cell features of benign and malignant prostate epithelial cells. *J Urol* 1998;160:2381–2392
100. Xin L, Lawson DA, Witte ON. The Sca-1 cell surface marker enriches for a prostate regenerating cells that initiate prostate tumorigenesis. *Proc Natl Acad Sci U S A* 2005;102:6942–6948
101. Li W, Wu C-L, Febbo PG, Olnvi AF. Stromally expressed c-jun regulates proliferation of prostate epithelial cells. *Am J Pathol* 2007;171:1189–1198

102. Wu CT, Altuwaijri S, Ricke WA et al. Increased prostate cell proliferation and loss of cell differentiation in mice lacking prostate epithelial androgen receptor. *Proc Natl Acad Sci U S A* 2007;104:12679–12684
103. Gamble SC, Odentiadis M, Waxman J et al. Androgens target prohibitin to regulate proliferation of prostate cancer cells. *Oncogene* 2004;23:2996–3005
104. Landstrom M, Eklow S, Colosetti P, Nillson S. Estrogen induces apoptosis in a rat prostatic adenocarcinoma: association with expression of TGF β 1 and its receptors. *Int J Cancer* 1996;67:573–576
105. Seone J. Escaping from TGF β antiproliferative control. *Carcinogenesis* 2006;27:2148–2156
- 106a. Assinder SJ, Dong Q, Kovacevic Z, Richardson DR. The TGF β , PI3K/Akt and PTEN pathways: established and proposed biochemical integration in prostate cancer. *Biochem J* 2009;417:411–421
- 106b. Matthews E, Yang T, Janulis L et al. Down-regulation of TGF β 1 production restores immunogenicity in prostate cancer cells: *Br J Cancer* 2000;83:519–525
107. Ngn ESW, Hashimoto Y, Ma Z-Q. Overexpression of cdc25B in prostate cancer. *Oncogene* 2004;23:734–739
108. Uzgar AR, Isaacs JT. Prostate cancer: potential targets of anti-proliferative and apoptotic signalling pathways. *Int J Biochem* 2005;37:707–714
109. Hong H, Kao C, Jeng M-H et al. Aberrant expression of CARM1 in the development of prostate cancer. *Cancer* 2004;101:83–89
110. Foster CS, Falcner A, Dodson AR et al. Transcription factor E2F3 overexpressed on prostate cancer predicts clinical outcome. *Oncogene* 2004;23:5871–5879
111. Tang DG, Bhatia B, Tang S, Broussard RS. 15-LOX-2 is a tumor suppressor that regulates human prostate epithelial cell differentiation and growth *Prosta. Lipid Med* 2007;82:135–146
112. His LC, Wilson LC, Eling TE. Opposing effects of 15Lox-1 and -2 metabolites on MAPK signaling in prostate. *J Biol Chem* 2002;277:40549–40556
113. DeMarzo AM, Nelson WG, Isaacs WB, Epstein JI. Pathological and molecular aspects of prostate cancer. *Lancet* 2003;361:955–963
114. Narla G, Heath KE, Reeves HL et al. KLF6 a candidate tumor suppressor gene mutated in prostate cancer. *Science* 2001;361:955–963
115. Santamaria A, Fernandez PL, Farre X. PTOU-1, a novel protein overexpressed in prostate cancer. *Am J Pathol* 2003;162:897–905
116. Petrovics G, Liu A, Shaheduzzaman S et al. Frequent overexpression of ETS related gene (ERG1) in prostate cancer transcriptosome. *Oncogene* 2005;24:3847–3852
117. Tomlins SA, Rhodes DR, Perner S et al. Recurrent fusion of TMPRSS2 and Ets transcription factor genes in prostate cancer. *Science* 2005;310:644–648
118. Chan H-H, Xue-cheng BI, Qi-Shan D et al. Detection of pim-1 mRNA in prostate cancer diagnosis. *Chin Med J* 2007;120:1491–1493
119. Erlich S, Tal-Or P, Liebling R et al. Ras inhibition results in growth arrest and death of androgen-dependent and independent prostate cancer cells. *Biochem Pharmacol* 2006;72:427–436
120. Xin L, Teitell MA, Lawson DA et al. Progression of prostate cancer by synergy of Akt with the androgen receptor. *Proc Natl Acad Sci U S A* 2006;103:7789–7794
121. Kremer CL, Klein RR, Mendelson J et al. Expression of mTOR signaling pathway markers in prostate cancer progression. *Prostate* 2006;66:1203–1212
122. Mirosevich J, Gao N, Gupta A et al. Expression and role of Foxo proteins in prostate cancer. *Prostate* 2006;66:1013–1028
123. Datta S, Pierce M, Datta MW. Perlecan signaling: helping hedgehog stimulate prostate cancer growth. *Int J Biochem Cell Biol* 2006;38:1855–1861
124. Yuan X, Li T, Wang H et al. Androgen receptor remains critical for cell-cycle progression in androgen-independent CWR22 prostate cancer cells: *Am J Pathol* 2006;119:682–696
125. Taichman RS, Loberg RD, Mehra R, Pienta K. The evolving biology and treatment of prostate cancer. *J Clin Invest* 2007;9:2351–2361

126. Wu C, Huang J. PI3kinase-Akt-mTOR pathway is essential for neuroendocrine differentiation of prostate cancer. *J Biol Chem* 2007;282:3571–3583
127. Shukla S, MacLennan GT, Hartmann DJ et al. Activation of PI3kinase-Akt signal pathway promotes prostate cancer cell invasion. *Int J Cancer* 2007;121:1424–1432
128. Xin W, Rhodes DR, Ingold C et al. Dysregulation of the annexin family and prostate cancer progression. *Am J Pathol* 2003;162:255–261
129. Wang L, Lin H-K, Hu Y-C et al. Suppression of androgen receptor mediated trans-activation and cell growth by GSK3 in prostate cells. *J Biol Chem* 2004;279:32444–324452
130. Yang F, Li X, Sharma M, Sasaki CT et al. Linking β -catenin to androgen signaling pathway. *J Biol Chem* 2002;277:11336–11344
131. Rao DS, Gui D, Koski NE et al. Inverse relation between cyclooxygenase-2 and E-cadherin expression correlates with aggressive histological features in prostate cancer. *Appl Immunohistochem Mol Morphol* 2006;14:375–383
132. Chen JZ, Kadlubar FF. Mitochondrial mutagenesis and oxidative stress in human prostate cancer. *J Environ Sci Health* 2004;22:1–12
133. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. *Nat Rev Cancer* 2001;1:34–45
134. Gioeli D. Signal transduction in prostate cancer. *Clin Sci* 2005;108:293–308
135. Febbo PG, Lowenberg M, Thorner AR et al. Androgen mediated regulation and functional implications of fkbp51 expression in prostate cancer. *J Urol* 2005;173:1772–1777
136. Zhang Y, Wang X-W, Jelovac D et al. The ErbB3 binding protein Ebp1 suppresses androgen receptor mediated gene transcription and tumorigenesis of prostate cancer cells. *Proc Natl Acad Sci U S A* 2005;102:98990–9895
137. OlapadeOlaopa EO, Moscatello DK, Mackay EH et al. Alterations in the expression of androgen receptor, wild type EGF receptor and a mutant EGF receptor in human prostate cancer. *Afr J Med Sci* 2004;33:245–253
138. Gao H, Ouyang X, Banach-Petrosky WA et al. Combinatorial activities of Akt and B-Raf/Erk signaling in a mouse model of androgen independent prostate cancer. *Proc Natl Acad Sci U S A* 2006;103:14477–14482
139. Bakin RE, Gioeli D, Sikes RA, Bissonette EA, Weber MJ. Constitutive activation of the Ras/MAPkinase signaling pathway promotes androgen hypersensitivity in LNCaP prostate cancer cells. *Cancer Res* 2003;63:1981–1989
140. Debes JD, Tindall DJ. Mechanisms of androgen refractory prostate cancer. *N Engl J Med* 2004;351:1488–1490
141. Wegiel B, Bjartell A, Culig Z, Persson J. Il-6 activates the PI3kinase/Akt pathway and cyclin A1 to promote prostate cancer cell survival. *Int J Cancer* 2008;122:1521–1529
142. Hermeking H, Benzinger A. 14-3-3 proteins in cell cycle regulation. *Semin Cancer Biol* 2006;16:183–192
143. Luo Y, He DL, Ning L et al. HIF1 α induces the epithelial-mesenchymal transition of human prostate cancer cells. *Chin Med J* 2006;119:713–718
144. Kelly P, Stemmler LN, Madden JF et al. A role for the G12 family of G-proteins in prostate cancer invasion. *J Biol Chem* 2006;281:26483–26490
145. Meyer-Siegler KL, Iczkowski KA, Leng L et al. Inhibition of macrophage MIF or its receptor(CD74) attenuates growth and invasion of DU145 prostate cancer cells. *J Immunol* 2006;177:8730–8739
146. Xu L, Bergan RC. Genistein inhibits MMP type 2 activation and prostate cancer cell invasion. *Mol Pharmacol* 2006;70:869–877
147. Miyamoto H, Altuwaijri S, Cai Y et al. Inhibition of the Akt,Cox-2 and MMP-9 pathways in combination with androgen deprivation therapy: potential therapeutic approaches for prostate cancer. *Mol Carcinog*. 2005;44:1–10
148. Li L, Chapman K, Hu X et al. Modulation of the oncogenic potential of β -catenin by the subcellular distribution of plakoglobin. *Mol Carcinog* 2007;46:824–838
149. Cowin P, Rowlands TM, Hatsell SJ. Cadherins and catenins in breast cancer. *Curr Opin Cell Biol* 2005;17:499–508

150. Hardy RG, Vicente-Duenas C, Gonzalez-Herrero I et al. Snail family transcription factors are implicated in thyroid carcinogenesis. *Am J Pathol* 2007;171:1037–1046
151. Morin PJ, Sparks AB, Korinek Y et al. Activation of β -catenin-Tcf signaling in colon cancer by mutations in β -catenin or APC: *Science* 1997;275:1787–1790
152. Taipale J, Beachy PA. The hedgehog and Wnt signalling pathways in cancer. *Nature* 2001;411:349–354
153. Campbell IG, Russell SE, Choong DY et al. Mutation of the PIK3CA gene in ovarian and breast cancer. *Cancer Res* 2004;64:7678–7681
154. Hennessy BT, Mills GB. Ovarian cancer: Homeobox genes, autocrine/paracrine growth and kinase signaling. *Int J Biochem Cell Biol* 2006;38:1450–1456
155. Dinulescu DM, Ince TA, Quade BJ et al. Role of K-Ras and Pten in development of mouse models of endometriosis and endometrial ovarian cancer. *Nat Med* 2005;11:63–70
156. Cheng W, Liu J, Yoshida H, Rosen D, Naora H. Lineage infidelity of epithelial ovarian cancers is controlled by HOX genes that specify regional identity in the reproductive tract. *Nat Med* 2005;11:531–537
157. Obermair A, Schmid BC, Packer LM et al. Expression of Muc1 splice variants in benign and malignant ovarian tumours. *Int J Cancer* 2002;100:166–171
158. Kamikihara T, Arima T, Kato K et al. Epigenetic silencing of the imprinted gene ZAC by DNA methylation is an early event in progression of human ovarian cancer. *Int J Cancer* 2005;115:690–700
159. Dimova DK, Dyson NJ. The E2F transcriptional network. *Oncogene* 2005;24:2810–28
160. Reimer D, Sadr S, Wiedemair A-M et al. Expression of the E2F family of transcription factors and its clinical relevance in ovarian cancer. *Ann N Y Acad Sci* 2006;1091:270–281
161. Mills GB, Moolenaar WH. The emerging role of lysophosphatidic acid in cancer. *Nat Rev Cancer* 2003;3:582–591
162. Kumar SR, Masood R, Spannuth WA et al. The receptor tyrosine kinase EphB4 is overexpressed in ovarian cancer and provides survival signals. *Br J Cancer* 2007;96:1083–1091
163. Sarrio D, Moreno-Bueno G, Sanchez-Estevéz C et al. Expression of cadherins and catenins correlates with types of ovarian carcinomas: *Hum Pathol* 2006;37:1042–1049
164. Cho EY, Choi Y, Chae SW, Sohn JH, Ahn GH. Immunohistochemical study of expression of adhesion molecules in ovarian serous neoplasms. *Pathol Int* 2006;56:62–72
165. Reddy P, Liu L, Ren C, Lindgren P et al. Formation of E-cadherin mediated cell-cell adhesion activates Akt and MAPK via PI3kinase and ligand-independent activation of EGF receptor in ovarian cancer cells. *Mol Endocrinol* 2005;19:2564–7258
166. Voutilainen KA, Anttila MA, Sillanpää SM et al. Prognostic significance of E-cadherin-catenin complex in epithelial ovarian cancer. *J Clin Pathol* 2006;59:460–467
167. Mackay CR, Terpe HJ, Stauder R. Expression and modulation of CD44 variant isoforms in human disease. *J Cell Biol* 1994;124:71–82
168. DiFeo A, Naria G, Camacho-Vanegas O et al. E-cadherin is a target of the KLF6 tumor suppressor. *Oncogene* 2006;25:6026–6031
169. Hamonishi J, Mandai M, Iwasaki M et al. Programmed cell death ligand-1 and tumor infiltrating CD8 T lymphocytes are prognostic factors of human ovarian cancer. *Proc Natl Acad Sci U S A* 2007;104:3360–3365
170. Shigeto T, Yokoyama Y, Xin B, Mizunuma H. PPAR alpha and gamma ligands inhibit the growth of human ovarian cancer. *Oncol Rep* 2007;18:833–40
171. Skolarikos AA, Papatsoris AG, Alivizatos G, Deliveiotis C. Molecular pathogenetics of renal cancer. *Am J Nephrol* 2006;26:218–231
172. Brugarolas J. Renal cell carcinoma-molecular pathways and therapies. *N Engl J Med* 2007;356:185–187
- 173a. Rosendahl AH, Forsberg G. IGF-1 and IGFBP-3 augment TGF β actions in human renal carcinoma cells. *Kidney Int* 2006;70:1584–1590
- 173b. Sjölund J, Johansson M, Manna S et al. Suppression of renal cell cancer growth by inhibition of Notch signaling. *J Clin Invest* 2008;118:217–228
174. Barker N, Cleaves H. Catenins, Wnt signaling and cancer: *Bioessays* 2000;22:961–965

175. Robinfeld B, Souza B, Albert I et al. Association of the APC gene with beta-catenin. *Science* 1993;262:1731–1733
176. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. *N Engl J Med* 2003;348:919–932
- 177a. Rosin-Arbesfeld R, Townsley P, Bienz M. The APC tumor suppressor has a nuclear export function. *Nature* 2000;406:1009–1012
- 177b. Marsh V, Winton DJ, Williams GT et al. Epithelial Pten suppresses adenoma development and progression after Apc mutation. *Nat Genet* 2008;40:1436–1444
178. Reddy BS, Burill C, Rigotty J. Effects of diets high in ω -3 and ω -6 fatty acids on initiation and post-initiation stages of colon carcinogenesis. *Cancer Res* 1991;51:487–491
179. Debruyne PR, Bryneel EA, Karaguni I-M et al. Bile acids stimulate invasion and haptotaxis in human colorectal cancer cells. *Oncogene* 2002;21:6740–6750
180. Oshima H, Oshima M, Kobayashi M et al. Morphologic and molecular processes of polyp formation in APC knockout mice. *Cancer Res* 1997;57:1644–1649
181. Tetsu O, McCormack F. β -Catenin regulates expression of cyclin D1 in colon cancer cells. *Nature* 1999;398:422–426
182. He TC, Sparks AB, Rago C et al. Identification of c-Myc as a target of the APC pathway. *Science* 1998;281:1509–1512
183. Koh TJ, Bulitta CJ, Fleming JV et al. Gastrin is a target of the β -catenin/TCF4 growth signalling pathway in a model of intestinal polyposis. *J Clin Invest* 2000;106:533–539
184. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990;61:L759–767
185. Oving IM, Clevus HC. Molecular causes of colon cancer. *Eur J Clin Invest* 2002;32:448–457
186. Sparks AB, Morin PJ, Vogelstein B, Kinzler KW. Mutational analysis of APC/beta-catenin/TCR path in colorectal cancer. *Cancer Res* 1998;58:1130–1134
187. Arends JW. Molecular interactions in the Vogelstein model of colorectal carcinoma. *J Pathol* 2000;190:412–416
188. Mazelin L, Bernet A, Bonod-Bidaud C et al. Nectin 1 controls colorectal tumorigenesis by regulating apoptosis. *Nature* 2004;431:80–83
189. Liu ESL, Shin VY. Nicotine promoted colon cancer growth via EGF receptor, and 5-lipoxygenase mediated signal pathway. *J Pharmacol Exp Ther* 2004;308:66–72
190. Klaunig JE, Kamendulis LM. The role of oxidative stress in carcinogenesis. *Annu Rev Pharm Toxicol* 2004;44:239–267
191. Rowley PT. Inherited susceptibility to colorectal cancer. *Annu Rev Med* 2005;56:539–554
192. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. *N Engl J Med* 2003;348:919–932
193. Mehlen P, Rabizadeh S, Snipas SJ et al. The DCC gene product induces apoptosis by a mechanisms requiring receptor proteolysis. *Nature* 1998;395:801–804
194. de Costa LT, He TC, Yu J, Sparks AB et al. CDK2 is mutated in colorectal cancer with normal APC/ β -catenin signaling. *Oncogene* 1999;18:5010–5016
195. Akiyama Y, Iwanage R, Saitoh K et al. TGF β type II receptor gene mutations in adenomas from hereditary non-polyposis colorectal cancer. *Gastroenterology* 1997;112:33–39
196. Liu Y-M, Furukawa Y, Tsunada T et al. Molecular diagnosis of colorectal tumors by expression profiles. *Oncogene* 2002;21:4120–4128
197. Hart IR, Saini R. Biology of tumour metastases. *Lancet* 1992;339:1453–1458
198. Tanake KT, Ellis LM, Saya H. Expression of CD44Ra adhesion molecule in colon carcinomas and metastases. *Lancet* 1993;341:725–726
199. Ding Y, Dale T. Wnt signal transmission. *Trends Biochem Sci* 2002;27:327–329
200. Conacci-Sorrell M, Zhurinsky J, Ben-Ze'ev A. The cadherin-catenin adhesion system in signaling and cancer. *J Clin Invest* 2002;108:987–1006
201. Wielenga V, van der Neut R, Offerhaus G, Pals ST. CD44 glycoprotein in colorectal cancer. *Adv Cancer Res* 2000;77:169–221
202. Wielenga VJM, van der Toort R, Fritz P et al. Expression of c-Met and heparin sulfate proteoglycan forms of CD44 in colon cancer. *Am J Pathol* 2000;157:1563–1574
203. Dumont M, Bakin AV, Artenga CL. Autocrine TGF β signaling mediates Smad-independent motility in human cancer cells. *J Biol Chem* 2003;278:3275–3285

204. Schmitz AAP, Govek E-E, Bottner B, van Aelst L. Rho-GTPases: signaling, migration and invasion. *Exp Cell Res* 2000;261:1–12
205. Deshayes F, Nahmias C. Angiotensin II receptors: a new role in cancer? *Trends Endocrinol Metab* 2005;16:293–299
206. Xu X-M, Wang D, Shen Q et al. RNA mediated gene silencing of the RON receptor tyrosine kinase alters oncogenic phenotypes of colorectal carcinoma cells. *Oncogene* 2004;23:8464–8474
207. Sheng H, Shao J, Kirland SC et al. Inhibition of human colon cancer cell growth by selective inhibition of Cox2. *J Clin Invest* 1997;99:2254–2258
208. Strak LA, Din FVN, Zwacka RM, Dunlop MG. Aspirin induced activation of NFkB signaling pathway: a novel mechanism for aspirin mediated apoptosis on colon cancer cells. *FASEB J* 2001;15:1273–1275
209. Imperiale TF. Aspirin and prevention of colorectal cancer. *N Engl J Med* 2003;348:879–880
210. Dihshit P, Chatterjee M, Goswami A et al. Aspirin induces apoptosis through inhibition of proteasome function. *J Biol Chem* 2006;281:29228–29235
211. Furstenberger G, Krieg P, Muller-Decker K, Habenicht AJR. What are cyclo-oxygenases and lipoxygenases doing in carcinogenesis? *Int J Cancer* 2006;119:2247–2254
212. Hull MA, Ko SC, Hawcroft G. Prostaglandin EP receptors: targets for treatment and prevention of colorectal cancer. *Mol Cancer Ther* 2004;3:1031–1039
213. Shao J, Lee SB, Guo H et al. PGE2 stimulates growth of colon cancer cells via induction of amphiregulin. *Circ Res* 2003;63:5218–5223
214. Zhang L, Yu J, Park BH, Kinzler KW, Vogelstein B. Role of Bax in apoptotic response to anticancer agents. *Science* 2000;290:289–292
215. Shao J, Jung C, Liu C, Sheng H. PGE2 stimulates the β -catenin/TCF dependent transcription in colon cancer. *J Biol Chem* 2005;280:26565–26572
216. Pa R, Soreghan B, Szabo IL et al. PGE2 transactivates EGF receptors: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. *Nat Med* 2002;8:289–229
217. Burstein E, Fearon ER. Colitis and cancer: a tale of inflammatory cells and their cytokines. *J Clin Invest* 2008;118:464–467
218. Dihlmann S, Siemann A, van Knebel Doeberitz M. The NSAIDs, aspirin and indomethacin, attenuate β -catenin/TCF4 signaling. *Oncogene* 2001;20:645–653
219. Chan T, Morin PJ, Vogelstein B, Kinzler KW. Mechanisms involving non-steroidal anti-inflammatory drug mediated apoptosis. *Proc Natl Acad Sci U S A* 1998;95:681–686
220. Turchanowa L, Dauletbaev N, Milovic V, Stein J. NSAIDs stimulate spermidine: spermine acetyltransferase and deplete polyamine content in colon cancer cells. *Eur J Clin Invest* 2001;31:887–893
221. Sharma S, Zhu L, Yang SC et al. Cox2 enzyme inhibition promotes IFN γ dependent enhancement of antitumor responses. *J Immunol* 2005;174:813–818
222. Langenbach R, Loftin CD, Lee C, Tiano H. Cox-deficient mice: susceptibilities to carcinogenesis. *Ann N Y Acad Sci* 1999;889:52–61
223. Oshima M, Dinchuk JE, Kargman SL et al. Suppression of intestinal polyposis in APC 716 knockout mice by inhibition of Cox-2. *Cell* 1996;87:803–809
224. Cha Y I, DuBois RN. NSAIDs and cancer prevention. *Annu Rev Med* 2007;58:239–252
225. Lim JW, Kim H, Kim KH. NF-kappaB regulates Cox2 expression and cell proliferation in human gastric cancer cells. *Lab Invest* 2001;81:349–360
226. Banu N, Buda A, Chell S et al. Inhibition of Cox2 decreases colon cancer cell motility through blocking EGF receptor transactivation. *Cell Prolif* 2007;40(5):768–779
227. Desreumaux P, Dubuquoy L, Nullen S et al. Attenuation of colon inflammation by RXR/PPAR heterodimer. *J Exp Med* 2001;193:827–838
228. Corton JC, Anderson SP, Stauter A. Central role of peroxisome proliferator activated receptors in the actions of peroxisome proliferators. *Annu Rev Pharmacol Toxicol* 2000;40:491–518

229. Crwoss HS, Lipkin M, Kalloy E. Nutrients regulate the colonic vitamin D synthesis in mice: relevance for human colonic malignancy. *J Nutrition* 2006;136:561–564
230. Jimenez-Lara AM. Colorectal cancer: potential therapeutic benefits of vitamin D. *Int J Biochem Cell Biol* 2007;37:672–677
231. Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nat Rev Cancer* 2007;7:684–696
232. Harkins L, Volk AL, Samanta M et al. Specific localisation of human CMV nucleic acids and proteins in human colorectal cancer. *Lancet* 2002;360:1557–1563
233. Guarino M. Epithelial-mesenchymal transition and tumour invasion. *Int J Biochem Cell Biol* 2007;39:2153–2160
- 234a. Sidani M, Wessels D, Mouneimne G et al. Cofilin determines migration behaviour of metastatic cancer cells. *J Cell Biol* 2007;179:777–791
- 234b. Sanchez-Garcia I. The crossroads of oncogenesis and metastasis. *New Engl J Med* 2009;360:297–300
235. Kitamura T, Taketo M. Keeping out the bad guys. *Cancer Res* 2007;67:10099–10102
236. Chiang A, Massague J. Molecular basis of metastasis. *New Engl J Med* 2008;359:2814–2823
237. Manavathi B, Kumar R. Metastasis tumor antigens. *J Biol Chem* 2007;282:1529–1533
238. Xu L, Chen S, Bergab RC. p38 activates mitogen activated protein kinase activated protein kinase 2 which activates hsp27 and both regulate cell invasion. *Oncogene* 2006;25:2987–2998
239. Knutson KL, Lu H, Stone B et al. Immunoediting of cancers may lead to epithelial to mesenchymal transition. *J Immunol* 2006;177:1526–1533
240. Kaplan RN, Riba RD, Zacharoutis S et al. VEGF.RI positive hematopoietic bone marrow progenitors initiate the premetastatic niche. *Nature* 2005;438:820–827
241. Rafii S, Lyden D. S100 chemokines mediate bookmarking of premetastatic niches. *Nat Cell Biol* 2006;8(12):1321–1323
242. Boccaccio C, Comoglio PM. Invasive growth: a MET-driven genetic programme for cancer and stem cells. *Nat Rev Cancer* 2006;6:637–644
243. Mazzone M, Comoglio PM. The Met pathway: master switch and drug target in cancer progression. *FASEB J* 2006;20:1611–1621
244. Arribas J. Matrix metalloproteinases and tumor invasion. *N Engl J Med* 2005;352:2020–2021
245. Mahanivang C, Yu J, Huang S. Elevated urokinase specific surface receptor expression is maintained through its interaction with urokinase plasminogen activator. *Mol Carcinog* 2007;46:165–176
246. Crippa MP. Urokinase type plasminogen activator. *Int J Biochem Cell Biol* 2007;39:690–694
247. Balkwill F. Cancer and the chemokine network. *Nat Rev Cancer* 2004;4:540–550

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