THE IKK NF-κB SYSTEM: A TREASURE TROVE FOR DRUG DEVELOPMENT

Michael Karin, Yumi Yamamoto‡ and Q. May Wang‡*

Nuclear factor-κB (NF-κB)/Rel transcription factors have been suspected since their discovery to play a pivotal role in chronic and acute inflammatory diseases. It now seems that aberrant regulation of NF-κB could also underlie autoimmune diseases and different types of cancer. Recently, NF-κB and the signalling pathways that regulate its activity have become a focal point for intense drug discovery and development efforts. Given the large number of major ailments in which aberrant regulation of NF-κB has been observed or is suspected, such efforts seem well justified. This review will discuss recent progress in the development of drugs that inhibit NF-κB activation, and consider their potential applications in inflammatory and autoimmune diseases, as well as cancer.

ANKYRIN
A type of protein structural motif composed of a helix-turn-helix that mediates protein–protein interactions.

PHOSPHORYLATION
A type of protein modification involving the covalent addition of phosphate groups to serine, threonine or tyrosine residues.

**Laboratory of Gene Regulation and Signal Transduction Department of Pharmacology, School of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0636, USA.*
‡Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285, USA.
e-mails: KarinOffice@ucsd.edu; qmwang@lilly.com
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The nuclear factor-κB (NF-κB) proteins are a small group of closely related transcription factors, which in mammals consists of five members: Rel (also known as c-Rel), RelA (also known as p65 and NF-κB3), RelB, NF-κB1 (also known as p50) and NF-κB2 (also known as p52). All five proteins have a Rel homology domain (RHD), which serves as their dimerization, DNA-binding and principal regulatory domain[1] (FIG. 1). The RHD contains at its C terminus a nuclear-localization sequence (NLS), which is rendered inactive in non-stimulated cells through binding of specific NF-κB inhibitors, known as the IκB proteins[1]. The IκBs use a core domain composed of six to seven ANKYRIN repeats to bind to the RHD and thereby mask the NLS. Interestingly, NF-κB1 and NF-κB2 are initially made as the larger precursors p105 and p100, respectively[2]. The two precursors are in essence an RHD fused through its C terminus to an auto-inhibitory IκB-like domain[1]. So the two precursors, which can dimerize with the different Rel proteins, are trapped in the cytoplasm and can therefore function both as reservoirs for the mature p50 and p52 subunits and as IκBs. Usually, p105 undergoes constitutive (non-regulated) processing to p50, causing the release of dimers containing the p50 subunit, which translocate to the nucleus unless met by another IκB protein. Unlike p105, which is not particularly selective in its choice of partners, p100 is found in the cytoplasm mostly dimerized with RelB[3]. Furthermore, unlike p105, p100 is subjected to regulated, signal-dependent processing that results in the preferential release of p52–RelB dimers[4] (FIG. 2).

Activation of most forms of NF-κB, especially the most common form — the p50–RelA dimer — depends on PHOSPHORYLATION-induced UBIQUITINATION of the IκB proteins (FIG. 2). This sequential modification depends on two protein complexes: the IκB kinase (IKK) complex and the $E3^{IκB}$ ubiquitin ligase complex[2]. Once poly-ubiquitinated, the IκBs undergo rapid degradation through the 26S proteasome and the liberated NF-κB dimers translocate to the nucleus, where they participate in transcriptional activation of specific target genes[4]. The IKK complex is composed of three subunits: the catalytic subunits IKK-α and IKK-β, and the regulatory subunit IKK-γ (also known as NEMO)[5]. Gene-disruption experiments indicate that IKK activity and classical NF-κB activation are absolutely dependent on the integrity of IKK-γ[6]. Interestingly, however, IKK-γ is not required for activation of the alternative NF-κB signalling pathway, which leads to nuclear translocation of p52–RelB dimers[7]. Of the two catalytic subunits, the most important for activation of the classical NF-κB signalling

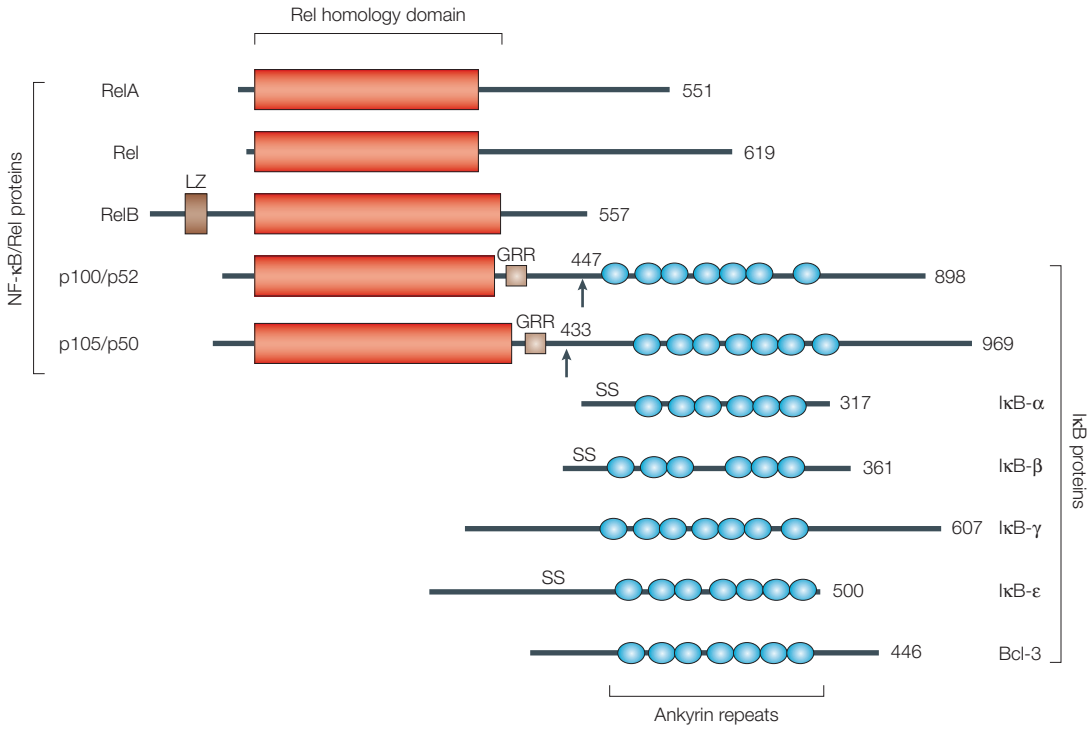


Figure 1 | **Schematic structure of NF-κB and IκB proteins.** The NF-κB proteins are related to each other by the presence of the c-Rel homology domain (RHD, red box) whereas IκB proteins share six to seven ankyrin repeats (AR, blue beads). The ARs of the inhibitors dock onto the RHDs of the NF-κB proteins and cause their cytoplasmic retention. In the case of the p105 and p100 precursors, these interactions can occur intramolecularly or with the RHD of the partner to which the precursor is bound. GRR, glycine-rich repeat; IκB, inhibitor of NF-κB; LZ, leucine zipper; NF-κB, nuclear factor-κB; SS, two conserved serines in IκB.

pathway is IKK-β[8–10]. Interestingly, cells lacking IKK-α show normal induction of NF-κB DNA-binding activity in response to most stimuli[11,12]. Nonetheless, IKK-α is required for activation of NF-κB DNA-binding activity in response to engagement of receptor activator of NF-κB (RANK), a member of the tumour-necrosis factor (TNF) receptor (TNFR) family[13]. Recent experiments indicate that IKK-α might also contribute to induction of NF-κB-dependent gene expression in fibroblasts stimulated with TNF-α, by acting as a histone H3 kinase[14]. However, mice that lack IKK-α kinase activity do not show any major defects that are consistent with aberrant activation of NF-κB target genes in response to TNF-α[13]. Therefore the histone kinase activity of IKK-α might be required only in certain cell types, such as fibroblasts[15]. As discussed below, the inhibition of IKK-α activity does not have the same pathophysiological outcomes as the inhibition of IKK-β activity.

IKK-α kinase activity, however, is indispensable for activation of the alternative NF-κB signalling pathway (FIG. 2), as it is essential for inducible p100 processing[4,7,16]. This function of IKK-α cannot be provided by IKK-β, despite the close structural similarity between the two catalytic subunits. Another unique function of IKK-α is its role in the induction of keratinocyte differentiation[12]. This function, however, does not depend on the protein kinase activity of IKK-α, its ability to bind IKK-γ or the activation of NF-κB[12].

Recent experiments, based on the use of conditional IKK-β loss-of-function mutations, indicate that IKK-β activity is required for the inactivation of a severe inflammatory reaction that leads to multi-organ failure in response to ischaemia-reperfusion[10]. IKK-β activity is also required for protection of a large number of different cell types from apoptosis[17]. These results indicate that IKK-β inhibitors could be useful for the treatment of inflammatory diseases[1,18,19]; however, an expected side effect inherent to such inhibitors would be increased susceptibility to the induction of programmed cell death. As such, a more likely application for IKK-β inhibitors could be in cancer therapy[20,21,22]. As previously discussed, there is ample circumstantial evidence that a variety of tumours, both solid and of haematological origin, use constitutive activation of NF-κB to suppress their susceptibility to both inherent and drug-induced apoptosis[17]. Inhibition of IKK-α activity, however, can be expected to have different results. For instance, mice in which IKK-α has been rendered inactivatable show defective RANK signalling, but normal TNFR1 signalling[13]. These mice, termed *Ikk-α*^{AA} mice, also exhibit normal innate immunity but defective development of secondary lymphoid organs, and defective humoral response (antibody production) to T-cell-dependent antigens[16]. These findings indicate that selective IKK-α inhibitors might be useful for inhibiting OSTEOCLAST formation, which depends on RANK signalling, and for preventing B-cell-mediated autoimmune diseases.

UBIQUITINATION
A type of protein modification involving the covalent addition of ubiquitin, a small protein, to lysine groups. Poly-ubiquitination targets proteins for degradation.

OSTEOCLASTS
Bone-degrading cells, derived from macrophages.

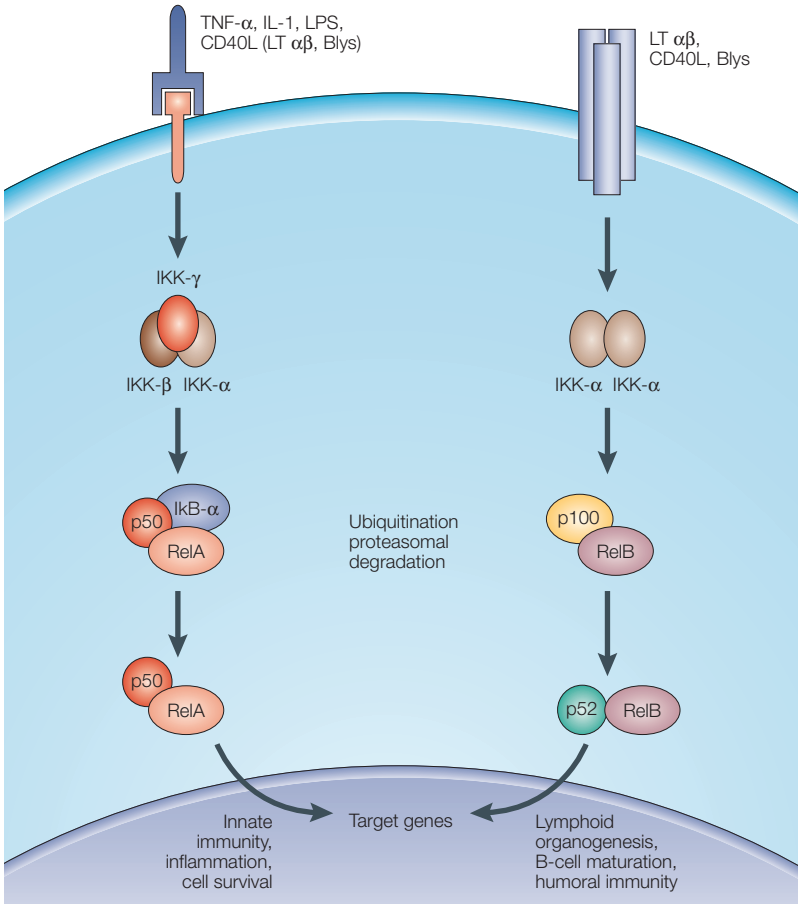


Figure 2 | **Schematic representation of the two NF-κB signalling pathways.** The classical pathway is depicted on the left. It is activated by TNF-α, IL-1, LPS, CD40 ligand (CD40L) and to a lesser extent by lymphotoxin α/β (LT α/β) and Blys/BAFF. Activation of this pathway depends on the three-subunit IKK holocomplex, which phosphorylates IκBs to induce their degradation. This pathway is crucial for the activation of innate immunity and inflammation, and for inhibition of apoptosis (increased cell survival). The alternative pathway is depicted on the right. It is activated by LT α/β, CD40L and Blys/BAFF, but not by TNF-α, IL-1 or LPS. Activation of this pathway depends on IKK-α homodimers, which induce processing of p100 and nuclear translocation of RelB–p52 dimers. This pathway is crucial for secondary lymphoid organ development, maturation of B cells and adaptive humoral immunity (that is, the production of high-affinity antibodies). IκB, inhibitor of NF-κB; IKK, IκB kinase; IL, interleukin; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; TNF, tumour-necrosis factor.

Strategies for inhibiting NF-κB

One can envision several different strategies for inhibiting NF-κB activation or function. One possibility is interfering with the binding of NF-κB to DNA. Although this can be accomplished through the use of decoy κB sites or their analogues, such molecules are quite large and polar, properties which are likely to hinder their cellular uptake and bioavailability. Given the large interaction surface mediating the binding of NF-κB to DNA, it is quite unlikely that small, non-polar molecules that specifically block NF-κB DNA binding can be found. The same logic applies for molecules that inhibit the dimerization of NF-κB proteins.

A strategy that is more likely to succeed is to interfere with the process of NF-κB activation. Indeed, inhibitors of the 26S proteasome were shown to inhibit IκB degradation and NF-κB nuclear translocation[23], as well as inducible p100 processing[24]. At least one proteasome inhibitor, bortezomib (Velcade; Millenium), has entered clinical development for the treatment of myeloma[25]. Nonetheless, it is not clear whether the therapeutic effects of bortezomib are due to inhibition of IκB degradation (and NF-κB activation) or to inhibition of other targets. After all, the proteasome is involved in the degradation of all poly-ubiquitinated proteins. A higher degree of specificity might be expected from inhibitors of the E3 ubiquitin ligases and the E2 ubiquitin-conjugating enzymes responsible for the phosphorylation-dependent poly-ubiquitination of IκBs and p100 (REF. 8). However, even these enzymes are involved in the poly-ubiquitination of several targets, and their inhibition is unlikely to result in very specific inhibition of NF-κB activation. For instance, the $E3^{IκB}$ complex has also been implicated in the degradation of β-catenin[26–28]. As accumulation of β-catenin can promote neoplastic transformation[29,30], inhibition of $E3^{IκB}$ activity might not offer the best approach for inhibiting NF-κB activation.

Given the genetic analysis described above, the most effective and selective approach for inhibition of NF-κB activation might be offered by inhibitors of IKK activity. So far there is little evidence that either IKK-α or IKK-β phosphorylate proteins that are not involved in NF-κB signalling. In addition, with the exception of the involvement of IKK-α in keratinocyte differentiation, all of the phenotypes caused by loss of IKK-α or IKK-β function can be attributed to defective activation of either the alternative or the classical NF-κB signalling pathway. Therefore, both IKK-α and IKK-β have been pursued by many groups as targets for the development of therapeutic agents to be used for the treatment of cancer, as well as inflammatory and metabolic diseases (BOX 1). The following sections summarize recent progress in the development of agents that specifically inhibit IKK enzymatic activity, and also discuss improvements in our understanding of the action of older drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs), which recent data indicate function as nonspecific IKK inhibitors.

Non-steroidal anti-inflammatory drugs

With the growing understanding of the importance of NF-κB in regulating the inflammatory process, the function of conventionally used anti-inflammatory agents has been re-evaluated and shown to be due, at least partially, to interference with the IKK–NF-κB system. In this section, we will discuss recent evidence for the ability of immunomodulating drugs to inhibit activation of IKK and/or NF-κB (BOX 1).

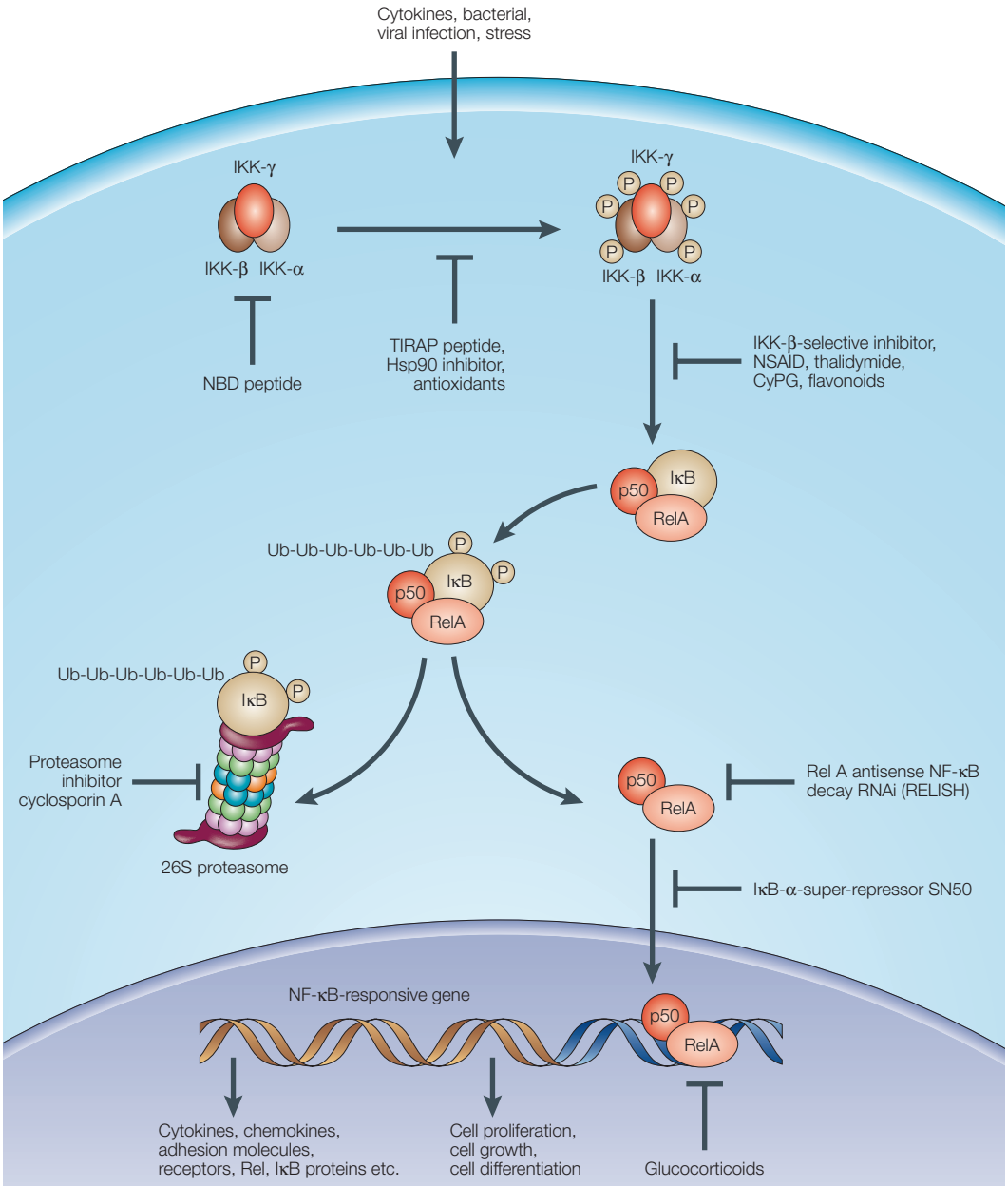
Several NSAIDs are capable of inhibiting NF-κB activation. These agents include aspirin and salicylates[31–33], sulindac and its analogues[34–36], and sulphasalazine and its metabolites[37–39]. The most commonly accepted mechanism by which NSAIDs exert their anti-inflammatory activities is by inhibition of cyclooxygenases (COX), which are essential for the production of PROSTAGLANDINS. However, the effects of these agents on the NF-κB pathway are independent of COX inhibition, as suggested by the fact that indomethacin, a potent inhibitor of

PROSTAGLANDINS
Derivatives of arachidonic acid (a fatty acid) that can trigger various physiological responses, including inflammation and pain.

prostaglandin synthesis, does not inhibit the NF-κB pathway[33,34]. Aspirin and sodium salicylate inhibit TNF-α-induced endothelial expression of the adhesion molecules vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) (REF. 32), which are encoded by NF-κB target genes. Treatment

Box 1 | **Other tools to interfere with the NF-κB pathway**

Although major efforts to develop inhibitors of the nuclear factor-κB (NF-κB) pathway in the pharmaceutical industry have focused on selective IKK-β inhibitors, there are a number of other different potential approaches. As summarized in the figure, targets of these approaches can be categorized into seven different groups on the basis of unique events in regulation of NF-κB proteins: regulation of NF-κB protein expression and binding to DNA (RelA antisense, NF-κB decoy and RNAi); interference with the formation of the IKK complex (NBD peptide, which corresponds to the IKK-γ-binding domain of IKK-β); blockade of IKK-β activation process (TIRAP peptide, which corresponds to the Toll-interleukin-1 receptor (TIR) domain-containing adapter domain (TIRAP), Hsp90 inhibitor: Hsp90 stabilizes RIP proteins that are the components of the TNF-α receptor signalling complex and antioxidants); inhibitors of IKK-β kinase activity; proteasome inhibitors; NF-κB nuclear translocation inhibitors (IκB-α super-repressor and SN50: a peptide consisting of the nuclear localization sequence (NLS) of the p50 NF-κB subunit); and last, inhibitors of NF-κB transcriptional activity (glucocorticoids). Given the diverse processes involved in regulation of the NF-κB pathways, a better basic understanding of these crucial signalling pathways will ultimately lead to new avenues in the development of efficient and clinically useful inhibitors.



of endothelial monolayers with sodium salicylate also inhibits the transendothelial migration of leukocytes[32], which depends on VCAM-1 and ICAM-1 expression. Such findings indicate that part of the anti-inflammatory properties of salicylates can be accounted for by inhibition of the NF-κB pathway. Furthermore, aspirin and sodium salicylate are competitive inhibitors of the ATP-binding site of IKK-β, thereby impairing the phosphorylation of IκBs and subsequent activation of NF-κB[33].

Sulindac, which is structurally related to indomethacin, and its derivatives (sulindac sulphide and sulindac sulphone) are also able to bind IKK-β and inhibit its catalytic activity and thereby prevent NF-κB activation in response to TNF-α stimulation[34]. In the colon cancer cell line HCT-15, which is defective in prostaglandin synthesis, sulindac, and to a lesser extent aspirin, enhance TNF-α-mediated apoptosis, suggesting that the pro-apoptotic response seen in these cells is independent of COX inhibition[37]. Sulindac also blocks TNF-α-induced NF-κB DNA binding, potentiates TNF-α-mediated cell killing in pulmonary carcinoma cell lines[35], and suppresses tumour growth of gastric carcinoma cells in nude mice[36]. These data indicate that treatment with sulindac in combination with cytokines that both induce apoptosis and activate the NF-κB pathway might result in enhanced cell death.

Sulphasalazine, another NSAID that is widely used to treat inflammatory bowel disease, is cleaved following oral administration to 5-amino-salicylic acid (5-ASA) and sulphapyridine. The treatment of human colonic epithelial cells with sulphasalazine, but not 5-ASA or sulphapyridine, prevents NF-κB activation through blocking IκB phosphorylation and degradation in response to TNF-α, lipopolysaccharide (LPS) or phorbol esters[37]. However, in a more recent study, 5-ASA was shown to block NF-κB activation by inhibiting both IKK-α and IKK-β kinase activity in mouse colonic cells[38]. This discrepancy might simply result from different permeability or uptake of 5-ASA in different cells. Mesalamine, a related aminosalicylate, can block the phosphorylation of p65 without affecting IκB degradation[39]. Although the effects of sulphasalazine and its metabolites are partially contradictory, these data indicate that these agents can block the NF-κB activation pathway at multiple steps.

Immunomodulatory drugs

Thalidomide and its analogues, which are known as immunomodulatory drugs (IMiDs), have anticancer, anti-inflammatory, anti-angiogenic and immunosuppressive effects that are achieved by modulating the levels of cytokines, including TNF-α, interleukin-6 (IL-6), IL-12 and vascular endothelial growth factor (VEGF). Recently, these agents, including IMiD CC-5013 (Phase III for multiple myeloma and metastatic melanoma) and IMiD CC-4047 (Phase I/II for multiple myeloma and prostate cancer)[40], have shown promise in clinical trials for the treatment of different cancers. Among several different hypotheses, the inhibition of NF-κB activation has been proposed to explain the therapeutic activity of thalidomide and related agents[41].

In endothelial cells, thalidomide prevents the degradation of IκB-α by inhibiting IKK-β, which is consistent with its role in inhibiting cytokine-induced NF-κB activation[41]. The inhibitory effect of thalidomide on TNF-α and H_2O_2-induced NF-κB activation is also seen in other cell types, including T lymphocytes, and myeloid and epithelial cells[42]. IMiD-induced apoptosis in multiple myeloma cells is associated with downregulation of NF-κB DNA-binding activity, as well as the reduced expression of NF-κB-dependent proteins, including the cellular inhibitor of apoptosis protein 2 (c-IAP-2) and FLICE inhibitory protein (c-FLIP)[43]. Therefore, a portion of the immunosuppressive effects of thalidomide might be due to inhibition of NF-κB activation.

Cyclopentenone prostaglandins (cyPGs) are naturally occurring prostaglandin metabolites[44]. These molecules are synthesized during the late phase of an inflammatory response and are thought to be key regulators in the resolution of inflammation. The anti-inflammatory activity of CyPGs has been attributed to their ability to inhibit NF-κB activation or activity. This effect could be partly due to the ability of cyPGs to activate the peroxisome proliferation-activated receptor-γ (PPAR-γ), which has been shown to antagonize NF-κB transcriptional activity[45]. The treatment of peritoneal macrophages with the cyPG 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) inhibits the expression of inducible nitric oxide synthase (iNOS), as well as NF-κB activity in a PPAR-γ-dependent manner. The synthetic PPAR-γ ligand BRL-49653 can also inhibit NF-κB activity. However, cyPGs can directly inhibit activation of NF-κB pathway by blocking IKK-β activity[46].

Both 15d-PGJ_2 and PGA_1 inhibit IκB-α degradation through inhibition of IKK activity by direct covalent modification of IKK-β at cysteine 179 within its activation loop. Cysteine residues in the DNA-binding domain of p50 and p65 might also be targets of cyPGs[47]. The substitution of these cysteines with serines abolishes the inhibitory effects of 15d-PGJ_2 on NF-κB DNA binding, suggesting that modification of p50 and/or p65 by cyPGs might be important for the inhibition of NF-κB activation. Interestingly, NF-κB might be involved not only in the onset of inflammation, but also in its resolution by being able to activate genes encoding both pro- and anti-inflammatory mediators[48]. For example, NF-κB activity is associated with increased iNOS expression during the onset of inflammation, whereas in the late phase of this process NF-κB activation is associated with expression of COX2, which directs the synthesis of anti-inflammatory cyPGs[48]. As such, the inhibition of NF-κB by cyPGs might be part of a negative-feedback loop that contributes to the resolution of inflammation.

Dietary supplements and herbs are commonly used to reduce the risk of atherosclerosis, neurodegenerative disorders and cancer. Several studies have recently suggested that the potential benefits of these agents might result from inhibition of the NF-κB signalling pathways along one or several steps in their activation cascade. Antioxidants, including vitamin C[49,50], and flavonoids[51,52] are examples of such agents. Antioxidants can reduce the balance of reactive oxygen species (ROS) generated by

phagocytic leukocytes during chronic and acute inflammatory diseases or by environmental stresses[53]. It was initially reported that oxidative stress enhances the expression of pro-inflammatory genes regulated by NF-κB, and that NF-κB activation can also increase the levels of intracellular ROS. However, recent reports show that inhibition of NF-κB activation actually promotes ROS production[54] and that ROS might not play a crucial role in NF-κB activation[55]. Therefore the mechanism of antioxidant action is far from being clearly understood.

Nevertheless, the administration of the antioxidant *N*-acetyl-L-cysteine (NAC) suppresses LPS-induced NF-κB activity and neutrophilic alveolitis in rats[56]. Vitamin C inhibits TNF-α- and IL-1β-induced IKK phosphorylation of IκB-α and subsequent NF-κB DNA binding in endothelial cell lines[49]. The inhibitory effect of vitamin C is relieved by treatment with a p38 mitogen-activated protein kinase (MAPK) inhibitor, suggesting that vitamin C enhances the activity of p38 MAPK and apparently exerts an indirect negative regulatory effect that acts between the TNF-α receptor and IKK complex[49]. In a study of dehydroasocorbic acid (DHA), which is the oxidized form of ascorbic acid generated in the biosynthetic pathway of vitamin C, suppression of TNF-α-induced NF-κB activation was proposed to result from the direct inhibition of IKK-β kinase activity independent of p38 MAPK[50]. It is important to realize that antioxidants can also inhibit the activity of other components of NF-κB signalling pathways, including TNF receptors and the proteasome, without exerting any direct effect on IKK[54].

Flavonoids are naturally occurring phenolic compounds that are ubiquitous in plants, and which have been used to suppress inflammation, prevent the development of cancer and protect against vascular disease. Several studies demonstrate that flavonoids mediate their effects by inhibiting NF-κB signalling[51,52]. For example, resveratrol inhibits expression of iNOS and decreases nitric oxide production in activated macrophages, which is associated with inhibition of LPS-induced IκB-α phosphorylation and the NF-κB DNA-binding activity[51]. Resveratrol is also able to induce apoptosis in Rat-1 cells by inhibiting Ras-mediated activation of NF-κB[52]. These results indicate that at least some of the biological activities of flavonoids are mediated by inhibition of NF-κB pathways. It remains to be examined, however, whether flavonoids act as direct IKK inhibitors.

The re-evaluation of the function of commonly used anti-inflammatory and dietary agents illustrates that inhibition of the NF-κB pathway could be an important part of their therapeutic efficacy, as well their potential toxicity. A better understanding of the target specificity, and the determination of the serum levels of these agents required for inhibition of NF-κB signalling, will allow a more rational use of these agents. In addition, greater knowledge of the molecular determinants used by these compounds to inhibit IKK or other components of the NF-κB pathway should provide clues for the development of more specific and efficacious NF-κB inhibitors.

Development of selective IKK inhibitors

A major effort towards the development of selective IKK or NF-κB inhibitors has been undertaken by the pharmaceutical industry. Much of this effort entails the screening of large compound libraries, or the use of combinational chemistry, to identify inhibitors of IKK-α and/or IKK-β catalytic activities. No potent IKK-α-specific inhibitors have been described to date. This might stem, in part, from an incomplete understanding of the role for IKK-α in NF-κB activation. Several compounds, which are summarized in TABLES 1 and 2, can inhibit IKK-α kinase activity in the low micromolar range, although these agents were initially identified as IKK-β inhibitors. The unique role of IKK-α in the activation of the alternative pathway, which is important for B-cell-mediated responses, and the recent demonstration of the auxiliary role of IKK-α in the classical pathway, indicate that IKK-α might be an attractive target for therapeutic intervention in autoimmune diseases and cancer[13,14,16,57]. It is therefore anticipated that recent developments in the understanding of the IKK-α-dependent alternative pathway[4] will provide better cell-based assays for the identification of IKK-α-selective inhibitors.

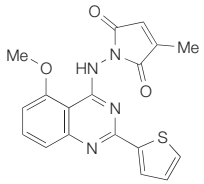
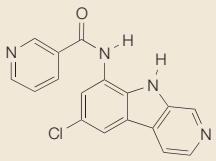
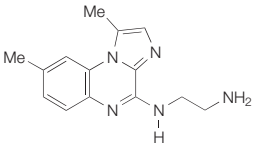
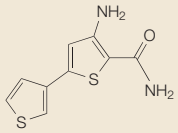
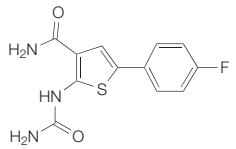
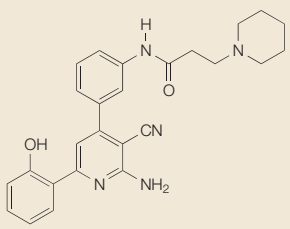
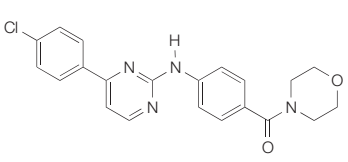
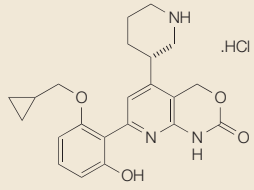
By comparison, the development of specific IKK-β inhibitors has progressed rather rapidly. Although most IKK-β inhibitors reported so far are still in pre-clinical stages of development, a number of novel small-molecule inhibitors of IKK-β have been disclosed (TABLES 1 and 2). For example, SPC-839 (compound 1), a member of a series of quinazoline analogues developed by Celgene[58–60], is one of the more extensively studied IKK-β inhibitors. SPC-839 inhibits IKK-β with an IC_{50} of 62 nM, and has a 200-fold selectivity for IKK-β over IKK-α (IC_{50} = 13 μM). This compound also inhibits IL-6 and IL-8 production in Jurkat T cells. When tested in animal models, SPC-839 blocks TNF-α production in LPS-challenged rats at 10 mg per kg and reduces paw oedema in a rat arthritis model at 30 mg per kg[59,60].

Several groups have reported the inhibition of IKK-β activity by β-carboline derivatives[61–63]. Of these, PS-1145 (compound 2), which was developed from a β-carboline natural product that inhibited several different kinases[62], has been extensively evaluated in various *in vitro* assays by different groups[61–63]. PS-1145 inhibits the IKK complex with an IC_{50} of 150 nM, blocks TNF-α-induced IκB phosphorylation and degradation in HeLa cells, and reduces the production of TNF-α in LPS-challenged mice[62]. In a separate study, PS-1145 was shown to interfere with NF-κB activation, abrogate cytokine production and secretion, and inhibit cell proliferation when tested in multiple myeloma cells[63].

Another well-studied molecule that inhibits IKK-β is BMS-345541 (compound 3), along with its related analogues[64,65]. BMS-345541 shows greater than tenfold selectivity for IKK-β (IC_{50} = 0.3 μM) over IKK-α (IC_{50} = 4 μM) and fails to inhibit a panel of 15 other cellular protein kinases at concentrations as high as 100 μM. Unlike other reported IKK inhibitors, BMS-345541 was found to bind at an allosteric site of IKK-β, and so behaves as an ATP-non-competitive inhibitor[65].

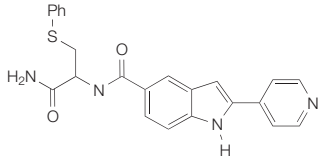
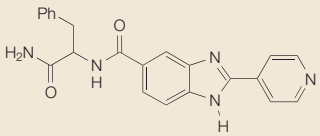
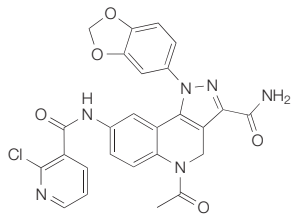
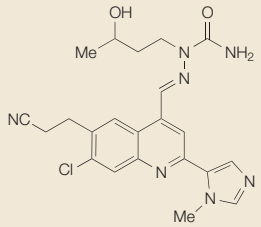
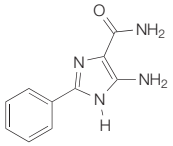
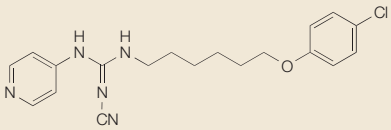
In addition, this molecule inhibits LPS-induced production of several cytokines, including TNF-α, IL-1, IL-6 and IL-8 in THP-1 monocytic cells, with IC_{50} values in the range 1–5 μM[65]. When tested in LPS-challenged mice, BMS-345541 blocked TNF-α production, measured in the serum of animals, with an EC_{50} of approximately 10

Table 1 | **Summary of selective IKK inhibitors in development**

Structure	Name	Comments	References
	Compound 1 (SPC-839) Quinazoline analogue	• Reversible ATP-competitive inhibitors • IC_{50} = 62 nM for IKK-β • IC_{50} = 13 μM for IKK-α • IC_{50} = 8 nM (NF-κB luciferase assay in Jurkat cells) • Inhibition of IL-2 and IL-8 production in Jurkat cells • Inhibition of NF-κB activation in LPS-challenged rat at 10 mg per kg • Paw oedema reduction in rat arthritis model at 30 mg per kg	58–60
	Compound 2 (PS-1145) β-carbolin analogue	• IC_{50} = 0.15 μM for IKK-β • IC_{50} = 5 μM (NF-κB activation in HeLa cells) • Inhibition of ICAM-1 transcription in primary cells • Reduction of TNF-α in LPS-challenged mice	61–63
	Compound 3 (BMS-345541) Imidazoquinoxaline derivative	• IC_{50} = 0.3 μM for IKK-β • IC_{50} = 4.0 μM for IKK-α • IC_{50} = 4 μM (IκB phosphorylation in cell-based assay) • Reduction of TNF-α in LPS-challenged mice • Effective in murine arthritis model	64–66
	Compound 4 (SC-514) Amino-thiophenecarboxamide derivative	• IKK-β inhibitor (IC_{50} = 2.7–11.2 μM) • Selective over IKK-α (IC_{50} >200 μM) and other kinases • Reversible ATP-competitive inhibitors • Blockade of IL-6, IL-8 and COX2 expression in cells (IC_{50} = 6–20 μM) • Inhibition of TNF-α production in LPS-challenged mice	67,68
	Compound 5 Ureido-thiophenecarboxamide derivative	• IC_{50} = 18 nM in IKK-β complex assay • Selective over IKK-α, JNK and p38 MAPK • Inhibition of TNF-α production in human monocytes (IC_{50} = 0.15–2.5 μM) • Blockade of IL-8 and IL-6 production by synovial fibroblasts (IC_{50} = 0.1 μM) • Paw oedema reduction in rat arthritis model	69–72
	Compound 6 Diarylpyridine derivative	• IC_{50} = 0.6 μM for IKK-β • IC_{50} = 20 μM for IKK-α • Inhibition of RANTES production in A549 cells (IC_{50} = 7 μM) • Reduction of LPS-induced TNF-α production in mice (ED_{50} = 2 mg per kg, ip)	73–75
	Compound 7 Anilino-pyrimidine derivative	• IC_{50} <0.5 μM for IKK-β • Inhibition of IκB degradation in cell-based assays • Block LPS-induced TNF-α production in mice (ED_{50} 1–30 mg per kg)	76
	Compound 8 Pyridooxazinone derivative	• Inhibition of IKK-β activity (IC_{50} = 4 nM) • Reduction of cytokine production in cell assays • Blockade of LPS-induced TNF-α in acute model	77

COX2, cyclooxygenase-2; ICAM-1, intercellular adhesion molecule-1; IκB, inhibitor of NF-κB; IKK, IκB kinase; IL, interleukin; ip, intraperitoneal; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; RANTES, regulated upon activation, normally T-expressed, and presumably secreted; TNF, tumour-necrosis factor.

Table 2 | **Summary of selective IKK inhibitors in development**

Structure	Name	Comments	References
	Compound 9 Indolecarboxamide derivative	• Inhibition of IKK activity (IC_{50} 0.05–32 μM)	78
	Compound 10 Benzoimidazole carboxamide derivative	• Inhibition of IKK activity (IC_{50} 0.07–70 μM)	79
	Compound 11 Pyrazolo[4,3-c]quinoline derivative	• IKK-β inhibitors (IC_{50} <1 μM)	80,81
	Compound 12 Imidazolylquinoline-carboxaldehyde semicarbazide derivative	• Selective against IKK-β (IC_{50} <30 μM) • Reduction of TNF-α in LPS-challenged mice • Efficacious in mouse arthritis model	82
	Compound 13 Amino-imidazolecarboxamide derivative	• IKK-β inhibitors	83
	Compound 14 Pyridyl cyanoguanidine derivative	• Antitumoral activity (Phase I/II evaluation) • Inhibition of IκB phosphorylation	84–88

IκB, inhibitor of nuclear factor-κB; IKK, IκB kinase; LPS, lipopolysaccharide; TNF, tumour-necrosis factor.

mg per kg. BMS345541 also shows dose-dependent efficacy in terms of reducing disease severity in a murine model of collagen-induced arthritis[66]. Interestingly, histopathological evaluation of various tissues, including liver, heart, lung and bone marrow of the mice treated with BMS345541 (six weeks of dosing at 100 mg per kg), revealed no toxicological changes[66].

More recently, Kishore and colleagues reported another IKK-β-selective inhibitor, SC-514 (compound 4)[67], which is similar to a group of amino-thiophenecarboxamides reported previously[68]. This compound inhibits various forms of recombinant IKK-β with IC_{50} values of 3–12 μM[67]. Unlike BMS-345541, SC-514 is a reversible ATP competitive inhibitor. Although it binds IKK-β at the conserved ATP-binding pocket, SC-514 demonstrates good selectivity, in that it does not inhibit ~30 cellular protein kinases tested and has little effect on other members of the IKK family, including IKK-α, IKK-ε and TBK1 *in vitro*[67]. It is interesting to note that SC-514 inhibits expression of NF-κB-dependent cytokines, such as IL-6 and IL-8, through the inhibition of IKK-β-mediated phosphorylation of IκB-α and p65 (REF. 67). Although SC-514 has limited bioavailability (2%) and a poor half-life (0.2 hours), it is efficacious in acute inflammation model and blocks TNF-α production in LPS-challenged rats[67].

In addition, several other compounds have been reported as nanomolar-range inhibitors of IKK-β kinase activity, and have demonstrated inhibitory activity in functional cell-based assays and shown efficacy in experimental models. It is noteworthy that a group of ureidocarboxamido thiophenes[69–71], some of which inhibit IKK-β with an IC_{50} as low as 18 nM, were found to reduce paw oedema in a rat arthritis

model by 100% at a dose of 30 mg per kg (compound 5)[72], indicating a potential use in the treatment of inflammatory disorders.

A recent report described the development of a group of 2-amino-3-cyano-4,6,-diarylpyridines as selective IKK-β inhibitors[73–75]. For example, compound 6 has an IC_{50} of 0.6 μM and 20 μM against the IκB-α kinase activity of IKK-β and IKK-α, respectively[75]. When tested in an acute cytokine-release model (LPS-induced TNF-α in mice), this inhibitor demonstrated *in vivo* efficacy with an ED_{50} of 2 mg per kg[75]. In addition, Signal Pharmaceuticals disclosed a group of anilinopyrimidine derivatives (compound 7) that inhibit IKK-β-mediated IκB phosphorylation and block LPS-induced TNF-α production in mice with ED_{50} values in the range 1–30 mg per kg[76].

More recently, a group of optically active pyridine analogues were reported to inhibit IKK-β activity[77]. Compound 8 inhibits IKK-β with an IC_{50} of 4 nM, demonstrates activity in cell-based assays, and reduces TNF-α production in acute mouse and rat models[77]. As summarized in TABLES 1 and 2, a number of other small molecules with diversified structures have been disclosed (compounds 9–13)[78–83]; however, no detailed information has been discussed in these disclosures.

It is interesting to note that CHS-828 (compound 14) and a group of related pyridyl cyanoguanidines were reported in a recent patent as IKK inhibitors[84,85]. CHS-828 was originally identified and evaluated as an anti-tumoral agent in clinical trials[86–88]. It is possible that CHS-828 and its analogues act by inhibiting IKK activity and blocking NF-κB activation. These studies, taken together with results obtained with PS-1145 discussed above, provide a framework for considering the potential use of IKK-β inhibitors in cancer treatment. However, the safety and efficacy profiles of these compounds remain to be determined, and until then it is not clear whether they can be used in the treatment of chronic inflammatory disorders.

Other approaches

In addition to efforts that focus on the design of specific small-molecule inhibitors, the use of macromolecules to block the activity or expression of IKKs has also been explored. These approaches include the use of antisense oligonucleotides that target the nucleic acid sequence of IKK-β to inhibit its expression and thereby prevent NF-κB activation[89]. Numerous recent reports have described the use of small interfering RNAs (siRNA) that modulate the expression of IKK proteins through RNA interference (RNAi); however, these approaches seem to be more suitable for mechanistic and target-validation studies than for therapeutic applications[90]. In addition to antisense oligonucleotides and RNAi approaches, the development of cell-permeable peptides containing the IKK-γ-binding motif, which is located at the C termini of IKK-α and IKK-β, has also been reported[89,90]. These peptides compete with IKK-α and IKK-β for binding to IKK-γ, thereby preventing assembly of the IKK complex and blocking activation of the canonical pathway. As expected, these peptides were shown to inhibit TNF-α-induced NF-κB activation and reduce expression of NF-κB-dependent genes in human endothelial cells[91,92].

In summary, given the recent progress in the development of IKK inhibitors there is much hope that one or several of these inhibitors will enter clinical testing and prove useful in either cancer therapy as an apoptosis-sensitizing drug or in the therapy of inflammatory and autoimmune diseases.

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Competing interests statement

The authors declare that they have no competing financial interests.

Online links

DATABASES

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