

# NF- $\kappa$ B transcription factor: a key player in the generation of immune response

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**Activation of the immune system is a multistep process. The transcription factor nuclear factor-kappa B (NF- $\kappa$ B), has been one of the most widely studied molecules in the immune system, as it is activated by a variety of stimuli and it in turn controls diverse genes and biological responses. It is an evolutionarily conserved molecule that coordinates the organism's response to infection, stress and injury. It plays a major role in the induction of pro-inflammatory genes and affects the various cells involved in immune response as well as affects the generation of innate and adaptive immune response. It is also a target for drugs used in treatment of various inflammatory diseases.**

**Keywords:** Apoptosis, inflammatory diseases, nuclear factor-kappa B, signal transduction, toll-like receptors.

THE main function of the immune system is recognition and control of invading pathogens. Recognition of pathogens by innate or adaptive immune receptors leads to activation of immune cells, e.g. macrophages, dendritic cells and lymphocytes. The signal generated after recognition of a pathogen is communicated to the nucleus, resulting in enhanced expression of effector molecules such as cytokines and adhesion molecules. This process depends on activation of various inducible transcription factors, among which the nuclear factor-kappa B (NF- $\kappa$ B) transcription factors play an evolutionarily conserved and critical role in triggering and coordinating both innate and adaptive immune responses<sup>1</sup>.

NF- $\kappa$ B is a collective name for inducible dimeric transcription factors. They are made up of members of the Rel family of DNA binding proteins that recognize a common sequence motif. It is a ubiquitous transcription factor. Nevertheless, its properties seem to be most extensively exploited in cells of the immune system<sup>2</sup>. NF- $\kappa$ B plays a crucial role in immune and inflammatory responses through the regulation of genes encoding pro-inflammatory cytokines, adhesion molecules, chemokines, growth factors and inducible enzymes such as cyclo-oxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS)<sup>3-5</sup>.

NF- $\kappa$ B can be activated within minutes by a variety of stimuli, including inflammatory cytokines such as TNF- $\alpha$  and interleukin-1, T-cell activation signals, growth fac-

tors and stress inducers<sup>6</sup>. The generation of mouse models with defective NF- $\kappa$ B activation or lack of essential NF- $\kappa$ B subunits, such as Rel-A (also known as p65), has revealed an important role of NF- $\kappa$ B in innate and adaptive immunity, inflammation and lymphoid organ development<sup>7,8</sup>. Another exciting development has been the demonstration, that in certain situations, NF- $\kappa$ B acts as an anti-apoptotic protein. Furthermore, several studies have focused on other diverse functions of NF- $\kappa$ B, which clearly illustrate its 'good and evil' aspects, whereby NF- $\kappa$ B is required for immunological functions but is detrimental when it is dysregulated. This review aims to highlight the above functions of this intriguing molecule.

## Structure, functions and signalling pathways of NF- $\kappa$ B

### Structure

NF- $\kappa$ B was first identified as a regulator of the expression of the kappa light-chain gene in murine B-lymphocytes, but has subsequently been found in many different cells. NF- $\kappa$ B represents a group of structurally related and evolutionarily conserved proteins that belong to the Rel family and are regulated via shuttling from the cytoplasm to the nucleus in response to cell stimulation<sup>9</sup>. Mammals express 5 Rel (NF- $\kappa$ B) proteins that belong to two classes. The first class includes Rel A (p65), c-Rel and Rel-B proteins that are synthesized as mature products and do not require proteolytic processing. The second group is encoded by the *NF- $\kappa$ B1* and *NF- $\kappa$ B2* genes, whose products are first synthesized as large precursors, p105 and p100, respectively, that require proteolytic processing to produce the mature p50 and p52 NF- $\kappa$ B proteins<sup>10</sup> (Table 1).

**Table 1.** Members of the NF- $\kappa$ B/rel and I $\kappa$ B families of proteins

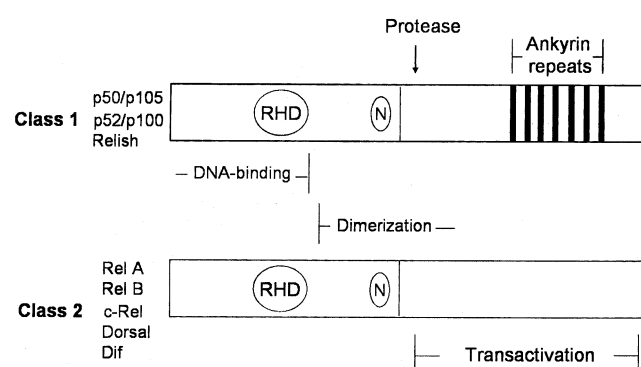
NF- $\kappa$ B/Rel proteins	I $\kappa$ -B proteins
Relish (971)	I $\kappa$ -B- <i>g</i> (607)
Dif (667)	Bcl-3 (421)
Dorsal (678)	I $\kappa$ B- <i>e</i> (500)
Rel B (558)	I $\kappa$ B- <i>a</i> (317)
c-Rel (587)	I $\kappa$ B- <i>b</i> (359)
p-65 (550)	Cactus (460)
P105/p50 (940)	

Values in brackets represent number of amino acids in each protein.

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**Table 2.** Functions and characteristics of NF- $\kappa$ B/rel and I $\kappa$ B families of proteins

NF- $\kappa$ B subunit	Size and special characteristics	Functions
RelA (p65)	65 kDa	Lack of Rel A causes embryonic lethality and liver degeneration in knockout mice due to apoptosis of foetal hepatocytes
RelB	68 kDa Does not possess phosphokinase A (PKA) phosphorylation site, but an additional N-terminal leucine zipper-like region, which affects its transcriptional activity	Essential for normal hemopoietic and immune cell function
NF- $\kappa$ B1 (p50; p105)	50 kDa Synthesized as the precursor I $\kappa$ B protein p105  p50 does not possess transactivation domain	Mice lacking p50 are immunodeficient, but otherwise develop normally to adulthood B-cells from p50 knockout mice show abnormal mitogen responses and antibody production Development and differentiation of dendritic cells, and a mutation disrupting relB impairs antigen presentation
NF- $\kappa$ B2 (p52; p100)	52 kDa Synthesized as the precursor I $\kappa$ B protein p100 p52 does not possess transactivation domain	Essential for normal hemopoietic and immune cell function
c-Rel	69 kDa	c-Rel-deficient mice develop normally, their mature B and T cells exhibit selective activation defects in response to certain mitogenic stimuli

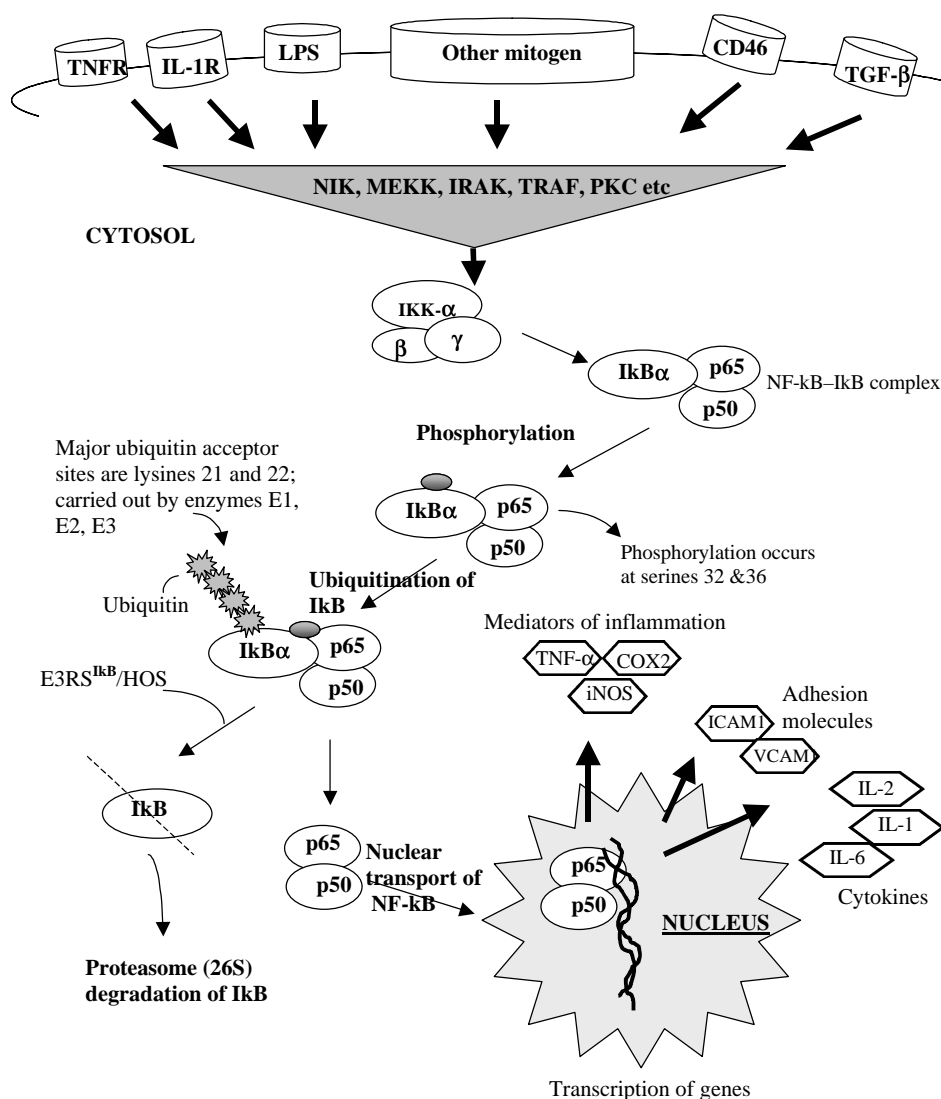
**Figure 1.** Structure of Rel family transcription factors. Adapted from [www.ncbi.nlm.nih.gov/entrez/query.fcgi](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi)

NF- $\kappa$ B dimers containing Rel A or c-Rel are held in the cytoplasm through interaction with specific inhibitors, the I $\kappa$ Bs. The I $\kappa$ Bs are also members of a gene family that contains seven known proteins, I $\kappa$ B $\alpha$ , I $\kappa$ B $\beta$ , I $\kappa$ B $\epsilon$ , I $\kappa$ B $\gamma$ , Bcl-3 and the precursor Rel proteins p100 and p105. Interestingly, p100 may serve both as an I $\kappa$ B and a specific heterodimeric partner for Rel B, such that its processing results in the release of p50 : Rel B heterodimers (Table 2). The I $\kappa$ Bs are characterized by the presence of multiple ankyrin repeats and interact with NF- $\kappa$ B via Rel homology domain (RHD). The RHD serves several functions: it is the dimerization and DNA-binding domain for this class of proteins, it contains the nuclear localization sequence (NLS), and most important for its regulation it is the site for binding of NF- $\kappa$ B inhibitors the I $\kappa$ Bs<sup>10-12</sup> (Figure 1).

### NF- $\kappa$ B activation by the I $\kappa$ B complex

I $\kappa$ Bs are a small family of related proteins with a core consisting of six or more ankyrin repeats, an N-terminal regulatory domain and a C-terminal domain that contains a PEST motif. The I $\kappa$ Bs undergo rapid ubiquitin-dependent degradation after exposure to a variety of agonists, which activate the I $\kappa$ B (IKK) complex<sup>10-12</sup> (Figure 2).

IKK is composed of three subunits, IKK $\alpha$  (IKK1), IKK $\beta$  (IKK2), and IKK $\gamma$  (also known as NF- $\kappa$ B essential modulator, NEMO)<sup>12</sup>. IKK $\alpha$  and IKK $\beta$  are the catalytic subunits of the complex, sharing 52% overall sequence identity and 65% identity in their catalytic domains. IKK $\alpha$  (previously identified as a protein kinase with unknown function named conserved helix-loop-helix ubiquitously kinase (CHUK)) was initially isolated as a protein that interacts in yeast cells with another protein kinase called the NF- $\kappa$ B-inducing kinase (NIK). NIK is so called due to its ability to potently stimulate NF- $\kappa$ B activity in transiently transfected cells. The third subunit, IKK $\gamma$ /NEMO, is the regulatory subunit and is not related to the catalytic subunits. IKK $\alpha$  and IKK $\beta$  have similar primary structures and contain protein kinase domains at their N-termini, and leucine zippers (LZ) and helix-loop-helix (HLH) motifs in their C-terminal portions. IKK $\gamma$  does not contain a recognizable catalytic domain, but is composed mostly of three large  $\alpha$ -helical regions, including an LZ. IKK $\alpha$  and IKK $\beta$  can form homodimers and heterodimers (or tetramers) *in vitro*, and purified recombinant forms of each can directly phosphorylate I $\kappa$ B $\alpha$  and I $\kappa$ B $\beta$  at the proper sites. Biochemical analysis of two human cell lines



**Figure 2.** Schematic representation of pathways of activation and regulation of I $\kappa$ B. Adapted from refs 1, 2, 5, 6.

indicates that the predominant form of IKK is an IKK $\alpha$ :IKK $\beta$  heterodimer associated with either a dimer or trimer of IKK $\gamma$ . The I $\kappa$ Bs bind to NF- $\kappa$ B dimers and sterically block the function of their NLSs, thereby causing their cytoplasmic retention<sup>10–12</sup>.

In addition to IKK $\alpha$ , IKK $\beta$ , NIK, IKB $\alpha$  and NF- $\kappa$ B/RelA, IKK complex contains a fourth protein, a 150-kDa IKK complex-associated protein called IKAP (IKK complex-associated protein). It is proposed to be involved in IKK activation and functions as a scaffold protein due to its ability to assemble IKK $\alpha$ , IKK $\beta$ , NIK, and NF- $\kappa$ B:I $\kappa$ B. However, since IKAP is not readily detected as a constituent of the IKK complex, its physiological significance and function are not yet clear. It also remains to be determined whether IKAP is required for IKK activation at all and, if so, whether it is involved in responses to all NF- $\kappa$ B-activating stimuli or to only a subset of them. It is possible that while IKK $\gamma$ , which is a stoichiometric com-

ponent of IKK, is required for IKK activation by all stimuli, proteins like IKAP may connect IKK to a specific set of upstream activators. Furthermore, sequence analysis suggests that IKAP is the human homologue of one of the components of the yeast transcriptional elongation 'Elongator' complex. Thus, IKAP is most probably a nuclear protein and, thus, is unlikely to serve as a component of the cytoplasmic IKK complex<sup>11–14</sup>.

Stimulation by a diverse array of pathogens and other inducers, including viruses, cytokines, and stress-inducing agents led to activation of signalling cascades that culminate with the activation of the IKK complex and phosphorylation of the I $\kappa$ B inhibitor. NF- $\kappa$ B DNA binding subunits are released and translocated to the nucleus, where they transactivate NF- $\kappa$ B responsive genes. Target genes are selectively regulated by the distinct transcriptional activation potential of different NF- $\kappa$ B subunit combinations<sup>15,16</sup>. The consensus pathway for NF- $\kappa$ B acti-

vation in response to pro-inflammatory stimuli such as TNF- $\alpha$  and IL-1 $\beta$  has been extensively characterized. These cytokines act through distinct signalling pathways that converge on the activation of an IKK; the subsequent phosphorylation of I $\kappa$ B molecules targets them for degradation by the proteasomes<sup>17</sup>.

### *Regulation and function of I $\kappa$ B*

Potent NF- $\kappa$ B activators, such as TNF $\alpha$  and IL-1, cause almost complete degradation of I $\kappa$ Bs (especially I $\kappa$ B $\alpha$ ) within minutes. The mechanisms by which these highly diverse stimuli activate IKK are, however, poorly understood. Structure prediction programs suggest the presence of numerous docking sites for interacting proteins on IKK $\alpha$ , IKK $\beta$ , IKK $\gamma$  and IKAP, but the search for signalling molecules that directly dock to these sites is in its infancy. Mitogen-activated protein kinase/ERK kinase kinases (MAP3Ks), such as NIK and MEK kinase 1 (MEKK1), activate IKK when overexpressed. NIK may potentially interact with IKK through IKAP. However, there is little evidence till now that NIK or MEKK1 is a physiological IKK activator. In the case of IKK $\gamma$  (but not IKAP), genetic and biochemical data clearly show that IKK $\gamma$  is essential for IKK activation in response to at least six different stimuli, but it is not known with which upstream activators IKK $\gamma$  interacts<sup>18,19</sup>.

Studies using mouse mutants that lack either IKK $\alpha$  or IKK $\beta$  have suggested that IKK $\beta$  is far more important than IKK $\alpha$  for activation of the IKK complex in response to pro-inflammatory stimuli. Most interestingly, however, these experiments indicate a new role for IKK $\alpha$  in controlling the proliferation and differentiation of epidermal keratinocytes as well as affecting (directly or indirectly) other developmental decisions, including skeletal patterning. IKK $\alpha$ -deficient mice are born alive, but die within 30 min. The mutant mice exhibit a plethora of developmental defects, the most striking of which are their taut, thick skin, highly rudimentary limbs and tail, and shorter head. It turns out, however, that IKK $\alpha$ <sup>-/-</sup> mice do have limbs and a tail whose proximal elements are almost normal, but they are hidden under their thickened skin. The distal elements of the limbs are mal-developed due to a defect in inter-digital apoptosis<sup>20,21</sup>.

Histochemical and microscopic examination of IKK $\alpha$ <sup>-/-</sup> skin reveals marked hyper-proliferation of the epidermal layer and almost complete absence of differentiation. Due to the absence of fully keratinized cells, the mutant skin appears to be rather sticky, which causes the limbs and tail to be 'glued' to the body instead of developing as well-separated outgrowths. Despite these marked alterations in morphology, the activation of IKK by TNF, IL-1 or lipopolysaccharide in the fibroblasts and liver of IKK $\alpha$ <sup>-/-</sup> mice seems to be normal. The loss of IKK $\beta$  results in an expected phenotype, which confirms its importance for IKK activation by TNF and other pro-inflam-

matory stimuli. IKK $\beta$ <sup>-/-</sup> mouse embryos die on day 12 to 13 of gestation due to massive liver apoptosis. This phenotype is essentially identical to that of p50 (RelA)-deficient mice, except that IKK $\beta$ <sup>-/-</sup> mice die a day or two earlier. This is probably due to the more severe decrease in NF- $\kappa$ B activity in IKK $\beta$ <sup>-/-</sup> cells than in RelA<sup>-/-</sup> cells. Indeed, protein kinase and mobility shift assays<sup>7,21,22</sup> indicate that IKK $\beta$ <sup>-/-</sup> cells are completely defective in activation of IKK and NF- $\kappa$ B in response to TNF or IL-1.

Despite the extensive sequence similarity between IKK $\alpha$  and IKK $\beta$  and their tight association in most cell types, these two protein kinases play different regulatory and functional roles. IKK $\beta$  is essential for IKK activation by pro-inflammatory cytokines and for I $\kappa$ B phosphorylation. Yet, IKK $\beta$  does not have an essential role in embryonic development. The hepatic apoptosis in IKK $\beta$ <sup>-/-</sup> embryos is simply due to a defect in NF- $\kappa$ B activation, which is required for protecting the liver from TNF-induced apoptosis. By contrast, IKK $\alpha$  is dispensable for IKK activation or I $\kappa$ B phosphorylation in response to pro-inflammatory stimuli, but plays an essential role in epidermal development<sup>19</sup>.

### *Functions of NF- $\kappa$ B*

NF- $\kappa$ B regulates the transcription of an exceptionally large number of genes, particularly those involved in immune and inflammatory responses. The regulation of inflammation, cell proliferation and apoptosis is at the centre of understanding of many diseases such as rheumatoid arthritis (RA), AIDS and cancer. It has become clear over the years that NF- $\kappa$ B is a pleiotropic transcription factor involved in the inducible expression of a wide variety of genes, particularly those that promote cell growth and survival.

### *NF- $\kappa$ B in apoptosis*

Apoptosis is an important process that regulates the development and selection of T- and B-lymphocytes and is critical for the maintenance of immunological homeostasis in the periphery. The finding that NF- $\kappa$ B is activated during or immediately before cell apoptosis under stimulatory conditions, has led to the suggestion that the activation of NF- $\kappa$ B is strongly linked to inhibition of apoptosis<sup>23-25</sup>. In most cells, NF- $\kappa$ B activation protects the cell from apoptosis, through induction<sup>26</sup> of survival genes such as TRAF1, TRAF2, c-IAP1, c-IAP2, IEX-IL, Bcl-x<sub>L</sub> and Bfl-1/A1<sup>26</sup>. *In vitro* studies<sup>27</sup>, where loss of RelA renders embryonic fibroblasts more susceptible to TNF-induced apoptosis and *in vivo* studies in which RelA<sup>-/-</sup> mice die as embryos as a consequence of TNF-mediated apoptosis in the livers of these animals, provide clear evidence for the role of NF- $\kappa$ B in preventing apoptosis<sup>28-30</sup>. In fact, NF- $\kappa$ B binding sites have been identified in the promoters of

interleukin-1 $\beta$  converting enzyme protease, *c-myc*, and TNF- $\alpha$  genes, which are commonly involved in signal-induced programmed cell death.

Although most studies link NF- $\kappa$ B with prevention of apoptosis, numerous recent studies have clearly demonstrated a pro-apoptotic role for NF- $\kappa$ B perhaps because it, along with AP-1, can induce FasL expression<sup>31</sup>. Treatment of human thymocytes and promyelocytic leukaemia cells with etoposide activates NF- $\kappa$ B and induces apoptosis<sup>32</sup>. Moreover, in other experimental systems, increased levels of NF- $\kappa$ B activation during development of avian embryos and in focal cerebral ischaemia have been correlated with apoptosis, but it is difficult to determine whether the increased NF- $\kappa$ B activity observed is a cause or consequence of apoptosis. How does NF- $\kappa$ B participate in a dual role, wherein it mediates both life and death signals in cells? It is possible that different NF- $\kappa$ B members mediate different signals and that the role of NF- $\kappa$ B in apoptosis depends on the cell type or the type of stimulation that determines which signalling pathways are activated<sup>25,33</sup>.

### *NF- $\kappa$ B in development of immune system*

The availability of NF- $\kappa$ B<sup>-/-</sup> cells and NF- $\kappa$ B<sup>-/-</sup> mice has elucidated the role of specific NF- $\kappa$ B proteins in development and function of immune cells. Initially, it was speculated as a key transcription factor for the development of B cell, but now it has become more evident that it is also required for the development and function of many other cells, including T cells thymocytes, dendritic cells, macrophages and fibroblasts<sup>34,35</sup>.

The targeted disruption (or knockout) of the p65 component of NF- $\kappa$ B is lethal because of the associated developmental abnormalities, whereas the lack of p50 components results in immune deficiencies and increased susceptibility to infection<sup>36</sup>. As mentioned above, RelA is essential to prevent TNF-mediated apoptosis in the liver of developing foetus, but RelA is not required for the development of T and B cells, as the transfer of foetal liver cells from RelA<sup>-/-</sup> mice to irradiated SCID mice leads to normal lymphocyte development. It appears that signalling through IKK $\beta$  is essential to protect T cells from TNF- $\alpha$  induced apoptosis during development<sup>37</sup>.

Further, the role of NF- $\kappa$ B in T-cell development and selection is evident from the transgenic expression of a degradation-deficient form of I $\kappa$ B $\alpha$  in T cells. Because this mutant protein is not degraded in response to signals that activate NF- $\kappa$ B, it acts as a global inhibitor of this signalling pathway. Several groups have generated similar transgenic mice, and while there are some differences between these mice, their phenotypes have included decreased thymic cellularity, reduced numbers of peripheral CD8<sup>+</sup> T cells, and increased levels of apoptosis<sup>38,39</sup>.

Although individual NF- $\kappa$ B proteins are not essential for the development of conventional, mature B cells, re-

cent gene knockout studies have provided evidence that a deficiency in B cells occurred when the genes *NF- $\kappa$ B1* and *NF- $\kappa$ B2* and *c-Rel* were disrupted<sup>40</sup>. In these models, B-cell maturation is blocked at the immature stage. The mechanism behind immaturity is not known. Perhaps NF- $\kappa$ B activation mediated by TNF superfamily member BlyS, is dysregulated in these mice. BlyS (B-lymphocyte stimulator) is a key molecule in B-cell proliferation<sup>41</sup>.

The role of NF- $\kappa$ B in the development of dendritic cells is illustrated by the lack of CD8 $\alpha$ <sup>-</sup> and thymic dendritic cells in RelB<sup>-/-</sup> mice<sup>42</sup>. Further, absence of NF- $\kappa$ B2 leads to lack of follicular dendritic cells in mice. Although the lack of thymic dendritic cells in RelB<sup>-/-</sup> mice is an indirect consequence of a defect in thymic epithelia, the lack of other dendritic cell populations in RelB<sup>-/-</sup> and NF- $\kappa$ B2<sup>-/-</sup> mice could be due to specific blocks in the development or maturation processes that give rise to these subsets<sup>43</sup>. In fact, previous studies have shown that inhibitors of NF- $\kappa$ B activation block maturation of dendritic cells<sup>44</sup>. Macrophages from RelB<sup>-/-</sup> mice are deficient in their ability to produce TNF- $\alpha$  and can produce normal levels of IL-6, IL-10, and IL-12, but overproduce IL-1 $\beta$ . Macrophages from c-Rel<sup>-/-</sup> mice overproduce GM-CSF and IL-6, but have a reduced ability<sup>38</sup> to produce TNF- $\alpha$ . The exact role of NF- $\kappa$ B family members in the development of cell lineages remains to be defined and future studies may provide answers to these crucial issues.

### *NF- $\kappa$ B in innate immunity*

The classical NF- $\kappa$ B pathway, based on IKK $\beta$ -dependent I $\kappa$ B degradation, is essential for innate immunity. The activation and nuclear translocation of NF- $\kappa$ B is associated with increased transcription of a number of different genes, including those coding for chemokines (IL-8), adhesion molecules (endothelial leukocyte adhesion molecule, vascular cell adhesion molecule, and intercellular adhesion molecule), and cytokines (IL-1, IL-2, TNF- $\alpha$ , and IL-12). These molecules are important components of the innate immune response to invading microorganisms and are required for migration of inflammatory and phagocytic cells to tissues. The activated phagocytic cells kill, ingest and degrade bacteria, and eventually present bacterial antigens once they re-migrate to secondary lymphoid organs<sup>45</sup>.

*Mycobacterium tuberculosis*, *Borrelia burgdorferi* and *Neisseria gonorrhoea* and bacterial products such as LPS and Shiga toxin activate NF- $\kappa$ B in macrophages as well as other cell types<sup>46-48</sup>. Enteroinvasive bacteria activate NF- $\kappa$ B in intestinal epithelial cells, a process which leads to increased production of inflammatory mediators such as chemokines (MCP-1 and IL-8) as well as TNF- $\alpha$ , intercellular adhesion molecule, and cyclooxygenase-2. Scrub typhus, *Chlamydia pneumoniae* and *Helicobacter pylori* also lead to NF- $\kappa$ B-dependent induction of chemokines<sup>49</sup>.

NF- $\kappa$ B-dependent responses are not restricted to bacteria, even protozoan parasite *Trypanosoma cruzi* can activate NF- $\kappa$ B on invasion of endothelial cells. There are also indirect pathways that lead to NF- $\kappa$ B activation, like release of IL-1 by pulmonary epithelial cells on infection with *M. tuberculosis*, which in turn activates the NF- $\kappa$ B pathway<sup>49</sup>. Though it has been recognized for long that many bacterial products can activate NF- $\kappa$ B, identification of Toll-like receptors (TLRs) as specific pattern recognition molecules and the recognition that stimulation of TLRs leads to activation of NF- $\kappa$ B has improved our understanding of how different pathogens activate NF- $\kappa$ B. Moreover, adjuvants used to boost immune response are also potent TLR agonists and therefore, activators of NF- $\kappa$ B. TLRs are widely distributed, and are mainly present on accessory cells (macrophage, dendritic cell, B cell), however, there is evidence that TLRs are also present on T and NK cells<sup>50</sup>.

Initial studies identified TLR4 as the receptor for the LPS component of Gram-negative bacteria, but later respiratory syncytial virus and heat shock proteins were also shown to be ligands for TLR4. TLR2 recognizes a larger variety of microbial products, including peptidoglycans and lipoproteins. TLR5 recognizes bacterial flagellin, TLR9 recognizes bacterial DNA, and TLR3 double-stranded RNA. TLR signalling leads to increased expression of a number of genes regulated by NF- $\kappa$ B, including cytokines, co-stimulatory molecules, nitric oxide, and susceptibility to apoptosis as well as an autocrine increase in Toll expression<sup>51,52</sup>. The activation of macrophages to produce reactive oxygen and nitrogen intermediates is important in the control of many bacterial and parasitic infections. This is a complex process in which IFN- $\gamma$  is important for the activation of macrophages, but alone is not sufficient to activate macrophages to kill intracellular organisms. Additional cofactors such as TNF- $\alpha$ , LPS or signalling through CD40, are required to provide a second signal for macrophage activation. These second signals activate NF- $\kappa$ B, and it has been shown that the induction of inducible nitric oxide synthase is regulated by NF- $\kappa$ B and that c-Rel is involved in this process<sup>52</sup>.

IL-12 is one of the most important cytokines involved in the activation of innate and adaptive responses to intracellular infections. Its production in response to numerous intracellular organisms leads to the innate activation of NK cells, and enhances their cytolytic activity and production of IFN- $\gamma$ . The link between IL-12 and NF- $\kappa$ B family of transcription factors was shown by studies in which the IL-12 p40 promoter was found to have NF- $\kappa$ B binding sites. Using macrophages from NF- $\kappa$ B<sup>-/-</sup> mice, researchers have shown that c-Rel and Rel-A are both important in the LPS-induced production of IL-12 by macrophages, although NF- $\kappa$ B<sub>1</sub> and NF- $\kappa$ B<sub>2</sub> or RelB appear to have no major role in the ability of macrophages to respond to LPS. Studies have shown that c-Rel also plays a role in the ability of CD8a<sup>+</sup> dendritic cells to make p35, but not

p40. Whether different stimuli that induce IL-12 have different requirements for specific NF- $\kappa$ B members is unknown, but recent studies indicate that there are c-Rel-independent pathways<sup>49</sup> that lead to the production of IL-12.

Although monocytes and macrophages constitute a major arm of the innate immune system, there are other cell types which play an important role in the initial recognition of many pathogens and may also provide effector activities. Nonimmune cells such as fibroblasts, endothelial and epithelial cells are also capable of responding to pathogens by activating NF- $\kappa$ B. Recently, it has been shown that fibroblasts respond to necrotic cells in a TLR2-dependent fashion to activate NF- $\kappa$ B and the production of chemokines. The latter studies provide a mechanism that allows the immune system to recognize the presence of pathogens that cause cellular necrosis<sup>52</sup>.

The ability of neutrophils, mast cells and eosinophils to recognize a diverse array of pathogens is thought to represent an important first line of defence against infection. However, little is known about the role of NF- $\kappa$ B in the regulation of these innate responses. Recent data suggest that TNF- $\alpha$ -mediated activation of NF- $\kappa$ B delays neutrophil apoptosis and thus provides survival signal to neutrophils. This allows neutrophils to mediate their antimicrobial activities at the local site. NF- $\kappa$ B is also involved in the ability of mast cells to make the T-cell and mast-cell growth factor IL-9 and their expression<sup>34,45</sup> of TLR4.

Although pathogens can stimulate activation of NF- $\kappa$ B, other immune molecules associated with innate immunity also activate NF- $\kappa$ B. IL-1, IL-18, TNF- $\alpha$ , and signalling through CD28 lead to activation of NF- $\kappa$ B, and these signals can augment the innate ability of NK cells to produce IFN- $\gamma$ . Since all of these cofactors are associated with activation of NF- $\kappa$ B and there are NF- $\kappa$ B sites in the promoter for IFN- $\gamma$ , it is likely that maximal activation of NK cells to produce IFN- $\gamma$  would be dependent on NF- $\kappa$ B. This argument is supported by studies<sup>35</sup> in which NK cells from mice which lack RelB have a defect in their ability to produce IFN- $\gamma$ .

Together, the above evidence associates activation of NF- $\kappa$ B with initial recognition of multiple pathogens, microbicidal mechanisms of macrophages, production of multiple pro-inflammatory cytokines, and activation of NK cells to produce IFN- $\gamma$ .

### NF- $\kappa$ B in adaptive immunity

Many of the events that are important in triggering innate immune response to infection are also important for the development of protective T-cell responses. Thus, the abilities of accessory cells to present antigen provide co-stimulation, and produce cytokines in response to infection are critical to the subsequent adaptive immune response. The previous section highlighted the role of NF- $\kappa$ B in the innate production of IL-12, which is critical to direct the

development of T-cell responses dominated by the production<sup>34</sup> of IFN- $\gamma$ .

Additional studies have shown a role for NF- $\kappa$ B in other accessory cell functions involved in adaptive immunity. For example, RelA is required by embryonic fibroblasts to express optimal levels of major histocompatibility complex class-I and CD40 molecules, that help in the development of CD8<sup>+</sup> T-cell responses. Chemical inhibitors of NF- $\kappa$ B activation block maturation of dendritic cells, and their ability to up-regulate expression of MHC class II and B7 co-stimulatory molecules, which are also required for efficient CD4<sup>+</sup> T-cell responses. NF- $\kappa$ B is also involved in the regulation of the co-stimulatory molecule B7, the ligand for the T-cell co-stimulatory molecule ICOS. Collectively, these studies indicate the importance of NF- $\kappa$ B in the regulation of accessory cell functions which affect adaptive responses<sup>34,35</sup>.

The expression of most of the NF- $\kappa$ B family members in T cells indicates that they are likely to be involved in T-cell functions. The development of an adaptive T-cell response is balanced by the proliferation and expansion of antigen-specific T cells during the initiation of the response and the loss of excess T cells as the response resolves. In addition, maintenance of long-term memory cells is the hallmark of adaptive immunity, and NF- $\kappa$ B has recently been implicated in signals that allow memory to develop. There are many studies which link NF- $\kappa$ B to T-cell proliferation, and there are clear links between the activation of NF- $\kappa$ B and the expression of cyclin D1, which is important in the commitment of the cell to DNA synthesis. Direct evidence for a role of NF- $\kappa$ B in T-cell proliferation is evident from transgenic mice that express the degradation-deficient  $\Delta$ I $\kappa$ B transgene, which acts as a global inhibitor of NF- $\kappa$ B activity, under the control of a T-cell-specific promoter. T cells from these mice had severely impaired proliferative responses. Moreover, mice lacking the polypeptide p105 precursor of NF- $\kappa$ B<sub>1</sub>, but which express p50, as well as c-Rel<sup>-/-</sup> mice have impaired T-cell proliferative responses. The basis for these proliferative defects is still unclear, although there are many potential explanations<sup>35</sup>.

The recognition that T-cell responses could be broadly divided into functional subsets of Th1 (dominated by the production of IFN- $\gamma$  and associated with cell-mediated immunity) or Th2 (characterized by production of IL-4, IL-5 and associated with humoral immunity), was important because it provided a basis for understanding how T cells contribute to resistance or susceptibility to different types of pathogens. While NF- $\kappa$ B is involved in the production of IL-12 required for the generation of Th1 responses, these transcription factors may also play a direct role in the development of polarized T-cell responses. Early studies suggested that although Th1 and Th2 cells expressed similar levels of RelA and c-Rel, Th1 cells could activate RelA in response to TCR stimulation, whereas Th2 cells could not. Evidence of a role for NF-

$\kappa$ B in the production of IFN- $\gamma$  by T cells is provided by the description of a functional NF- $\kappa$ B site in the IFN- $\gamma$  promoter and by studies that linked the ability of IL-18 to activate NF- $\kappa$ B and cause IFN- $\gamma$  production by T cells. Moreover, transgenic expression of the degradation-deficient  $\Delta$ I $\kappa$ B $\alpha$  mutant in T cells resulted in reduced production of IFN- $\gamma$  following TCR stimulation. That mice deficient in c-Rel or RelB have defects in their ability to produce IFN- $\gamma$  implicates these two members in the regulation of Th1-cell responses. Regardless of whether NF- $\kappa$ B has a direct or indirect role in the regulation of IFN- $\gamma$  production, there are several *in vivo* systems which demonstrate a role for NF- $\kappa$ B in disease states in which IFN- $\gamma$  plays an important role. Experimental allergic encephalomyelitis is an autoimmune condition mediated by a Th1-type T-cell response, and NF- $\kappa$ B<sub>1</sub><sup>-/-</sup> mice are more resistant to the development of this condition. Similarly, collagen-induced arthritis is also mediated by Th1 cells, and the inhibition of NF- $\kappa$ B in this experimental system ameliorates this inflammatory disease<sup>53-56</sup>.

While there are many studies that link NF- $\kappa$ B to the development of Th1-type responses, less is known about the role of NF- $\kappa$ B in the regulation of Th2-type responses. *In vitro* studies have revealed that Th2 cells do activate NF- $\kappa$ B and that the binding of RelA to sites in the IL-4 promoter leads to inhibition of NF-AT binding required for IL-4 production. Initial studies using transgenic mice which express the  $\Delta$ I $\kappa$ B mutant in T cells indicated that it did not alter the ability of these mice to develop Th2-type responses *in vivo*, although there was some decrease in their capacity to produce IL-4 upon primary TCR stimulation *in vitro*<sup>57</sup>. Subsequent studies have provided evidence that NF- $\kappa$ B<sub>1</sub> is required for development of Th2 responses in experimental allergic encephalomyelitis and pulmonary inflammation. A potential mechanism that explains the requirement for NF- $\kappa$ B<sub>1</sub> in Th2 responses is increased expression of the transcription factor GATA3, which has an important role in differentiation of Th2 cells and their production<sup>58-60</sup> of IL-4 and IL-5.

The IL-1 family (IL-1 $\alpha/\beta$  and IL-18) of proteins activate NF- $\kappa$ B and are associated with development of Th1 as well as Th2 responses. Related to those findings are studies, which identified the ST2/T1 protein as a homologue of the IL-1 receptor which activates NF- $\kappa$ B and is required for the development of Th2-mediated responses in models of infection and inflammation. However, generation of mice deficient in ST2/T1 has revealed that this protein is not essential for Th2 responses. c-Rel is required for optimal production of IL-4 as well as in the development of Th2 responses associated with allergic pulmonary inflammation. Interpretation of many of the studies which examine the role of NF- $\kappa$ B in Th1 and Th2 development is frequently complicated by the linkage between NF- $\kappa$ B and its role in proliferation and cell survival, which can have a profound influence on the development of Th1 and Th2 responses<sup>61</sup>.

*B-cell activation and effector function*

The original identification of NF- $\kappa$ B as a nuclear factor able to bind to the  $\kappa$ B site in the immunoglobulin kappa light chain enhancer and the presence of constitutive NF- $\kappa$ B activity observed in B cells, indicate the importance of NF- $\kappa$ B in the control of B-cell functions. This was confirmed by multiple studies in which mice deficient in NF- $\kappa$ B<sub>1</sub>, NF- $\kappa$ B<sub>2</sub>, RelA, RelB, c-Rel or Bcl-3 or in which the  $\Delta$ I $\kappa$ B mutant was expressed in B cells, were shown to have compromised humoral immune responses. During B-cell maturation, the composition of the  $\kappa$ B binding activity changes, with the p50/c-Rel heterodimer being the dominant complex present in mature B cells, consistent with a requirement for NF- $\kappa$ B<sub>1</sub> in immunoglobulin class switching<sup>62</sup>.

The basis for the defects in humoral immunity in the NF- $\kappa$ B-deficient mice is not well understood and may be intrinsic to the B-cell compartment or due to compromised accessory cell or T-cell functions. For example, NF- $\kappa$ B<sub>2</sub> has functions in cells of the hemopoietic and nonhemopoietic lineages that regulate splenic microarchitecture, and as a consequence of the disrupted splenic architecture and lack of germinal centres, B-cell responses in NF- $\kappa$ B<sub>2</sub><sup>-/-</sup> mice are compromised. There is also a role for RelB in the development of radiation-resistant stromal cells, but not in bone marrow-derived hemopoietic cells, which are required for proper formation of germinal centres. c-Rel may also have an indirect role in the regulation of B-cell functions due to its ability to regulate the production of Jagged1, a ligand for Notch receptors, which have a critical role in B-cell proliferation and differentiation. Similarly, c-Rel is thought to regulate interferon regulatory factor 4, which is required for the ability of the interferon to inhibit B-cell proliferation. Nevertheless, there is a direct role for NF- $\kappa$ B in B-cell function, and an understanding of the role of individual family members in B-cell function is provided by studies which showed that NF- $\kappa$ B<sub>1</sub> is required for survival of quiescent B cells and, in combination with c-Rel, prevents apoptosis of mitogen-activated B cells. Similarly, c-Rel, RelA and RelB are necessary for normal proliferative responses upon stimulation through the B-cell receptor, CD40 and LPS. Together, these findings indicate that the NF- $\kappa$ B proteins are important regulatory factors that control the ability of B cells to survive, progress through the cell cycle and mediate their effector functions<sup>63-65</sup>.

*NF- $\kappa$ B in immuno-inflammatory diseases*

The inflammatory response involves the sequential release of mediators and recruitment of circulating leukocytes, which become activated at the inflammatory site and release further mediators. This response is self-limiting and resolves through the release of endogenous anti-inflam-

matory mediators and clearance of inflammatory cells. The persistent accumulation and activation of leukocytes is a hallmark of chronic inflammation. Current approaches to the treatment of inflammation rely on the inhibition of pro-inflammatory mediator production and of mechanisms that initiate the inflammatory response. However, the mechanisms by which the inflammatory response resolves might provide new targets in the treatment of chronic inflammation<sup>66,67</sup>.

NF- $\kappa$ B increases the expression of genes for many cytokines, enzymes and adhesion molecules in chronic inflammatory diseases. One of them is inducible nitric oxide synthase<sup>68</sup>, the expression of which is increased in airway epithelial cells<sup>69</sup> and macrophages in patients with asthma<sup>70</sup>, in colonic epithelial cells in patients with ulcerative colitis, and in synovial cells in inflamed joints<sup>71</sup>. In all chronic inflammatory diseases, adhesion molecules recruit inflammatory cells such as neutrophils, eosinophils and T lymphocytes, from the circulation to the site of inflammation. NF- $\kappa$ B regulates the expression of several genes that encode adhesion molecules such as intercellular adhesion molecule 1, vascular-cell adhesion 1 and E-selectin<sup>72</sup>.

Production of IL-1 $\beta$ , TNF- $\alpha$ , IL-6, granulocyte-macrophage colony stimulating factor and many chemokines is increased in patients with asthma, RA, psoriasis and inflammatory bowel disease. All these cytokines have an important role in the inflammatory process. IL-1 $\beta$  and TNF- $\alpha$  may influence the severity of the disease, possibly by the persistent activation of NF- $\kappa$ B. The treatment of patients with RA with antibodies to TNF- $\alpha$  can control refractory disease<sup>73-75</sup>.

*Role in rheumatoid arthritis*

NF- $\kappa$ B has been shown to play diverse roles in the initiation and perpetuation of rheumatoid arthritis. Activated NF- $\kappa$ B is a common feature in human rheumatoid arthritis synovium and in various animal models of rheumatoid arthritis such as adjuvant arthritis in rats, collagen-induced arthritis in mice, and streptococcal cell wall-induced arthritis in rats. Studies have shown that NF- $\kappa$ B activation increases expression of inflammatory molecules in synovocytes and protects cells against TNF- $\alpha$  and Fas ligand-induced apoptosis. Studies with cultured cells in which NF- $\kappa$ B was inactivated or over-expressed showed a protective function of NF- $\kappa$ B against the cytotoxicity of TNF and other agents. NF- $\kappa$ B activation leads to expression of several genes that prevent apoptosis in a number of cell types. Inhibition of NF- $\kappa$ B induces apoptosis in TNF- $\alpha$  or IL-1 $\beta$ -stimulated synovial cells and prevents their hyperproliferation and *in vivo* suppression of NF- $\kappa$ B enhanced apoptosis in the synovium of experimentally induced arthritis of rats. Intra-articular administration of NF- $\kappa$ B 'decoy' oligodeoxynucleotides prevented the recurrence of streptococcal cell wall arthritis in treated joints, suggesting the



beneficial effects of local inhibition of NF- $\kappa$ B in arthritis<sup>76–78</sup>.

### *Role in allergic diseases*

Although there are many similarities between the inflammatory responses in patients with arthritis, asthma, inflammatory bowel disease and other inflammatory diseases, there are also important differences in the type of inflammatory cells involved and in the perpetuation of inflammation. These differences may relate to the secretion of specific cytokines such as IL-5, which in patients with asthma promotes eosinophilic inflammation. NF- $\kappa$ B should be considered as an amplifying and perpetuating mechanism that can exaggerate the disease-specific inflammatory process through the coordinated activation of several inflammatory genes. Thus, the presence of IL-5 alone results in relatively little accumulation of eosinophils in the tissue, but action of IL-5 can be amplified by local injection of the eosinophil-specific chemokine, eotaxin. The transcription of the genes for both IL-5 and eotaxin is influenced by NF- $\kappa$ B<sup>66,67</sup>.

### *NF- $\kappa$ B in malignancies*

Further, several tumour systems have constitutive activation of NF- $\kappa$ B. This enhanced NF- $\kappa$ B activity allows tumour cells to constitutively express angiogenic and angiostatic chemokines, cytokines such as VEGF, IL-1 and IL-6, which affect tumour growth and escape from apoptosis. The expression of CCL5 in melanoma and breast cancer correlates with the metastatic capacity of the tumour and the expression of CCL5 is induced by NF- $\kappa$ B. Down-modulation of CCL5 through the inhibition of NF- $\kappa$ B offers promise for therapeutic intervention. Researchers have observed that once the expression of CXCL1 and CXCL8 has been induced, these secreted chemokines lead to positive feedback on the activation of NF- $\kappa$ B. Endogenous activation of NF- $\kappa$ B in melanoma tumour cells can be inhibited by 50% with an antibody specific for CXCL1, indicating that chemokine autocrine loops may be important for perpetuating the constitutive activation of NF- $\kappa$ B in melanoma. In addition, cytokines or alterations in the signal-transduction pathway may also contribute to the dysregulation of NF- $\kappa$ B in melanoma<sup>79</sup>.

### *NF- $\kappa$ B in oxidative stress*

Recently, a possible role for oxidative stress in the activation of NF- $\kappa$ B has been implicated. NF- $\kappa$ B is recognized as a redox-sensitive transcription factor, and has been implicated in cellular response to oxidative stress<sup>80</sup>. Molecular oxygen presents a paradox to aerobic organisms in that it is both essential and at the same time extremely toxic. Al-

though non-reactive in its ground state, molecular oxygen is reduced via normal metabolic processes, yielding a variety of highly reactive oxygen intermediates (ROIs) in the process. These ROIs include the superoxide radical ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), and the most potent oxidant, the hydroxyl radical (OH). ROIs can readily cause oxidative damage to various biological macromolecules resulting in DNA damage, DNA strand breaks, oxidation of key amino acid side chains, formation of protein–protein cross-links, oxidation of the polypeptide backbone resulting in protein fragmentation, and lipid peroxidation<sup>81,82</sup>. These oxidation-induced lesions have been suggested to contribute to various diseases and degenerative processes such as aging, carcinogenesis, immunodeficiencies and cardiovascular diseases and have also been implicated in the pathogenesis of most inflammatory diseases, including cerebral vascular disease. Since pro-inflammatory molecules are involved in the pathogenesis of these inflammatory diseases, interactions between ROS and NF- $\kappa$ B might be a component of the intracellular signalling process that leads to activation.

In this regard, the mouse hepatocyte iNOS promoter has NF- $\kappa$ B-binding sites at nt –1044 to nt –1034 and at nt –114 to nt –104, which are considered to be critical for iNOS expression in response to pro-inflammatory cytokine stimulation. Activity of the transcription factor NF- $\kappa$ B is redox-modulated and peroxide-mediated oxidative stress induces hyper-acetylation and enhanced accessibility of the restriction enzyme to nt-114 NF- $\kappa$ B region. Thus, chromatin structural changes activate the NF- $\kappa$ B site and increase interleukin-1 $\beta$ -stimulated iNOS expression in the presence of oxidative stress<sup>83</sup>.

Four areas of evidence indicate that NF- $\kappa$ B activation is linked to the generation of ROS. First, treatment with hydrogen peroxide directly activates NF- $\kappa$ B in some cells. In addition, over-expression of superoxide dismutase, the enzyme that converts superoxide anion to hydrogen peroxide, enhances the TNF-induced activation of NF- $\kappa$ B. Second, most of the known stimuli for NF- $\kappa$ B activation, including LPS, TNF- $\alpha$ , and IL-1 $\beta$ , produce oxidative stress in cells. Third, treatment with N-acetylcysteine,  $\alpha$ -lipoic acid, membrane-permeable hydroxyl scavengers, metallothionein and the iron chelator, PDTC, blocks NF- $\kappa$ B activation induced by a wide variety of stimuli. These antioxidants are also effective in attenuating inflammation in rodent models of neutrophilic lung and cerebral inflammation. Fourth, over-expression of catalase or glutathione peroxidase, enzymes that scavenge hydrogen peroxide as well as organic peroxides using glutathione (GSH) as the electron donor, inhibits the cytokine-induced activation of NF- $\kappa$ B. Further, over-expression of  $\gamma$ -glutamylcysteine synthetase, the rate-limiting enzyme for GSH synthesis attenuates TNF- $\alpha$ -induced NF- $\kappa$ B activation. These observations, together, suggest that ROS act as a common second messenger following cellular exposure to agents that induce NF- $\kappa$ B activation<sup>84–87</sup>.

**Table 3.** Therapeutic targetting of NF- $\kappa$ B

Inhibitors of Rel/NF- $\kappa$ B	Molecule	Mechanism
Degradation-resistant I $\kappa$ B proteins	*I $\kappa$ B $\alpha$ mutant/ super-repressor	Sequesters NF- $\kappa$ B in the cytoplasm and thus prevents the induction of specific NF- $\kappa$ B target genes
Glucocorticoids	Dexamethasone Prednisone	Induce expression of I $\kappa$ B $\alpha$ to enhance the cytosolic retention of NF- $\kappa$ B Maintain NF- $\kappa$ B in an inactive cytoplasmic complex so that the expression of genes involved in the pathogenesis of the immune response is reduced Reduce NF- $\kappa$ B-mediated transcriptional activity without altering I $\kappa$ B protein levels or the nuclear translocation of NF- $\kappa$ B Direct protein–protein interactions between the activated glucocorticoid receptor and NF- $\kappa$ B can also prevent activation of this pathway Competition between NF- $\kappa$ B and the glucocorticoid receptor for limiting amounts of the coactivators CREB-binding protein (CBP) and steroid receptor coactivator-1 (SRC-1) Glucocorticoid receptor directly disrupts p65 interactions with the basal transcription machinery, by direct interactions either with p65 or with components of the basal transcription complex
NSAIDs	Aspirin Sodium salicylate Sulfasalazine Sulindac	Specific inhibition of ATP-binding to IKK $\beta$ . Thus, IKK $\beta$ -dependent phosphorylation of I $\kappa$ B $\beta$ is markedly reduced, preventing its degradation by the proteasome and activation of the NF- $\kappa$ B pathway Suppression of I $\kappa$ B $\alpha$ phosphorylation and its subsequent degradation Activation of the NF- $\kappa$ B pathway by inhibiting IKK activity
Immunosuppressive agents	CsA Tacrolimus (FK-506)	CsA serves as a noncompetitive inhibitor of the chymotrypsin-like activity of the 20S proteasome and thus prevents I $\kappa$ B $\alpha$ degradation and activation of the NF- $\kappa$ B pathway Specifically blocks translocation of c-Rel from the cytoplasm to the nucleus FK-506 blocks both antigen receptor-induced and phorbol ester and ionomycin-induced c-Rel nuclear translocation in both B and T cells; neither RelB induction nor p50 expression is altered by FK-506 treatment
CyPGs		Directly inhibits NF- $\kappa$ B activity by modification of a cysteine residue in the activation loop of IKK $\beta$
Inhibition of proteasome function prevents I $\kappa$ B degradation	Peptide aldehydes: MG101, MG132, and MG115 Lactacystin  Boronic acid peptides: PS-341	Inhibitors of proteasome function reduce the degradation of I $\kappa$ B to prevent activation of the NF- $\kappa$ B pathway  Block protein degradation activity by acylating a threonine residue in one of the key proteasome subunits Potent inhibitors of proteasome function
Natural products	Flavonoids Rsvratrol  Myricetin, etc.	Ability to reduce IKK activity Inhibit NF- $\kappa$ B activity and induce apoptosis in transformed cells, which may contribute to the ability of red wine to reduce mortality from coronary heart diseases and certain cancers Has strong inhibitory effects on iNOS expression and NO generation in activated macrophages

\*Is I $\kappa$ B $\alpha$  protein with mutations at serine residues 32 and 36 is not subject to phosphorylation by IKK and is not degraded by the proteasome.  
Cyclopentenone prostaglandins, CyPGs; Cyclosporin A, CsA; Nonsteroidal anti-inflammatory drugs, NSAIDs.

However, the common point of the interaction between ROS on the NF- $\kappa$ B activation pathway is unknown. The most likely scenario is that ROS promote the activation pathway by activating a critical redox-sensitive kinase, probably NIK or the IKK signals, since these molecules lead to phosphorylation of critical serine residues in I $\kappa$ B, resulting in the liberation of cytoplasmic RelA/p50 heterodimers. However, the exact biochemical nature of the interaction between ROS and kinase activity of IKK $\alpha$ , IKK $\beta$ , or NIK has not yet been delineated. It is possible, though not proven, that the redox state of critical cysteine residues could reversibly modulate kinase activity and regulate NF- $\kappa$ B activation.

### *NF- $\kappa$ B as a therapeutic target*

Consistent with the pivotal role of NF- $\kappa$ B in inflammation, it is an attractive target for various anti-inflammatory compounds (Table 3). It can be blocked at various steps, including its activation through different pathways, its translocation to the nucleus and its binding to DNA. Indeed the most commonly used compounds like aspirin and other nonsteroidal anti-inflammatory drugs do block NF- $\kappa$ B<sup>88</sup>. They inhibit IKK $\beta$ -dependent phosphorylation of I $\kappa$ B $\alpha$ , thus preventing its degradation and subsequent activation of NF- $\kappa$ B.

Glucocorticoids such as prednisone are one of the most potent anti-inflammatory agents used in therapy, and they

cause transcriptional repression of several cytokines and adhesion molecules. While glucocorticoids down-regulate the expression of genes relevant during inflammation, NF- $\kappa$ B/Rel proteins function as important positive regulators of these genes. Glucocorticoids inhibit NF- $\kappa$ B at multiple steps, including induction of I $\kappa$ B $\alpha$  leading to enhanced binding to NF- $\kappa$ B and retention in the cytoplasm, direct interaction of activated glucocorticoid receptor (GnR) in cytosol with NF- $\kappa$ B, competition between GnR and NF- $\kappa$ B for coactivators and inhibition of basal transcriptional complex<sup>89</sup>. Even other anti-inflammatory compounds used in therapy of rheumatoid arthritis have been shown to inhibit NF- $\kappa$ B<sup>90,91</sup>. For example, gold compounds block NF- $\kappa$ B activation. Sulphasalazine<sup>92</sup> inhibits TNF- $\alpha$ -induced NF- $\kappa$ B activation by decreasing I $\kappa$ B $\alpha$  phosphorylation like NSAIDs. Other immunosuppressive drugs like cyclosporin and tacrolimus also inhibit the NF- $\kappa$ B pathway<sup>93</sup>.

Other ways to block NF- $\kappa$ B is to use synthetic peptides that inhibit proteasome degradation of I $\kappa$ B<sup>94</sup>, anti-cytokine therapy to prevent cytokine-mediated activation and introduction of I $\kappa$ B $\alpha$  super-repressor. Recent studies<sup>95</sup> have shown that during the resolution of inflammation, cyclopentenolone prostaglandins (cyPG) induced NF- $\kappa$ B activation in leukocytes and protracted the inflammatory response. Notably, the selective COX2 inhibitor NS398 did not reduce NF- $\kappa$ B DNA-binding activity in leukocytes, which suggests that cyPGs may act downstream of NF- $\kappa$ B in the resolution of inflammation *in vivo*. There are concerns with the use of these inhibitors of NF- $\kappa$ B, which may have effects independent of the NF- $\kappa$ B pathway. However, use of inhibitors allows modulation of NF- $\kappa$ B at specific stages of the inflammatory response. It is important to note that such inhibitors may prevent the proper resolution of inflammation *in vivo*. Thus, the identification of NF- $\kappa$ B as a key player in the pathogenesis of inflammation suggests that NF- $\kappa$ B-targeted therapeutics might be effective in diseases like RA, inflammatory bowel disease, and various other animal models of inflammatory disease<sup>96,97</sup>.

The ultimate benefit of such targeted therapy will depend on the delicate balance between suppression of inflammation and interference with normal cellular functions. Unfortunately, currently available molecular genetics tools do not allow modulation of the NF- $\kappa$ B pathway at different stages of the inflammatory response *in vivo*. It is to be hoped that the development of such tools will help define the role of NF- $\kappa$ B pathway in inflammation.

## Conclusion

NF- $\kappa$ B is a central regulator of innate and adaptive immune responses. This function is accomplished through the induction of genes, some of which promote inflammation, leukocyte migration and activation, whereas others act as potent inhibitors of apoptosis. Finally, the role of NF- $\kappa$ B

in modulating the expression of different cytokines strongly supports its proposed role as a coordinating element in the body's response to stress, infection or inflammation. It may be desirable under certain circumstances, such as during cancer therapy, to dissociate the immunoregulatory function of NF- $\kappa$ B from its anti-apoptotic activity. However, this may not be easily achieved without a better understanding of all the mechanisms involved in the activation of specific and physiologically relevant NF- $\kappa$ B targets. The complete understanding of NF- $\kappa$ B gene regulation is therefore a major challenge for future research.

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