Chapter 11
Cell Defence and Survival

Abstract Central to immune defence mechanisms is the role of transcription factor nuclear factor kappa B (NF-κB). This is a complex biochemical topic with ever more controls revealed. NF-κB 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 perioxisome 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-κB) was identified in 1986 as a transcription factor (TF) that binds to the enhancer of the B cell immunoglobulin-κ light chain gene. NF-κB proteins are cell homologues of viral v-Rel. NF-κB 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 (IκB). The IκB proteins have ankyrin repeats that attach to the RHDs. When the IκB units are phosphorylated on two serine residues, they are ubiquitinated and degraded by the 26S proteasome. Thus, the IκBs 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′GGGRNNYYCC-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-κB 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-κB [8, 9]: (a) proinflammatory cytokines interleukin 1 (IL-1) and tumour necrosis factor-α (TNFα), (b) oxidants like H_2O_2, (c) ceramide, (d) protein kinase

a

surface receptor   eg p38 or
upstream kinases   ERK1/2

IKK1/α | IKK2/β
Nemo IKKγ

P   IκBα   P   IκBα-P2 inhibitor
                          destroyed by ubiquitination
                          liberating NFkB p50/p65
p50 | p65   (helped by oxidants)

                          amplifying loop via
                          Il-1β , TNFα

            50   65

chemokines(MCP-1) and cytokines   enzymes eg iNO synthase, Cox2
adhesion molecules (CAMs)   tissue factor and PAI-1
cyclin D1 + cMyc→ cell growth   apoptosis protection:survivin,TRAF1,IAP1/2,bcl-2,bclXl

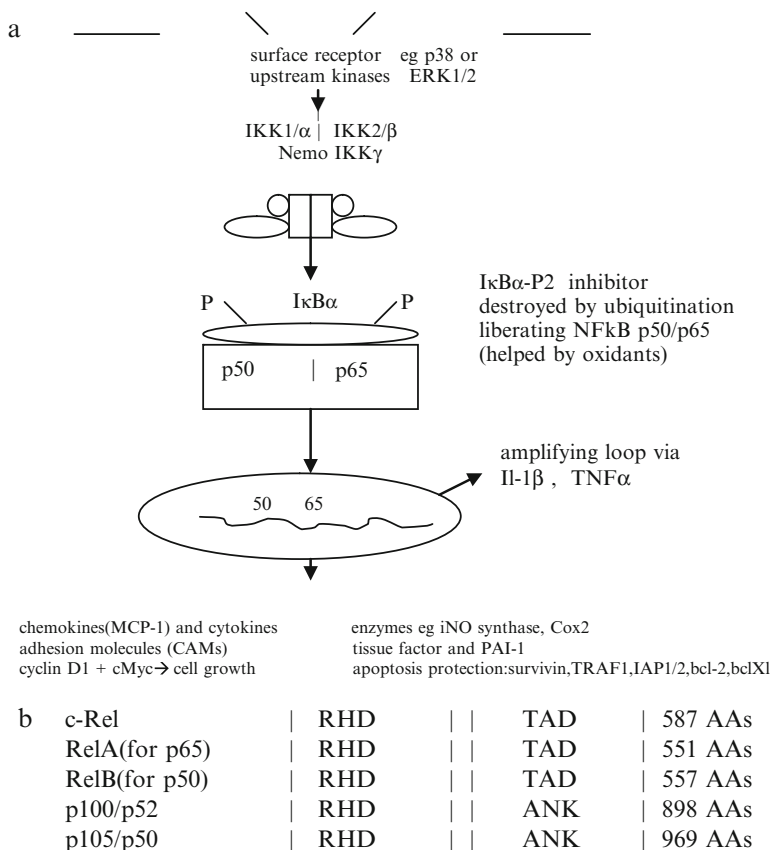| b				
c-Rel	RHD	| |	TAD	587 AAs
RelA(for p65)	RHD	| |	TAD	551 AAs
RelB(for p50)	RHD	| |	TAD	557 AAs
p100/p52	RHD	| |	ANK	898 AAs
p105/p50	RHD	| |	ANK	969 AAs

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 IκB inhibitors to be dissociated from the NF-κB units. Hence, intracellular reductants like glutathione and thioredoxin have differential roles in the redox regulation of target proteins, and S-nitrosylation can inhibit NF-κB DNA binding [13]. Yet, within nuclei it is the more reducing environment there that enables NF-κB RHDs to bind onto the DNA.

As you will now expect, zymosan proinflammatory agent stimulates NF-κB, 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-κB [15]. Examination of synovial tissue in rheumatoid arthritis shows marked expression of NF-κB1 at sites adjacent to the cartilage-pannus junction. NF-κB activation upregulates chemokines and adhesion molecules and neutrophils and macrophones are then recruited.

Extra comment about Nemo (IκB kinase-γ, IKKγ) 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-κB 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-κB Pathway

The IL-1β and TNFα cytokine receptors connect via upstream kinases to the activation of NF-κB [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 TNFα. Cytokine IL-1 can activate either IKKβ or the IKKα.

As shown in Fig. 11.1a, serine-threonine kinases IKKα and IKKβ form homo- or heterodimers in conjunction with stabilising protein Nemo (the NF-κB essential modulator), also termed IKKγ [4, 19]. Binding of Nemo to Lys63-polyUb-RIP1 forms the active kinase complex IKKγ, IKKβ-P, IKKα-P that phosphorylates the inhibitor unit IκBα [20], which then dissociates and is destroyed by ubiquitination. Accordingly, NF-κB as p50/RelA is free to enter nuclei. Oscillations in intranuclear NF-κB coupled to cycles of phosphorylation of RelA and IκB are required for NF-κB-dependent gene expression [21]. As revealed in Table 11.1, NF-κB 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-κB, such as IκBα 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 TNFα-induced NF-κB activation, a step that requires Richard TAXIBI [21b].

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

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

Class	Target gene
Immunoreceptors	Immunoglobulin κ light chain
	Interleukin 2 receptor α-chain
	Major histocompatability 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 (TNFα)
	Interleukin 1β (IL-1β), 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) 1α, 1β
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-κB in activated cells [22b]. Fortunately, there is control by heat shock proteins HSP70 and HSP90 which regulate the IKK complex [22c].

NF-κB plays a crucial role in inflammatory processes. Activation of p38 mitogen-activated protein kinase (MAP) kinase is well recognised to activate NF-κB [23]. If a single injection of lipopolysaccharide (LPS) is given intraperitoneally to mice, there is transient NF-κB activation in lung cell types. When the LPS is infused by a pump, there is widespread NF-κB 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-κB 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-κB activation. In resting neutrophils, RhoA GTPase inhibits the NF-κB to prevent TNFα production, and yet in LPS-stimulated neutrophils RhoA activates NF-κB and supports TNFα formation [27].

11.1.1.1 The Roles of Subunits: subtle feedback control mechanisms

The various homodimers and heterodimers of NF-κB are kept inactive by association with cytoplasmic inhibitory proteins [28]. They are IκBα, IκBβ and IκBε 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]. IKKα is a histone H3 kinase that moves into nuclei to associate with the promoters of NF-κB-responsive genes [30, 31]. IKKα

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

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

As for IKKβ, it can increase the activity of hypoxia-inducible factor 2α (HIF2α) [33b], so linking hypoxia to innate immunity and inflammation, and it activates a subset of (IFNγ)-stimulated genes. For sure IKKβ promotes the canonical pathway of NF-κB activation (Fig. 11.1a) [29]. IKKβ promotes the MAP kinases and cell proliferation (e.g. in skin keratinocytes) [29]. All the actions are tissue specific. Yet, IKKβ exerts negative control over the production of IL-1β [34]. Furthermore in some situations IKKβ 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-κB activation [35]. Lymphotoxin, BAFF (B cell-activating factor), CD40L or RANKL (receptor activator of NF-κB ligand) activate NIK (NF-κB-inducing kinase), and it then acts on IKKα homodimer, whose target is NF-κB2, 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-κB2 is also required for establishment of thymic central tolerance through an Aire-dependent pathway [37]. AIRE is the autoimmune regulator gene, and NF-κB2 is required in medullary thymic epithelial cells.

Whenever Rankl stimulates osteoclastogenesis, the NF-κB2 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 ultraviolet light acts on skin to cause immunosuppression by induction of Treg cells, Rankl on keratinocytes first stimulates Rank on Langerhans cells. Vitamin D$_3$, which is immunosuppressive, also induces Rankl [40].
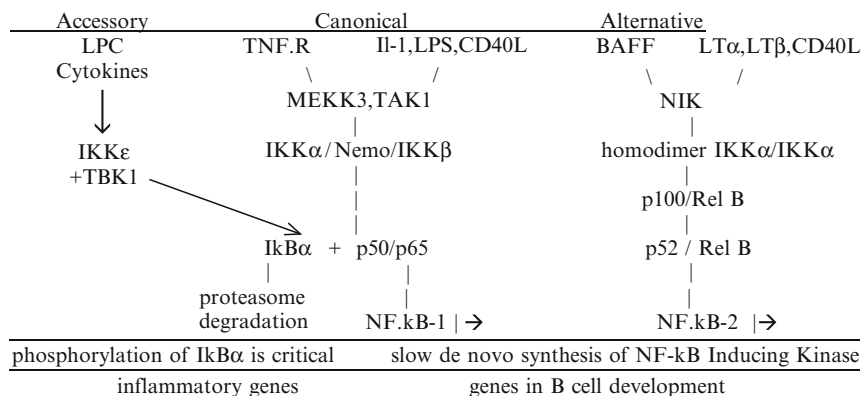
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        Accessory              Canonical                  Alternative
          LPC          TNF.R        Il-1,LPS,CD40L        BAFF      LTα,LTβ,CD40L
        Cytokines          \          /                      \          /
                          MEKK3,TAK1                            NIK
           |                  |                                  |
          IKKε         IKKα / Nemo/IKKβ          homodimer IKKα/IKKα
          +TBK1 \            |                                  |
                   \         |                               p100/Rel B
                     \       |                                  |
                      IκBα  +  p50/p65                      p52 / Rel B
                       |        |                                  |
                   proteasome   |                                  |
                   degradation  NF.kB-1 | →              NF.kB-2  | →
    ─────────────────────────────────────────────────────────────────────────
    phosphorylation of IκBα is critical    slow de novo synthesis of NF-kB Inducing Kinase
             inflammatory genes                  genes in B cell development
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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 H_2O_2, 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-κB 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 IFNγ. In fact, p53 downregulates Stat1 and proinflammatory cytokines [49]. In some cells, like leucocytes, IκBζ negatively regulates intranuclear NF-κB activity [50]. We have noted how LPS exposure can attenuate TNFα gene transcription because NF-κB p50 homodimers bind to the TNFα 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-κB1 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-κB (Table 11.2). Commensal gut bacteria like *Bacteroides* reduce the activity of NF-κB in gut

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

Physiological
Interleukins 10 and 13 transforming growth factor-β
IKBNS 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, acetylsalicyclic acid, sulindac (nonsteroidal anti-inflammatory, NSAID), sulfasalazine, mesalamine
Antioxidants: vitamin E, *N*-acetylcysteine, taurine-chloramine, lipoic acid
Lipoxin A4, pyrolidinedithiocarbamate, conjugated linoleic acid (CLA)
PPARγ agonists like the thiazolidinediones (TZDs) and PPARα
Immunosuppressant cyclosporin 1,25(OH)2 vitamin D$_3$ 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, epoxomycin, peptide aldehydes

[a]Early in macrophages, nitric oxide induces NF-κB, but later it inhibits it

mucosal epithelial cells. Actually, *Bacteroides* species activate perioxisome prolif-erator-activated receptor-γ (PPARγ) receptors, which take RelA out of cell nuclei of the gastrointestinal (GI) epithelium. Hence, cytokine production via NF-κB 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 trans-forming growth factor-β (TGFβ) [59].

Enhanced NF-κB 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 → p50 and enhanced degradation of inhibitor IκBα. Then p50/c-Rel is important for IFNγ 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 IKKε phosphorylate the C-terminal of cRel so that p50/cRel can bind at NF-κB sites [61]. TBK also phosphorylates IRF3. This means that interferors IFNα/β are induced (Chap. 14) [62].

11.2   How to Inhibit NF-κB

Anti-inflammatory cytokines IL-10 and IL-13 suppress the activation of NF-κB. Unlike many cytokines, IL-10 does not activate the NF-κB. Heat shock proteins inhibit NF-κB by stopping the degradation of IκB [22c]. There is accumulating information on drugs and antioxidants like N-acetylcysteine [63], resveratrol of red wine or the polyunsaturated fatty acid (PUFA) conjugated lineoleic acid (CLA) [64] that inhibit NF-κB 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-κB (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-κB are used by the biochem-ist. A recent review of pharmacological aspects of NF-κB [66] listed inhibitory pep-tides for NF-κB that have been used in anti-inflammatory exploration. The cyclopentenone 15-deoxyPGJ2 is anti-inflammatory by virtue of its ability to modu-late 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) lym-phocytes act, in part, by inhibition of NF-κB. Thus, Li et al. [68] demonstrated how suppressor lymphocytes stop the presentation of CD86 on antigen-presenting cells (APCs). IκBNS is an inhibitor that binds to nuclear Rel/p65. It accounts for nega-tive selection of thymocytes [69] and T lymphocytes or macrophages [32b].

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

on NF-κB p50-cRel [70]. Not surprisingly, we know of numerous microorganisms that are able to subvert NF-κB activation to gain entry into the body [71]. Conversely, if there is p38 MAP kinase activation, that will lead to NF-κB induction of inflammatory genes [72]. NF-κB 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 IκBα, and ineffectual p50/p50 homodimers form [74]. It turns out that 1,25(OH)2 vitamin D_3 and 1,24(OH)2D2 inhibit TNFα expression by macrophages. What is more, these forms of vitamin D increase the activity of IκBα; thus, they inhibit NF-κB activity [75].

Obviously, using PPARγ and -α agonists (Fig 4.14) is a way of dealing with inflammation. PPARα knockout mice show augmented inflammation. In them, there is marked expression of TNFα and IL-1β 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. Gαq-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 Gαq 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-κB controls are gradually being elucidated by techniques like this (cf. [79]). B lymphocytes with RelA^{−/−} deficiency were killed easily by exposure to TNFα since they had reduced expression of Flice (i.e. caspase 8) and of Bcl-2 antiapoptotic protein. Clearly, NF-κB enables B cell survival by eliminating the effects of TNFα toxicity. This may remind one that c-Flip, inhibitor of the extrinsic apoptotic pathway, is known to promote NF-κB 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-κB) supports antiapoptosis/cell survival

NFkB 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
GADD45β, 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-κB1 protein in the maturation of monocyte-derived DCs. siRNA for p50 reduces the transcription of IL-12 p40. Absence of NF-κB1 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 IFNγ receptor or the TNFRI are removed by gene deletions in mice. NF-κB2, 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-κB 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-κB, 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/EBPβ binding to the Cox-2 promoter. Sphingosine-1-P, by stimulation of NF-κB, also stimulates Cox-2 activity [86].

Cox-2 expression in neutrophils is promoted by ROS induction of NF-κB in response to cell membrane events [87]. Likewise, when monocytes interact with
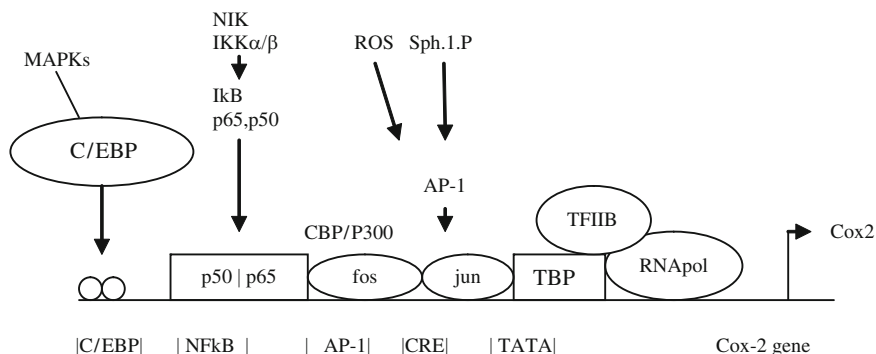
Fig 11.3 Transcription factors affecting the cyclooxygenase 2 (*Cox-2*) promoter. (as in airway smooth muscle cells) C/EBP = CCAAT/enhancer binding protein

platelets or P-selectin-coated surfaces, there is activation of NF-κB 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/EBPβ and C/EBPδ TFs [89]. Conserved elements in the macrophage Cox-2 gene promoter as revealed by analysis after exposure to LPS are –CRE2/NF-κB-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-κB [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-1β or by bradykinin. Whereas IL-1-induced transcription utilises NF-IL-6, NF-κB and CRE, bradykinin uses CRE [91]. Induction of Cox-2 in macrophages is complicated. Normally, any response to NF-κB activation is rapid. Full Cox-2 expression in macrophages is delayed since there first has to be induction of TNFα by IFNγ [92]. In mesangial cells, TGFβ 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-κB that activates Cox-2 [94].

As a lesson, consider how the peptidoglycan of bacterial cell walls stimulates TLR2 receptors; hence, there is NF-κB 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-κB. The consequence is that peptidoglycan elicits the secretion of IL-6 [95]. PGE2 induction of Stat3 is operative in this process [96].

11.5 PPARγ and PPARα 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). PPARγ receptors are highly expressed in macrophage-derived foam cells in atheroma. It was found that PPARγ receptors are involved in the differentiation of monocyte macrophages, and that PPARγ receptors help to regulate their inflammatory activities [97]. In macrophages, PPARγ serves as a stimulus for the anti-inflammatory action of carbon monoxide.

PPARγ 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 PPARγ can inhibit proliferation of activated T lymphocytes through inhibition of IL-2 formation and induced T cell apoptosis. The PPARγ associates with NFAT, so the DNA binding and transcriptional activation of the IL-2 promoter is blocked. Also, PPARγ receptors are expressed on DCs, and PPARγ ligands inhibit the release of IL-12 [99]. DCs treated with synthetic PPARγ ligands have reduced ability to stimulate lymphocytes. People are getting excited about the potential use of PPARγ ligands in conditions like rheumatoid arthritis [100]. PPARγ agents will decrease synovial production of TNFα, 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-κB and AP-1, maintain the repression of inflammatory response genes (cf. Fig. 11.4). PPARα receptors respond to a different set of ligands/drugs. PPARα receptors are also expressed on monocyte macrophages, and ligands can induce apoptosis of those cells. PPARα receptors dampen endothelial cell inflammatory responses. In aged mice, oxidative stress-induced NF-κB is apparent in many tissues. Fortunately, PPARα agonists restore the cellular redox balance, so there is amelioration of the production of inflammatory cytokines [103] since NF-κB 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.
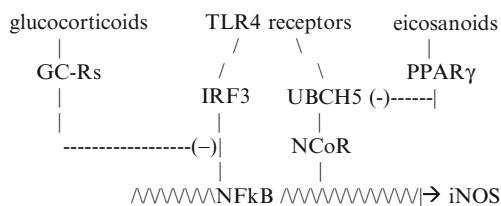
```
glucocorticoids        TLR4 receptors      eicosanoids
       |                    /    \               |
     GC-Rs                 /      \           PPARγ
       |                 IRF3    UBCH5 (-)------|
       |                   |       |
       ------------------(–)|     NCoR
                           |       |
            /\/\/\/\/\/NFkB /\/\/\/\/\/\/\/\|→ iNOS
```

Fig 11.4 Perioxisome proliferator-activated receptor-γ (*PPARγ*) 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 TNFα will activate Akt, which then associates with and phosphorylates IKK so that NF-κB 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 PKCζ and protein phosphatase 2A (PP2A) negatively regulate the Akt survival pathway [108], this explains how ceramide often mediates cell apoptosis. Nevertheless, the α4-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-κB. NF-κB supports antioxidant enzymes. Akt phosphorylates glycogen synthase kinase 3β (GSK3β), 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. GSK3β is essential for cell survival, but too much or too little will cause apoptosis. On the one hand, components of the NF-κB system are phosphorylated by GSK3 to promote NF-κB 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-κB 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-κB for cell survival. NF-κB suppresses apoptosis (Fig. 8.2) since NF-κB 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-κB in some cells enables them to stay alive long enough to acquire genetic errors. Hence, NF-κB activity can promote carcinogenesis [5] and help metastases [123]. Such carcinogenesis is oxygen radical mediated [124]. Yet generally, NF-κB 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-κB 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-κB, 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 $\alpha2\beta2$ 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 −SH2 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 TNFα 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 TNFα acting on the adipocytes is a cause of

```
                          Insulin receptor
                          tyrosine kinase
       SOCS-1----(- )\ IRS.1(2,3,4)-Shc-Grb2-Sos -----------Ras
                          |                                  |
   S6kinase ←PDK1←PI3K     AS160                        MAPkinases
       |                    |    /        \                  |
   protein              Akt(PKBβ)   (O)             mitogenesis
   synthesis                |            vesicle
                            |         mobilisation →(O)
   glycogen ← GSK3 /   GLUT4 mobilisation
```
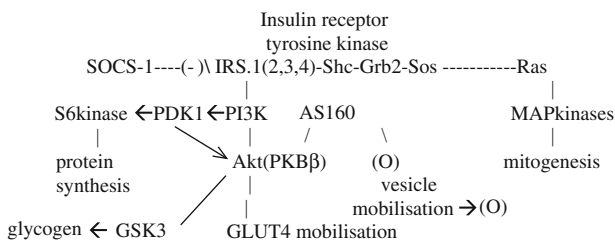
Fig. 11.5 Scheme for insulin receptor activation. *PDK-1* 3-phosphoinositide-dependent kinase 1. Active JNK opposes IRS 1, 2 action. In this scheme, phosphatase Src homology inositol polyphosphate 5-phosphatase 2 (*SHIP2*) regulates the PI3 kinase-dependent signalling. Insulin-like growth factor 1 receptor (*IGF-1R*) 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. TNFα-induced insulin resistance involves activation of inhibitory serine kinases or tyrosine phosphatases, which inhibit the insulin signalling path. It works through H_2O_2 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, TNFα inhibits Akt phosphorylation, causing insulin resistance [136]. Ceramide, by blocking insulin stimulation of Akt, leads to insulin resistance [137]. TNFα can also produces insulin resistance in skeletal muscle by activation of IκB kinase inhibitor of NF-κB 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 PPARα 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 p85α 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 PPARγ-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].
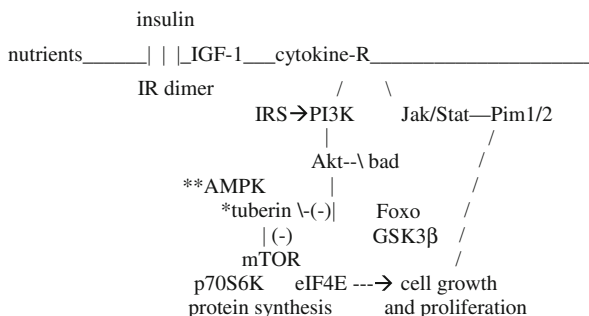
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                        insulin
        nutrients_____| | |_IGF-1___cytokine-R_____
                IR dimer              /     \
                        IRS→PI3K       Jak/Stat—Pim1/2
                            |                /
                        Akt--\ bad          /
            **AMPK        |                /
              *tuberin \-(-)|   Foxo     /
              | (-)           GSK3β    /
              mTOR                    /
        p70S6K    eIF4E ---→ cell growth
        protein synthesis       and proliferation
```

Fig 11.6 Model of the PI3 kinase-Akt-tuberose sclerosis (*TSC*)-mammalian target of rapamycin (m*TOR*) 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 → 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 → eIF4E and (b) by the path mTOR → 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 hvps34 (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].

GSK3β 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 AMPKα1, 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 TNFα 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 $\rightarrow$ 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) eIF2α. Actually, ERK decreases proapoptotic JNK activity by stabilizing MPK7 dual phosphatase. When JNK activity is low, there is higher activity of phosphatase PP1α. Accordingly, there is hypophosphorylation of translation initiation factor eIF2α, so there is ongoing protein translation that means survival of the AMs.

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