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The non-canonical NF-κB pathway in immunity and inflammation

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Abstract

The nuclear factor-κB (NF-κB) family of transcription factors is activated by canonical and non-canonical signalling pathways, which differ in both signalling components and biological functions. Recent studies have revealed important roles for the non-canonical NF-κB pathway in regulating different aspects of immune functions. Defects in non-canonical NF-κB signalling are associated with severe immune deficiencies, whereas dysregulated activation of this pathway contributes to the pathogenesis of various autoimmune and inflammatory diseases. Here we review the signalling mechanisms and the biological function of the non-canonical NF-κB pathway. We also discuss recent progress in elucidating the molecular mechanisms regulating non-canonical NF-κB pathway activation, which may provide new opportunities for therapeutic strategies.

The nuclear factor-κB (NF-κB) family of inducible transcription factors includes NF-κB1 p50, NF-κB2 p52, RELA (also called p65), RELB and c-REL[1]. NF-κB proteins normally exist as components of inactive cytoplasmic complexes bound by members of the inhibitor of κB (IκB) family. This family includes the prototypical member IκBα and several structurally related proteins[2]. NF-κB1 p50 and NF-κB2 p52 are produced as pre-cursor proteins, p105 and p100, both of which contain a carboxy-terminal IκB-homologous region and function as IκB-like NF-κB inhibitors. Proteasome-mediated selective degradation of the C-terminal region of p105 and p100 (termed p100 processing) yields mature NF-κB1 p50 and NF-κB2 p52 and also interrupts the IκB-like function of these precursor proteins[2]. The processing of p105 is constitutive and coupled with its translation, although a large proportion of p105 remains unprocessed and functions as an IκB-like molecule[2,3]. By contrast, the processing of p100 is tightly regulated in a signal-induced manner[4].

NF-κB activation occurs via two major signalling pathways: the canonical and the non-canonical NF-κB signalling pathways (FIG. 1). The canonical pathway mediates the activation of NF-κB1 p50, RELA and c-REL (which are also referred to as canonical NF-κB family members), whereas the non-canonical NF-κB pathway selectively activates p100-sequestered NF-κB members, predominantly NF-κB2 p52 and RELB (also referred to as non-canonical NF-κB family members).

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The canonical NF-κB pathway responds to stimuli from diverse immune receptors and leads to rapid but transient NF-κB activation[1,5,6]. A first step in the intracellular signalling cascade is the activation of TGFβ-activated kinase 1 (TAK1; also known as MAP3K7), which, in turn, activates a trimeric IκB kinase (IKK) complex, composed of catalytic (IKKα and IKKβ) and regulatory (IKKγ; also known as NEMO) subunits (FIG. 1). The IKK complex then phosphorylates IκBα or other IκB family members, such as p105. Phosphorylated IκB family members undergo ubiquitylation and proteasomal degradation, resulting in the release and nuclear translocation of the canonical NF-κB family members, predominantly the NF-κB1 p50-RELA and NF-κB1 p50-c-REL dimers. IKK-mediated phosphorylation of p105 targets this IκB-like molecule for complete degradation, although it may also promote p105 processing (that is, the generation of NF-κB1 p50) in some cell types, contributing to the induction of nuclear translocation of p105-sequestered NF-κB members, including NF-κB1 p50, RELA and c-REL[2,7,8] (FIG. 1). Genetic evidence suggests that IKKγ and IKKβ are crucial for mediating phosphorylation-dependent IκBα deg radation and canonical NF-κB nuclear translocation, whereas IKKα appears to play a supporting role in activating the canonical NF-κB pathway[9].

By contrast, the activation of the non-canonical NF-κB pathway is based on the processing of p100 (REF 4) (FIG. 1). Unlike the constitutive processing of p105, the processing of p100 is tightly controlled and occurs in a strictly inducible manner[10]. Signal-induced p100 processing leads to the generation of NF-κB2 p52 as well as nuclear translocation of p100-associated NF-κB members, predominantly RELB and NF-κB2 p52 (REF. 4). The processing of p100 is induced via its phosphorylation at specific C-terminal serine residues (serines 866 and 870), which triggers p100 ubiquitylation via recruitment of the E3 ubiquitin ligase βTrCP[10–12]. A central signalling component of the non-canonical NF-κB pathway is NF-κB-inducing kinase (NIK; also known as MAP3K14), which induces p100 phosphorylation via the activation of the kinase IKKα[10,13]. In contrast to the rapid and transient activation of the canonical NF-κB pathway, the activation of the non- canonical NF-κB pathway is characteristically slow and persistent. Typical inducers of the non-canonical NF-κB pathway are ligands of a subset of tumour necrosis factor receptor (TNFR) superfamily members. Most of these receptors also stimulate the canonical NF-κB pathway and mediate biological processes that involve the functional cooperation between the two NF-κB pathways (FIG. 1).

Here, we review the activation, signalling mechanisms and biological function of the non-canonical NF-κB pathway. Initially thought to be mainly required for lymphoid organ development and B cell maturation, the non-canonical NF-κB pathway has recently been shown to also regulate different aspects of innate and adaptive immune responses. Dysregulated non-canonical NF-κB activation also contributes to the pathogenesis of inflammatory diseases.

Non-canonical NF-κB signaling

Signalling components

Genetic evidence has established that NIK is a central and specific component of the non-canonical NF-κB pathway[10]. Although NIK may also regulate NF-κB-independent cellular

processes[14–16], its predominant function is to mediate non-canonical NF-κB activation, as suggested by the phenotypic similarities between mice carrying mutations or deficiencies in NIK and mice with mutated or deficient NF-κB2 p52 (REF. 17). Another essential component of the non-canonical NF-κB pathway is IKKα, which, as described above, is activated by NIK and directly phosphorylates p100 (REF. 13). However, unlike NIK, IKKα responds to both canonical and non-canonical NF-κB stimuli and possesses many functions beyond non-canonical NF-κB signalling[18]. For example, IKKα mediates canonical NF-κB pathway activation by certain inducers, although IKKβ is the predominant IKK catalytic subunit for canonical NF-κB pathway activation. IKKα also directly phosphorylates canonical NF-κB family members and thereby activates their function as transcriptional activators. Moreover, IKKα modulates general gene transcription by phosphorylating chromatin components and transcriptional co-activators[18]. Another well-recognized function of IKKα is to regulate an epidermal growth factor receptor (EGFR) signalling loop in keratinocytes, thereby affecting skin homeostasis and malignancy[19]. Moreover, IKKα also has a role in the development of lung squamous cell carcinoma. This seems to involve transcriptional suppression of the oncogenic factors ΔNp63 (an amino-terminally truncated isoform of p63) and tripartite motif-containing protein 29 (TRIM29) in $K5^+$ lung epithelial cells[20]. *In vitro* experiments suggest that IKKα exerts this function epi-genetically by associating with the promoter regions of p63 and TRIM29, and mediating the downregulation of the repressive histone mark H3K27me3 and the upregulation of the active histone mark H3K4me3 (REF. 20).

IKKα activation by signals that do not activate NIK is insufficient for triggering non-canonical NF-κB pathway activation, the reasons for which are as yet unclear. A possible explanation is that NIK may both activate IKKα and facilitate the binding of IKKα to its substrate p100 (REF. 21). Another intriguing question is whether IKKα functions in the typical IKKα-IKKβ-IKKγ complex or a distinct complex containing NIK during non-canonical NF-κB signalling. However, it is also possible that NIK induces additional signalling factors that functionally cooperate with IKKα in the induction of p100 processing.

Non-canonical NF-κB signalling predominantly activates NF-κB2 p52 and RELB, although additional NF-κB members, such as RELA, are associated with p100 and, in some cell types, are activated via the induction of p100 processing[22–24]. NF-κB2 p52 and RELB typically function as a heterodimer to induce target gene transcription, but they may also function independently under some conditions. In T cells, NF-κB2 p52 cooperates with the canonical NF-κB member c-REL to induce the expression of the inflammatory cytokine GM-CSF (granulocyte–macrophage colony-stimulating factor)[25]. In B cells, the combined deletion of *Relb* and *Nfkb2* results in more severe B cell defects than the single deletions, further supporting the idea that these two NF-κB members have both common and unique functions[26]. It is important to note, however, that the phenotype of *Nfkb2* knockout mice is difficult to interpret, as the *Nfkb2* deficiency eliminates both NF-κB2 p52 and the IκB-family molecule p100. Caution should also prevail when interpreting the phenotypes of RELB-deficient mice, as RELB activation is not always dependent on non-canonical NF-κB signalling. For example, in dendritic cells (DCs), a large proportion of RELB is regulated by IκBα and activated via the canonical NF-κB pathway[27]. Moreover, RELB activation by the

T cell receptor (TCR) is only partially dependent on the non-canonical NF-κB pathway[25]. Mouse models with defective p100 processing, such as NF-κB2^{Lym1} mice (which express a processing-defective p100 mutant)[23], are more appropriate than RELB-deficient mice for functional studies of the non-canonical NF-κB pathway.

Cellular and pathogenic stimulators

Initial clues as to which receptors stimulate the non-canonical NF-κB pathway came from studies of alymphoplasia (Aly) mice, which carry a loss-of-function mutation in NIK[28]. Most notably, the *Aly/Aly* homo zygous mice resemble mice lacking the TNFR superfamily member lymphotoxin-β receptor (LTβR) with regard to impaired development of secondary lymphoid organs. Overexpression of LTβR stimulates NIK-dependent p100 processing[10], and crosslinking of LTβR with an agonistic antibody stimulates p100 processing in a NIK- and IKKα-dependent manner[29]. This demonstrates that LTβR acts as a non-canonical NF-κB pathway stimulating receptor[29]. Around the same time that these observations were made, two other TNFR members — B cell activating factor receptor (BAFFR) and CD40 — were identified as non-canonical NF-κB pathway stimulating receptors in B cells[30–32]. It is now clear that several additional TNFR members mediate non-canonical NF-κB pathway activation (TABLE 1); these include receptor activator for NF-κB (RANK), TNFR2, fibroblast growth factor-inducible factor 14 (FN14), CD27, CD30 and OX40 (also known as CD134)[17,33–39]. A common feature of these TNFRs is the presence of cytoplasmic motifs that bind TNFR associated factor 2 (TRAF2), TRAF3 or both TRAF family members. As discussed in the following section, TRAF2 and TRAF3 facilitate the ubiquitylation of NIK and thereby act as negative regulators, and their degradation serves as a major mechanism of non-canonical NF-κB pathway activation.

Some non-TNFR receptors have also been shown to mediate non-canonical NF-κB pathway activation (TABLE 1). One example is macrophage colony-stimulating factor (MCSF) receptor (MCSFR), a growth factor receptor that mediates the differentiation and proliferation of macrophages[40]. MCSF-induced macrophage differentiation from bone marrow cells is associated with the induction of p100 processing, and like TNFRs, MCSFR is capable of associating with TRAF2 and TRAF3 (REF 40). In T cells, ligation of the TCR and the co-stimulatory receptor CD28 stimulates p100 processing and nuclear translocation of p52 and RELB, which is dependent on NIK and the presence of the C-terminal phosphorylation site of p100 (REF 25). However, as T cell activation is associated with the expression of several TNFR members and their ligands[41], it seems likely that the TCR–CD28 signals activate the non- canonical NF-κB pathway indirectly through the induction of TNFRs and their ligands. In support of this hypothesis, ligation of the TNFR family member OX40 promotes TCR–CD28-induced activation of the non-canonical NF-κB pathway[38,42]. Future studies will examine whether TCR–CD28 signalling acts synergistically with TNFR signalling in the induction of the non-canonical NF-κB pathway.

The non-canonical NF-κB pathway is also a target of some pathogens (TABLE 2). These include several RNA viruses[40,43–45], which seem to induce non- canonical NF-κB pathway activation by stimulating the cytoplasmic RNA sensor retinoic acid inducible gene I (RIG-I)[45]. Some viral oncoproteins are capable of directly stimulating non-canonical NF-κB

pathway activation; these include the Tax protein of human T cell leukaemia virus type I, Epstein–Barr virus latent membrane protein 1 (LMP1), the Kaposi sarcoma-associated herpesvirus protein vFLIP, Herpesvirus saimiri transforming protein STP-A11 and the Tio protein of Herpesvirus ateles[46–50]. Tio is a transmembrane protein that resembles TNFRs and mediates NIK-dependent non-canonical NF-κB activation by interacting with, and inducing the degradation of, TRAF3 (REF. 50). LMP1 and STP-A11 are also transmembrane proteins that contain TRAF-binding sequences, which are required for non-canonical NF-κB activation in a NIK-dependent manner[47,49]. By contrast, non-canonical NF-κB activation by Tax and vFLIP is independent of NIK but requires IKKγ and IKKα[46,48]. The non-canonical NF-κB pathway is also activated by some bacteria; these include *Helicobacter pylori,* a Gram-negative bacterium associated with gastric inflammation and cancer[51], and *Legionella pneumophila,* a pathogen that infects lung macrophages and is associated with Legionnaires' disease[52].

Negative regulators

A yeast two-hybrid screen for NIK-binding proteins identified TRAF3 as a central negative regulator of the non-canonical NF-κB pathway[53]. In mammalian cells, newly synthesized NIK is rapidly bound by TRAF3 and targeted for ubiquitin-dependent degradation. Under steady-state conditions, this keeps the level of NIK extremely low, whereas signal-induced TRAF3 degradation leads to NIK accumulation and subsequent p100 processing[53] (FIG. 2). TRAF3 knockdown or knockout results in NIK stabilization, which is sufficient for the induction of p100 processing[53,54]. Moreover, a NIK mutant lacking the TRAF3-binding motif was shown to escape from TRAF3-mediated degradation and to turn into a ‘super-stimulator’ of the non-canonical NF-κB pathway[53,55]. These findings suggest that signal-induced NIK protein accumulation is sufficient for its activation, an idea that was corroborated by subsequent structural studies that suggested NIK is a kinase that is in a constitutively active conformation[56,57].

Proteasomal degradation of proteins typically involves their conjugation with lysine (K) 48-linked polyubiquitin chains. TRAF3 lacks K48-specific E3 ligase activity, but instead induces NIK K48 ubiquitylation by recruiting the E3 ligases cellular inhibitor of apoptosis 1 (cIAP1) and cIAP2 (hereafter collectively referred to as cIAP) to NIK, a mechanism that also requires TRAF2, which appears to serve as an adaptor for connecting TRAF3 with cIAP[4,58,59]. Thus, the deletion of either TRAF2 or TRAF3 or a simultaneous deletion of cIAP causes NIK accumulation and p100 processing[53,54,60–63]. Similarly, small-molecule antagonists of IAPs, which induce the degradation of cIAP, induce NIK accumulation and p100 processing[64,65].

Recent studies suggest that a member of NACHT, LRR and PYD domain-containing protein 12 (NLRP12; also known as Monarch 1) physically interacts with NIK and promotes ubiquitin-dependent NIK degradation in myeloid cells[66,67]. NLRP12 also binds to TRAF3 and maintains the steady level of TRAF3 stability, and NLRP12 deficiency causes a reduction in the basal level of TRAF3, as shown in DCs[66]. Thus, NLRP12 may regulate the steady level of non-canonical NF-κB signalling by targeting both TRAF3 and NIK, although the underlying mechanism has not been well defined (FIG. 2).

Signal-induced non-canonical NF-κB pathway activation is also subject to negative regulation. OTUD7B (also known as Cezanne) is a deubiquitinase that interacts with TRAF3 in response to CD40 and LTβR signals and inhibits signal-induced TRAF3 degradation by deconjugating K48-linked polyubiquitin chains from TRAF3 (REF. 68) (FIG. 2). The N-terminal ubiquitin-association domain of OTUD7B is required for its inducible binding to TRAF3, suggesting that TRAF3 ubiquitylation may facilitate the recruitment of OTUD7B and, thereby, allows OTUD7B to deubiquitylate TRAF3 and inhibit TRAF3 degradation[68].

NIK itself is also a target of negative regulators under signal-induced conditions. Upon activation by NIK, IKKα phosphorylates NIK at several C-terminal serine residues and thereby destabilizes it, thus serving as a negative feedback mechanism to prevent the aberrant accumulation of NIK following its liberation from TRAF3 (REF. 69). Similar to IKKα, the IKK-related kinase TANK-binding kinase 1 (TBK1) phosphorylates NIK to promote NIK degradation, which occurs in B cells in response to BAFFR and CD40 signalling[70].

Non-canonical NF-κB signalling is also regulated at the level of receptor trafficking and degradation, as demonstrated for LTβR[71]. RNA interference- mediated knockdown of major subunits of ESCRT (endosomal sorting complexes required for transport) causes endosomal accumulation of LTβR, which leads to non-canonical NF-κB pathway activation in a ligand-independent manner. These findings suggest that ESCRT prevents the aberrant sorting of LTβR and constitutive activation of the non-canonical NF-κB pathway[71]. Whether other non-canonical NF-κB pathway-stimulating receptors are also subject to ESCRT regulation remains to be examined.

Mechanism of activation

The principal mechanism of non-canonical NF-κB pathway activation by inducing receptors involves the disruption of the cIAP–TRAF2–TRAF3 complex, which typically occurs through inducible degradation of TRAF3, although some inducers trigger degradation of TRAF2 or cIAP[4] (FIG. 2). Early studies demonstrate that the induction of TRAF3 degradation and p100 processing by BAFFR requires its TRAF3-binding sequence[72]. Recruitment of TRAF2 and TRAF3, as well as their associated cIAP, which then leads to the disruption of the complex, seems to be a common mechanism mediating non-canonical NF-κB pathway activation by different non-canonical NF-κB inducing receptors[17]. Recruitment to the receptor is thought to promote the E3 ligase activity of cIAP, a process that may involve K63 ubiquitylation of cIAP by TRAF2, thereby allowing cIAP to mediate K48 ubiquitylation and degradation of TRAF3 (REF. 58). However, for some receptors, such as LTβR, the recruitment of TRAF3 is thought to induce the release of NIK from TRAF3 before TRAF3 degradation[73,74]. Similarly, HIV is thought to activate NIK by inducing the binding of MYD88 to TRAF3 and, thereby the dissociation of NIK from TRAF3 (REF. 44).

A very unusual mechanism of non-canonical NF-κB activation has recently been proposed for complement membrane attack complexes (MACs), which are structures formed as a result of complement activation in host defences against pathogens or in pathological immune reactions[75] (FIG. 2). In contrast to TNFRs, which typically take hours to trigger non-canonical NF-κB signalling, alloantibody-induced MACs have been shown to stimulate

NIK stabilization within as little as 30 minutes, and this mechanism does not require TRAF3 degradation but involves MAC-induced phosphorylation of the kinase AKT and the formation of a signalosome complex that includes AKT and NIK on RAB5^{+} endosomes, whereby activated AKT mediates NIK stabilization[75]. It remains to be further investigated how AKT mediates NIK stabilization and whether this involves the dissociation of NIK from the cIAP–TRAF2–TRAF3 complex.

Role in immune regulation

Well-characterized functions of the non-canonical NF-κB pathway include the regulation of lymphoid organ development, B cell survival and maturation, and the differentiation of osteoclasts, which are bone-absorbing cells of monocyte–macrophage lineage[4,76,77]. In addition, recent studies using conditional knockout mice and bone marrow chimeric mice have uncovered several novel functions of the non-canonical NF-κB pathway.

Lymphoid organ development

Primary and secondary lymphoid organs are the sites for lymphocyte development and activation, respectively. A well-characterized function of the non-canonical NF-κB pathway is to mediate the development and architectural organization of secondary lymphoid organs, including the spleen, lymph nodes and mucosal lymphoid tissues[4,78]. Loss-of-function mutation of NIK in *Aly*/*Aly* mice impairs the development of lymph nodes and Peyer's patches and causes disorganized spleen architecture[28,79]. Similar phenotypes have been detected in mice deficient in the downstream signalling components IKKα and RELB or mice that harbour a mutation in the *Nfkb2* gene (NF-κB2^{Lym1} mice) producing a non-processable p100 (REFS 13,23,80,81). Compared with these mutant mice, the *Nfkb2*-knockout mice have milder defects in secondary lymphoid organ development[82,83]. This is probably because the *Nfkb2* gene deletion does not block the activation of RELB, which may form a dimer with p50 to partially compensate the function of RELB–p52 dimer, as mice deficient in both *Nfkb1* and *Nfkb2* have more severe defects in lymphoid organ development[83]. The predominant NF-κB-inducing receptor that mediates lymphoid organ development is LTβR, which is expressed on stromal organizer cells and responds to stimulation by its ligand, LTα1β2, expressed on haematopoietic lymphoid tissue inducer (LTi) cells[84]. Through LTβR ligation, the LTi cells stimulate stromal organizer cells to express specific chemokines, including CXC-chemokine ligand 13 (CXCL13; also known as BLC), CC-chemokine ligand 21 (CCL21; also known as SLC) and CCL19 (also known as ELC), as well as cell adhesion molecules that mediate the recruitment and retention of more LTi cells required for the growth and development of lymph nodes[84]. Induction of these chemokine genes by LTβR requires the non-canonical NF-κB pathway[29].

The non-canonical NF-κB pathway also has a role in the development of a primary lymphoid organ, the thymus, where it regulates the development of thymic epithelial cells (TECs)[76]. The thymus has two different types of TECs: cortical TECs (cTECs) and medullary TECs (mTECs), which mediate the early and late phases of thymocyte development, respectively[85]. In particular, mTECs have a central role in mediating immune tolerance, as they are required for thymocyte negative selection and the generation of the

immunosuppressive regulatory T (T_{reg}) cells[85]. The development of mTECs involves several members of the TNFR superfamily, including LTβR, CD40 and RANK, all of which are known to mediate the activation of both the canonical and non-canonical NF-κB pathways[85]. RANK signalling is crucial for mTEC development during embryogenesis, but the different TNFR members seem to have functional redundancies in the postnatal development and maintenance of mTECs[86]. However, signalling downstream of both the canonical and non-canonical NF-κB pathway is essential for mTEC development. Mice deficient in non- canonical NF-κB signalling components, such as NIK, IKKα or RELB, or the canonical NF-κB signalling component TRAF6, have severe defects in mTEC development[87–91]. Recent studies suggest that the non-canonical NF-κB pathway is dispensable for initial mTEC lineage specification but is required for the further differentiation of mTEC progenitors into mature mTECs[92,93]. In addition to mediating mTEC development, non-canonical NF-κB signalling regulates the expression of autoimmune regulator (AIRE), an mTEC-specific transcription factor that is required for the expression of tissue-specific antigens and the induction of central tolerance[85]. Mice deficient in the non-canonical NF-κB family member NF-κB2 p52 have a defect in *Aire* expression in mTECs, which is associated with impaired central tolerance[94]. Although this phenotype may also involve defects in mTEC differentiation, this early study is corroborated by more recent studies demonstrating that the *Aire* gene contains a conserved non-coding sequence that binds and responds to non-canonical and canonical NF-κB family members and is required for *Aire* gene expression and immune tolerance[95,96].

DC function

DCs serve as the primary antigen-presenting cells (APCs) that connect innate immunity to adaptive immunity by facilitating the activation of antigen-specific naive T cells[97]. DCs sense infections and tissue damage via pattern recognition receptors (PRRs) and undergo maturation to become competent APCs, and this process requires signalling via the canonical NF-κB pathway. DCs also express TNFR superfamily members, such as CD40, LTβR and RANK, which are capable of stimulating the non-canonical NF-κB pathway[98]. Early studies using mice deficient in the NF-κB members RELB, c-REL, or both c-REL and NF-κB1 suggested an important role for these NF-κB members in the regulation of DC development and maturation[99]. In particular, DCs require RELB in order to induce T cell responses via both the conventional antigen presenting pathway and via cross-priming. Elevated levels of RELB expression and nuclear translocation are also associated with DC maturation[100]. However, as noted above, RELB activation is not solely dependent on the non-canonical NF-κB pathway. More recent studies using *Aly/Aly* mice and mice with a DC-specific knockout of NIK suggest that non-canonical NF-κB pathway signalling is dispensable for DC development and maturation or for antigen presentation to $CD4^+$ T cells but is required for DC cross-priming of $CD8^+$ T cells[101,102].

In addition to priming T cells, DCs have an important role in maintaining immune tolerance[103]. Non-canonical NF-κB pathway signalling has been implicated in the regulation of immune tolerance by mediating the induction of indoleamine 2,3-dioxygenase 1 (IDO1), an enzyme that catalyses the catabolism of an essential amino acid, tryptophan, to kynurenine and thereby inhibits T cell proliferation and survival[44,104]. Small interfering

RNA (siRNA)-mediated knockdown of NIK or IKKα in human DCs was shown to inhibit IDO1 induction *in vitro*[104,105]. It remains to be examined whether NIK and non- canonical NF-κB signalling have an *in vivo* role in regulating IDO1 expression and the tolerogenic function of DCs.

Antiviral innate immunity

Infection with RNA viruses, such as Sendai virus, vesicular stomatitis virus, HIV1 and respiratory syncytial virus, induces non-canonical NF-κB pathway activation[40,43–45,106] (TABLE 2). One study suggests that non-canonical NF-κB signalling negatively regulates virus-stimulated type I interferon (IFN) production[40]. Macrophages derived from *Map3k14-knockout* mice (which lack NIK) and *Nfkb2*^{Lym1} mice display elevated levels of type I IFN induction by vesicular stomatitis virus and Sendai virus, whereas macrophages expressing a stable form of NIK that lacks the TRAF3-binding sequence (NIKΔT3) have reduced type I IFN induction[40]. The non-canonical NF-κB family members p52 and RELB seem to suppress the transcription of *Ifnb* by competing with the canonical NF-κB family member RELA for binding to the *Ifnb* gene enhancer.

Humoral immunity and germinal centres

A crucial role of the non-canonical NF-κB pathway in humoral immune responses was demonstrated in studies of mouse models[60,62,76] and human patients with genetic mutations in genes encoding NIK and NF-κB2 (REFS 107–110). Non-canonical NF-κB signalling is required for the survival, maturation and homeostasis of B cells in peripheral lymphoid organs[60,62,76]. In addition, there is strong evidence that the non-canonical NF-κB pathway is involved in germinal centre (GC) formation, a hallmark of humoral immune responses against protein antigens[111]. GCs are sites within the B cell follicles of secondary lymphoid organs where antigen-stimulated B cells undergo clonal expansion and a series of differentiation events, including somatic hypermutation and immunoglobulin class switching, selection of B cells with high-affinity B cell receptors (affinity maturation), and deletion of self-reactive B cells (negative selection)[111] (FIG. 3). GC formation requires the activation of the non-canonical NF-κB family members p52 and RELB, as well as the upstream kinases NIK and IKKα, in both lymphoid stromal cells and B cells[112–116]. In stromal cells, the non-canonical NF-κB pathway responds to LTβR signalling and mediates the induction of specific chemokines, including CXCL13 and CCL21. These are required for the formation of B cell follicles and the follicular DC (FDC) network, which, in turn, are crucial for GC formation[114]. In B cells, the non-canonical NF-κB pathway not only mediates the survival of naive B cells but also regulates GC formation and maintenance as well as immunoglobulin class switching, as demonstrated by recent studies using GC B cell-specific NIK-deficient mice[117,118] (FIG. 3). It has also been shown that simultaneous deletion of *Nfkb2* and *RelB* in GC B cells causes the collapse of established GCs[119]. Interestingly, NIK is also important for the survival of B cells that have undergone class switching, as well as the survival of fully differentiated plasma cells[118].

GC reactions require a subset of $CD4^+$ effector T cells termed T follicular helper (T_{FH}) cells, which characteristically express the chemokine receptor CXC-chemokine receptor 5 (CXCR5) and can migrate to the GCs in B cell follicles, where they promote the activation,

proliferation and differentiation of antigen- stimulated B cells[120]. T_{FH} cell generation involves cognate interactions of antigen-stimulated T cells (thought to be the precursors of T_{FH} cells) and B cells at the border of the T cell zone and B cell follicles. A crucial event in this T cell–B cell interaction is the engagement of inducible co-stimulator (ICOS) on T cells by ICOS ligand (ICOSL) on B cells[120]. Non-canonical NF-κB activation by BAFFR and CD40 is important for maintaining high levels of ICOSL expression in B cells and, thus, antigen-stimulated T_{FH} cell differentiation[121] (FIG. 3). Patients with loss-of-function mutations in the gene encoding NIK have been found to have impaired ICOSL expression in B cells and impaired T_{FH} cell generation[107]. Similarly, patients with mutations in *NFKB2,* producing non-processable p100 mutants lacking C-terminal phosphorylation sites, have reduced numbers of B cells and T_{FH} cells and develop common variable immunodeficiency[108,110,122,123]. The non-canonical NF-κB pathway is also required to induce ICOSL expression on the $CD8^-$ subset of DCs, which contributes to the induction of T_{FH} cells[124]. Therefore, the non-canonical NF-κB pathway facilitates GC reactions via multiple mechanisms, including the survival of naive and GC B cells and the generation of T_{FH} cells (FIG. 3).

T cell responses

T cells serve as the central component of adaptive immunity and are also responsible for many auto-immune and inflammatory diseases. Upon activation, $CD4^+$ T cells differentiate into several subsets of effector T cells, including T helper 1 (T_H1), T_H2, T_H9, T_H17 and T_{FH} cells, each with specialized helper or regulatory functions[125]. The non-canonical NF-κB pathway is dispensable for the initial activation of naive T cells through TCR signalling but is crucial for the *in vivo* generation and maintenance of effector and memory T cells[25,126,127]. As described above, non-canonical NF-κB signalling facilitates the differentiation of T_{FH} cells involved in GC reactions, although this function of non-canonical NF-κB signalling is not T cell-intrinsic. Non-canonical NF-κB s ignalling has a cell-intrinsic role in mediating the induction of T_H9 cells stimulated by OX40, a member of the TNFR family, which contributes to the pathogenesis of airway inflammation[42].

Recent studies using NIK-deficient mice and NF-κB2^{Lym1} mice have also demonstrated a crucial role of the non-canonical NF-κB pathway in the induction of T_H1 and T_H17 effector cells in the neuroinflammation model experimental autoimmune encephalomyelitis (EAE)[25,126,127]. Compared with the situation *in vivo,* the non-canonical NF-κB pathway is much less important for T_H1 and T_H17 cell differentiation in an *in vitro* system, in which T cells are stimulated using agonistic TCR- and CD28-targeted antibodies in the presence of polarizing cytokines[25,100]. A likely explanation for this is that *in vivo,* the non-canonical NF-κB pathway may be activated by TNFR members that are stimulated by ligands expressed on APCs.

In addition to regulating T cell differentiation, the non-canonical NF-κB pathway may also modulate the effector function of differentiated T cells. For example, the non-canonical NF-κB family member p52 promotes the pathological function of T_H17 cells in neuroinflammation by mediating the induction of the cytokine GM-CSF[25]. Based on the current knowledge, a model can be proposed to explain how the non-canonical NF-κB

pathway regulates T cell responses (FIG. 4). The initial activation of naive T cells via the TCR and the CD28 co-stimulatory receptor relies on the canonical, but not the non-canonical, NF-κB pathway. Following T cell activation, several TNFRs are induced[41], which mediate the activation of the non-canonical NF-κB pathway upon ligation by their ligands on APCs. As activated T cells also express the ligands for TNFRs[41], it is likely that non-canonical NF-κB activation also involves T cell–T cell interactions. Upon activation, NF-κB family members induced by the non-canonical pathway regulate the expansion and survival of effector T cells as well as their production of effector cytokines (FIG. 4).

Non-canonical NF-κB signalling also has a role in regulating the development of FOXP3$^+$ T_{reg} cells. Early studies suggested that non-canonical NF-κB signalling mediates T_{reg} cell development indirectly through maintaining the integrity of the thymic stroma and the function of DCs[89,128]. However, more recent studies using bone marrow adoptive transfer and mice with a conditional depletion of NIK in T cells have demonstrated a T cell- intrinsic function for the non-canonical NF-κB pathway in regulating the peripheral maintenance of T cells[126,129]. T cell-specific deletion of NIK reduces the frequency and absolute number of T_{reg} cells in the spleen and lymph nodes, although not in the thymus[126]. Thus, non-canonical NF-κB signalling has paradoxical roles in regulating T cell responses. The non-canonical NF-κB pathway also has a role in regulating the development of natural killer T (NKT) cells and γδ T cells, and this function is indirectly mediated via the regulation of mTECs[130–132].

Role in inflammatory diseases

Inflammation is a protective host response to infections and tissue damage that serves as a mechanism to prevent pathogen spread and to promote pathogen destruction and wound healing[133]. A typical inflammatory response involves the production of pro-inflammatory cytokines, chemokines and other inflammatory mediators in the affected tissue, thereby causing a series of local reactions, such as vasodilation and recruitment of neutrophils and monocytes. Inflammation also involves T cells, especially the inflammatory T_H1 and T_H17 cells[133]. Although inflammation is usually resolved in a timely manner and beneficial to the host, dysregulated inflammation contributes to the pathogenesis of various chronic inflammatory diseases[134].

When aberrantly activated, non-canonical NF-κB signalling in different cell types can promote auto-immunity and inflammation (FIG. 5). As discussed below, dysregulated non-canonical NF-κB activation causes aberrant survival of self-reactive B cells, rendering them resistant to negative selection and leading to autoimmune antibody production associated with several inflammatory diseases. Furthermore, dysregulated non- canonical NF-κB signalling in endothelial cells can result in aberrant chemokine production and inflammatory cell recruitment, thereby causing excessive and/or chronic tissue damage and inflammation. The non-canonical NF-κB pathway is also activated in other cell types, such as T cells, monocytes and hepatocytes, whereby it promotes inflammation with different mechanisms (FIG. 5).

Rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune disease that is characterized by chronic inflammation and hyperplasia of synovial tissue associated with destruction of cartilage and bone[135]. The pathogenesis of RA involves the recruitment of innate immune cells and inflammatory T cells, particularly T_H17 cells, to the synovial tissue, where they release pro-inflammatory cytokines and induce synovitis. Canonical NF-κB signalling has long been recognized for its pro-inflammatory role in RA, but strong evidence suggests that non-canonical NF-κB signalling is also involved in different aspects of RA pathogenesis[136]. The aetiology of RA is linked to several TNF and TNFR superfamily members known to mediate non-canonical NF-κB activation[137]. Furthermore, NIK is highly expressed in the synovial endothelial cells of patients with RA and promotes pathogenic angiogenesis and synovial inflammation through the induction of chemokines such as CXCL12 (REFS 138,139). NIK activation in endothelial cells may also promote the formation of tertiary lymphoid structures, which are associated with chronic inflammatory diseases like RA[140].

Progressive joint damage in patients with RA involves the aberrant generation and activation of osteoclasts[141]. Osteoclastogenesis requires RANK, which can stimulate both the canonical and the non-canonical NF-κB pathways in osteoclast precursor cells[77]. Defects in non-canonical NF-κB signalling lead to an accumulation of p100, which serves as an IκB to prevent the activation of several NF-κB members, resulting in impaired osteoclast generation[77]. The non-canonical NF-κB pathway is also required for bone erosion in animal models of inflammatory arthritis[142,143]. Conversely, dysregulated non-canonical NF-κB activation, owing to TRAF3 deletion or the transgenic expression of NIKΔT3 (a stable form of NIK), causes increased osteoclast formation and bone loss[144,145].

RA pathogenesis also involves B cells, which are responsible for the production of autoantibodies and also have additional pathological functions, including the regulation of other immune cells, the formation of synovial tertiary lymphoid tissue and the production of cytokines[146]. The B cell survival factor BAFF has been linked to RA and explored as a therapeutic target in clinical trials[146]. Under physiological conditions, BAFF mediates the survival and maturation of B cells, which crucially involves the activation of the non-canonical NF-κB pathway. However, overproduction of BAFF, as revealed in BAFF-transgenic mice, promotes the aberrant survival of autoreactive B cells and the development of autoimmunity[147]. Patients with RA often have elevated levels of BAFF in the serum and synovial fluid, which is associated with disease severity[146]. These studies suggest a role for the non-canonical NF-κB pathway in regulating RA pathogenesis in different cell types.

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an auto immune and chronic inflammatory disease that affects multiple tissues and organs[148]. The aetiology of SLE is multifactorial, including genetic, environmental, hormonal and immunological factors. A hallmark of the immunopathology of SLE is the accumulation of autoantibodies as immune complexes with nucleic acids, which stimulate PRRs on innate immune cells for the aberrant production of type I IFNs. As the source of auto antibodies, B cells have a central role in the pathogenesis of SLE and have been exploited as a major target in SLE therapy[149]. As observed in patients

with RA, the pathological survival and differentiation of autoantibody-producing B cells in patients with SLE rely on the non-canonical NF-κB pathway, which is activated via TNFR superfamily members, particularly BAFFR and CD40. Patients with SLE frequently have elevated serum levels of BAFF, which is associated with disease activity[150,151]. Belimumab, a BAFF-neutralizing antibody, has been approved for SLE therapy, and several other BAFF inhibitors have been explored in clinical trials as therapeutic agents for SLE[152].

Kidney inflammation and injury

The non-canonical NF-κB pathway has been implicated in the pathogenesis of IgA nephropathy, a leading cause of primary glomerulonephritis kidney diseases, which are characterized by aberrant production of glycosylated IgA and the deposition of IgA-immune complexes in kidney glomeruli[153]. Studies of mouse models suggest that dysregulated non-canonical NF-κB pathway activation in B cells (owing to the loss of the negative regulator TBK1) is associated with elevated serum levels of IgA and nephropathy-like disease symptoms, including antibody deposition in kidney glomeruli and signs of kidney dysfunction[70]. Similar pathological phenotypes have been detected in transgenic mice that overexpress BAFF in B cells[154]. An increasing number of studies also show that non-canonical NF-κB signalling in kidney cells can regulate inflammation when activated by TNF-like weak inducer of apoptosis (TWEAK), a TNF superfamily member implicated in the pathogenesis of kidney diseases[153,155]. Elevated expression of TWEAK and its receptor, FN14, has been detected in patients with kidney diseases and in animal models of kidney injury, and it has been implicated in the pathogenesis of kidney diseases[155]. TWEAK engages FN14 on kidney cells, including tubular epithelial cells, mesangial cells and podocytes, and stimulates both the canonical and non-canonical NF-κB pathways, thereby inducing the expression of pro-inflammatory cytokines and chemo kines. The non-canonical NF-κB pathway mediates TWEAK-induced expression of CCL21, a chemokine involved in the recruitment of T cells and the induction of kidney inflammation and fibrosis[156–158].

Metabolic inflammation

Metabolic diseases, such as obesity and type 2 diabetes, are associated with dysregulated immune responses and chronic inflammation, and the targeting of the inflammatory mediators is considered an attractive therapeutic approach[159]. Recent studies have revealed the involvement of the non-canonical NF-κB pathway in metabolic diseases[160–162]. Upregulated levels of NIK protein are found in skeletal muscle cells of patients with metabolic syndrome[160]. Furthermore, gastric bypass surgery, leading to weight loss, has been shown to correlate with a decrease in skeletal muscle cell NIK protein levels[160]. The levels of NIK protein are also upregulated in cortical kidney cells in a mouse model of type 2 diabetes and in pancreatic islets of diet-induced obese mice[162,163]. In the pancreatic islands of obese mice, NIK is stimulated by RANK ligand (RANKL), a non-canonical NF-κB inducer that has also been shown to mediate the induction of type 2 diabetes in mouse models[163,164].

Direct evidence for a functional contribution of NIK to the development of metabolic diseases came from a study showing that NIK is activated in hepatocytes in a mouse model of dietary and genetic obesity[161]. Moreover, NIK deficiency or the liver-specific expression

of a kinase-dead NIK mutant results in reduced glucagon responses and hepatic glucose production, whereas hepatocyte-specific over expression of wild-type NIK increases glucagon responses and hepatic glucose production[161]. Mechanistically, NIK stabilizes cAMP-responsive element-binding protein (CREB1), a transcription factor that mediates glucagon-stimulated gluconeogenesis by inducing the expression of the gluconeogenesis enzymes phosphoenol pyruvate carboxykinase and glucose 6-phosphatase[161]. NIK phosphorylates CREB1 and stabilizes CREB1 via both phosphorylation-dependent and independent mechanisms. NIK is also implicated as an inflammatory mediator in liver inflammation and injury induced by hepatitis viruses and alcohol consumption, and NIK over expression in hepatocytes induces fatal liver injury and fibrosis[165,166]. Inhibition of NIK with a small-molecule inhibitor protects mice from toxin-induced liver inflammation and injury[167]. Furthermore, non-canonical NF-κB pathway activation in pancreatic β-cells via the conditional deletion of TRAF2 or TRAF3, which act as negative regulators of NIK, reduces insulin secretion and causes exacerbated glucose intolerance in a mouse model of diet-induced obesity[163]. Consistent with these findings, NIK induction by SMAC mimetics, which induce the degradation of the NIK-inhibitory cIAP proteins, impairs glucose-induced insulin secretion in mouse and human islets[163].

Other inflammatory diseases

Largely on the basis of studies of animal models, non-canonical NF-κB signalling has been implicated in the pathogenesis of several other inflammatory diseases. These include EAE, a widely used animal model of multiple sclerosis, which is a neuroinflammatory disease that is thought to involve autoreactive inflammatory T_H1 and T_H17 cells[168]. Several studies have demonstrated an essential role for NIK and non-canonical NF-κB signalling in mediating the pathogenesis of EAE[14,25,126,169]. Moreover, a recent genome-wide association study identified NIK as one of the candidate genes for multiple sclerosis susceptibility[170]. As described above, the non-canonical NF-κB pathway regulates the pathological effector function of T_H17 cells, as well as recall responses of autoreactive T cells[25,126]. Another study also proposed a DC-specific role for non-canonical NF-κB signalling in mediating the pathogenesis of EAE[169]; however, this finding was not supported by a more recent study using DC-conditional NIK-deficient mice[102].

In contrast to its essential role in EAE induction, NIK acts as a negative regulator in hypereosinophilic syndrome-like disease, an inflammatory disorder characterized by a persistent increase in eosinophil count in the blood and various organs[16]. NIK-deficient mice develop hypereosinophilic syndrome-like disease involving aberrant T_H2 cell responses. However, bone marrow adoptive transfer experiments suggest that the disease development in NIK-deficient mice depends on non-haematopoietic cells[16]. Furthermore, as mice carrying a mutation in the activation loop of IKKα (the phosphorylation site of NIK) do not develop hypereosinophilic syndrome, this pathological phenotype in NIK-deficient mice may be independent of IKKα and non-canonical NF-κB pathway signalling[16].

A recent study using epidermal cell-specific *Traf2*-knockout ($Traf2^{EKO}$) mice suggest that TRAF2 deficiency in keratinocytes causes NIK accumulation and constitutive non-canonical NF-κB activation, which is associated with heightened expression of inflammatory

cytokines and the development of psoriasis-like skin inflammation[171]. It seems that TNF-dependent cell death promotes early inflammation, whereas constitutive activation of the non-canonical NF-κB pathway may lead to the subsequent onset of skin inflammation in the *Traf2*^{EKO} mice. In support of this hypothesis, deletion of both *Nfkb2* and *Tnf* in the *Traf2*^{EKO} mice completely prevents skin inflammation[171]. Skin inflammation also involves IL-17-producing γδ T cells[172], the development of which requires NIK-mediated signalling in thymic epithelial cells[132,173].

A role for the non-canonical NF-κB pathway in colon inflammation was revealed in a study of mice deficient for NLRP12, which negatively regulates non- canonical NF-κB activation[66]. The NLRP12-deficient mice are hypersensitive to the induction of colitis and colitis-associated cancer, and this anti-inflammatory function of NLRP12 involves the inhibition of non- canonical NF-κB pathway activation. In line with this study, *Nfkb2-knockout* mice show resistance to dextran sulfate sodium (DSS)-induced colitis and colitis-associated colon cancer[174]. Non-canonical NF-κB signalling also has an important role in regulating mucosal immunity against infections, which may involve crosstalk with the canonical NF-κB pathway[175]. Defects in non- canonical NF-κB signalling render mice more sensitive to the intestinal pathogen *Citrobacter rodentium,* leading to gut inflammation, whereas enhanced activation of the non-canonical NF-κB pathway promotes host defence against infections[68,175–177]. Thus, physiologically regulated non-canonical NF-κB pathway function is important for maintaining immunity and inducing inflammation in the intestine.

Concluding remarks

The non-canonical NF-κB pathway has been a hot topic of studies in recent years, and substantial progress has been made in our understanding of its signalling mechanism and biological functions. It has become clear that NIK is a central and specific component that integrates various TNFR signals, resulting in the activation of non-canonical NF-κB family members. A paradigm has been established by demonstrating an unusual mechanism of NIK regulation[4,53] and by characterizing a NIK-specific E3 ubiquitin ligase complex, cIAP–TRAF2–TRAF3 (REFS 4,58,59,64,65). Recent studies have also demon- strated new functions of this pathway in the immune sys- tem, highlighting its involvement in both normal immune responses and inflammatory diseases, as discussed above.

Despite these advances, several outstanding questions remain to be addressed. For example, the mechanism by which TNFR family members stimulate NIK-dependent p100 processing is only partially understood. Although IKKα has been established as a downstream kinase of NIK that mediates p100 phosphorylation, IKKα can also be activated by NIK- independent signals that do not induce p100 processing[18]. Does IKKα need to be assembled into a NIK-specific complex for accessing the substrate p100? Does NIK activate additional signalling factors that functionally cooperate with IKKα? Another question concerns the mechanism of NIK activation. Although signal-induced NIK accumulation typically involves the disruption of the cIAP–TRAF2–TRAF3 complex, recent studies suggest that some stimuli activate NIK without involving cIAP-TRAF degradation. Thus, the biochemical mechanism of NIK activation warrants further studies. Furthermore, we are just beginning to understand the cell type-specific functions of the non-canonical NF-κB pathway in the regulation of immune

and inflammatory responses. Further studies along this line, with the help of the recently generated conditional knockout mice, will provide new insights into the immunoregulatory roles of this NF-κB pathway. Finally, an exciting area for future studies is to explore the therapeutic efficacies of small-molecule inhibitors of NIK in the treatment of inflammatory diseases.

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Glossary

Tumour necrosis factor receptor (TNFR) superfamily
A large family of cytokine receptors that are engaged by members of the TNF superfamily of cytokines and mediate signal transduction

Epidermal growth factor receptor (EGFR)
A receptor tyrosine kinase that responds to the growth factor EGF and mediates cell growth and survival by triggering several intracellular signalling pathways

p63
A p53 homologue produced as two main isoforms, TAp63 and ΔNp63, with ΔNp63 lacking the N-terminal typical transactivation domain and functioning as a dominant-negative form to promote oncogenesis

Tripartite motif-containing protein 29 (TRIM29)
A member of the TRIM family implicated in oncogenesis

Stromal organizer cells
Matrix cells of mesenchymal origin that characteristically express the cell adhesion molecules VCAM1 and ICAM1 and the TNFR member LTβR and are required for lymphoid organ development

Cross-priming
A mechanism of $CD8^+$ T cell priming, in which antigen-presenting cells take up extracellular antigens, process and present them with MHC class I molecules to $CD8^+$ T cells

Small interfering RNA (siRNA)

Short double-stranded RNA molecules, typically 20–25 bp in length, which bind to and induce degradation of mRNAs with complementary sequences, thereby interfering with production of the corresponding proteins

Common variable immunodeficiency
A frequently diagnosed and heterogeneous type of primary immunodeficiency characterized by low to undetectable levels of antibodies and increased susceptibility to infections

Experimental autoimmune encephalomyelitis (EAE)
A commonly used animal model of the autoimmune neuroinflammatory disease multiple sclerosis, characterized by infiltration of the central nervous system with T cells and monocytes that cause inflammation and demyelination leading to limb paralysis

Rheumatoid arthritis(RA)
An autoimmune disease characterized by chronic inflammation in the joints and destruction of cartilage and bone

Systemic lupus erythematosus (SLE)
An autoimmune disease characterized by chronic inflammation throughout the body, causing tissue damage in multiple organs

SMAC mimetics
A class of small-molecule compounds that bind to and antagonize cIAP by mimicking the endogenous IAP antagonist SMAC

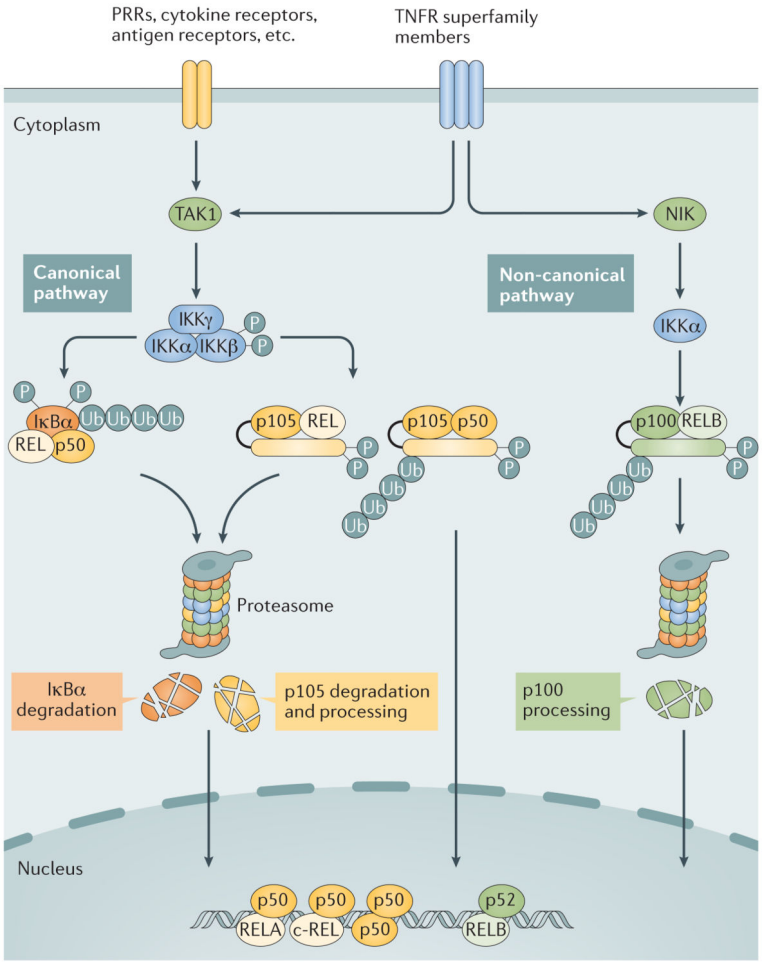

Figure 1. Canonical and non-canonical NF-κB pathways

The canonical nuclear factor-κB (NF-κB) pathway is triggered by signals from a large variety of immune receptors, which activate the kinase TGFβ -activated kinase 1 (TAK1). TAK1 then activates a trimeric IκB kinase (IKK) complex, composed of catalytic (IKKα and IKKβ) and regulatory (IKKγ) subunits, via phosphorylation of IKKβ. Upon stimulation, the IKK complex, largely through IKKβ, phosphorylates members of the inhibitor of κB (IκB) family, such as the prototypical IκB member IκBα and the I κB-like molecule p105, which sequester NF-κB members in the cytoplasm. IκBα associates with dimers of p50 and members of the REL family (RELA or c-REL), whereas p105 associates with p50 or REL (RELA or c-REL). Upon phosphorylation by IKK, IκBα and p105 are targeted for ubiquitin (Ub)-dependent degradation in the proteasome, resulting in the nuclear translocation of canonical NF-κB family members, which bind to specific DNA elements, termed κB enhancers of target genes, in the form of various dimeric complexes, including RELA–p50, c-REL–p50, and p50–p50 (REF. 1). By contrast, non-canonical NF- κB signalling is based on the processing of p100, an IκB-like molecule that predominantly, although not exclusively, regulates RELB. The non-canonical NF- κB pathway selectively responds to a subset of tumour necrosis factor receptor (TNFR) superfamily members that target the activation of the kinase NFκB-inducing kinase (NIK)[4]. NIK phosphorylates and activates IKKα, which in turn phosphorylates carboxy-terminal serine residues of p100, triggering

selective degradation of the C-terminal IκB-like structure of p100 and leading to the generation of p52 and the nuclear translocation of p52 and RELB[4]. PRRs, pattern recognition receptors.

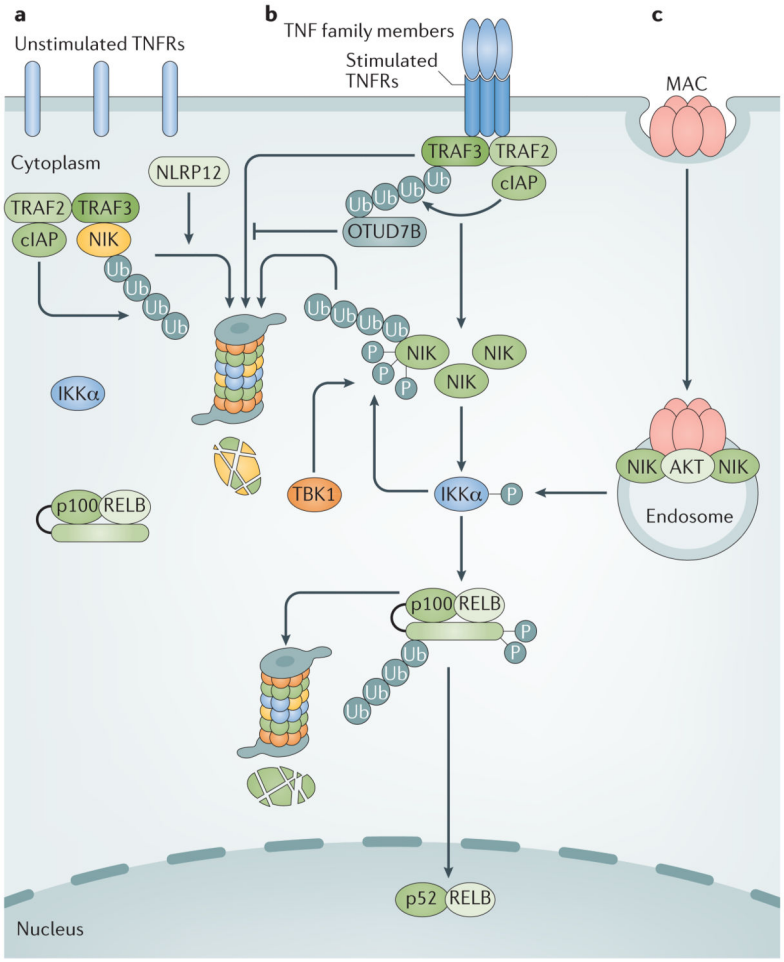

Figure 2. Regulation and activation of the non-canonical NF-κB pathway

a | Under unstimulated conditions, newly synthesized NF-κB-inducing kinase (NIK) is rapidly bound by TNFR associated factor 3 (TRAF3) and targeted for ubiquitylation (Ub) by the cellular inhibitor of apoptosis (cIAP)–TRAF2–TRAF3 E3 ubiquitin ligase complex[4]. The continuous degradation of NIK in the proteasome prevents NIK accumulation and non-canonical nuclear factor-κB (NF-κB) pathway activation. **b** | Ligation of specific TNFR superfamily members by their ligands (TNF family members) stimulates the recruitment of TRAF2, TRAF3, cIAP1 and cIAP2 (shown as cIAP in the figure) to the receptor complex, in which activated cIAP mediates K48 ubiquitylation and proteasomal degradation of TRAF3, resulting in stabilization and accumulation of NIK and the induction of p100 processing, followed by the translocation of NF-κB p52–RELB heterodimers into the nucleus[4]. Receptor-mediated non-canonical NF-κB activation is negatively regulated via TRAF3 deubiquitylation by OTUD7B and phosphorylation-dependent degradation of NIK is mediated by IκB kinase-α (IKKα) and TANK-binding kinase 1 (TBK1)[68–70]. **c** | Complement membrane activation complex (MAC) activates the non-canonical NF-κB pathway via a TRAF-independent mechanism that involves the formation of an endosome-based signalling complex containing MAC, the kinase AKT and NIK, in which activated

AKT mediates NIK stabilization[75]. NLRP12, NACHT, LRR and PYD domain-containing protein 12.

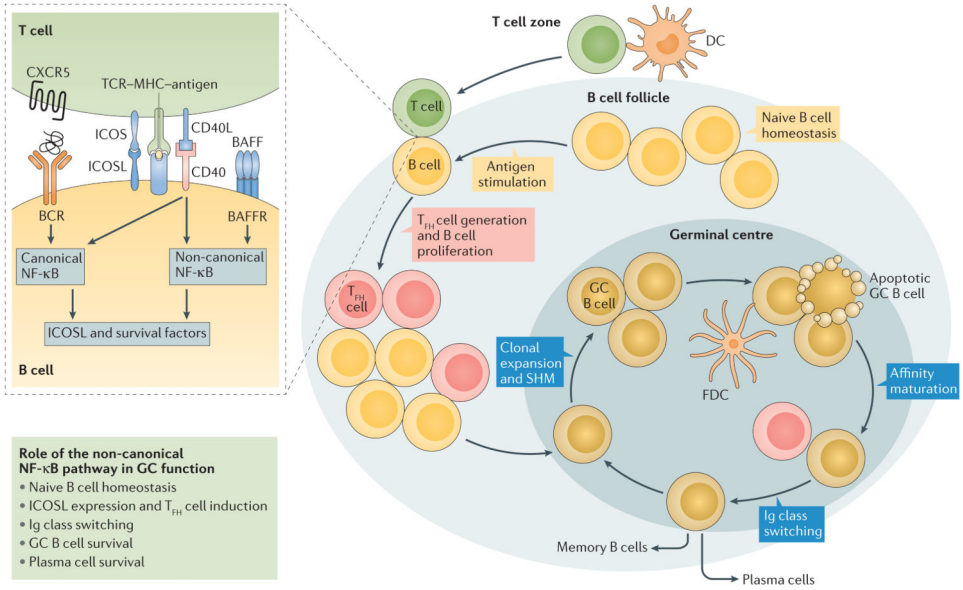

Figure 3. The non-canonical NF-κB pathway regulates GC reactions in multiple steps

Immune responses to protein antigens involve the formation of germinal centres (GCs) within B cell follicles, in which activated B cells undergo clonal expansion and a series of differentiation events, which leads to the generation of antibody-secreting plasma cells or memory B cells. The initial step of GC formation involves interactions between antigen-primed T cells and B cells on the border of the T cell zone and the B cell follicle, which are required for differentiation of the $CD4^+$ T cells into follicular helper T (T_{FH}) cells. The T cell–B cell interaction is initiated through TCR interaction with the MHC–antigen complex and also requires the ICOS–ICOSL interaction. Ligation of CD40 and BAFFR by their ligands, CD40L and BAFF, induces non-canonical NF-κB signalling in B cells, which is important for maintaining high levels of ICOSL expression and, thus, T_{FH} cell differentiation. T_{FH} cells, in turn, promote the clonal expansion and differentiation of B cells in the subsequent steps of GC reactions. Non-canonical nuclear factor-κB (NF-κB) signalling regulates GC reactions through promoting the survival of naive B cells, the differentiation of T_{FH} cells by inducing the expression of inducible co-stimulator ligand (ICOSL) in B cells, GC cell expansion, GC B cell survival, immunoglobulin (Ig) class switching, somatic hypermutation (SHM) and plasma cell survival[60,62,76,117–119,121]. In addition, non-canonical NF-κB signalling also functions in stromal cells to mediate the induction of chemokines required for the formation of B cell follicles and the follicular dendritic cell network, which in turn are crucial for GC formation[114] (not shown in Figure). BAFF, B cell activating factor; BAFFR, BAFF receptor; BCR, B cell receptor; CXCR5, CXC-chemokine ligand 5; DC, dendritic cell; FDC, follicular dendritic cell; TCR, T cell receptor.

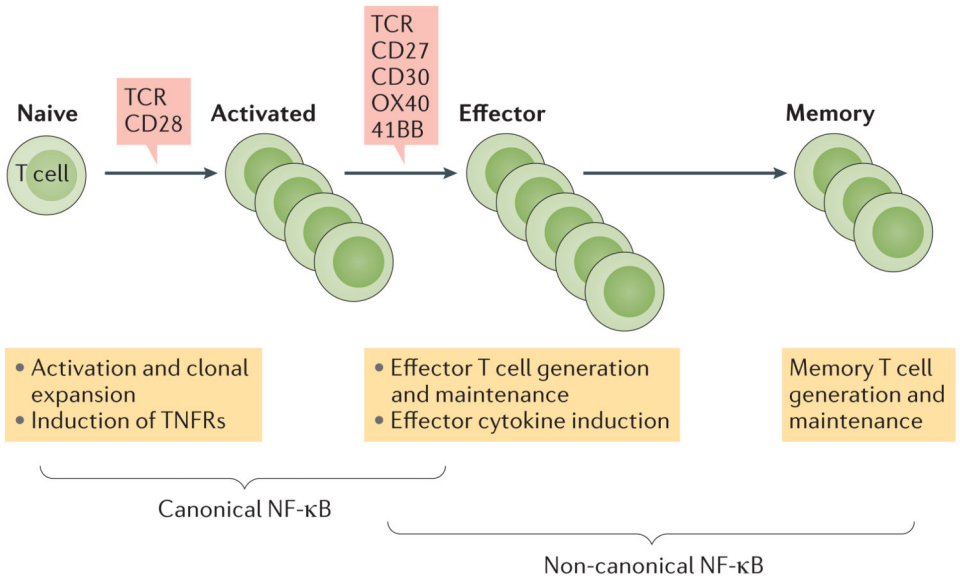

Figure 4. Regulation of T cell responses by canonical and non-canonical NF-κB pathways

Naive T cell activation via the T cell receptor (TCR) and signalling through the co-receptor CD28 leads to the rapid activation of the canonical nuclear factor-κB (NF-κB) pathway, which contributes to the initial activation and clonal expansion of T cells[2]. T cell activation is associated with TCR stimulation and the inducible expression of several TNF receptor (TNFR) superfamily members such as CD27, CD30, OX40 and 41BB, which mediate the activation of non-canonical NF-κB signalling upon ligation by their ligands on antigen-presenting cells and activated T cells. Non-canonical NF-κB signalling is crucial for the generation and maintenance of effector and memory T cells and also has a role in mediating the induction of effector cytokines[25,126,127]. Since TNFRs also activate canonical NF-κB signalling, it is likely that the two pathways function cooperatively during the effector and memory phases of T cell responses.

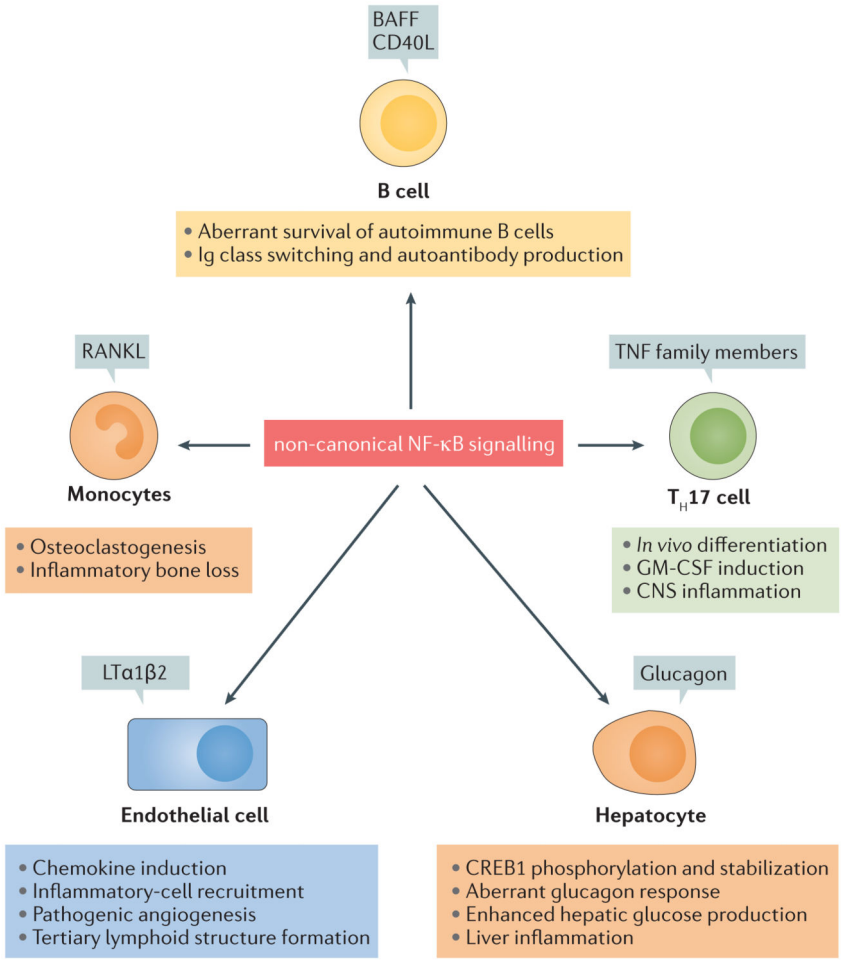

Figure 5. The non-canonical NF-κB pathway regulates inflammation in different cell types

Under physiological conditions, non-canonical nuclear factor-κB (NF-κB) signalling mediates the survival and homeostasis of B cells, the generation and effector function of T helper 17 (T_H17) cells, the differentiation of osteoclasts from monocytes, chemokine production in endothelial cells, and glucagon responses in hepatocytes[25,60,62,76,77,126,139,161]. These cellular events become pathogenic in situations in which the non-canonical NF-κB pathway is aberrantly activated owing to the uncontrolled production of its inducing agents or genetic deficiencies in its negative regulators. Aberrant B cell survival renders self-reactive B cells resistant to negative selection, contributing to the accumulation of autoantibodies associated with inflammatory diseases[147]. Autoimmune T_H17 cells mediate inflammation in the central nervous system (CNS) and other tissues. Excessive and chronic production of chemokines by endothelial cells promotes the recruitment of inflammatory cells and the formation of tertiary lymphoid structures in tissue-specific inflammation[138,139]. Aberrant osteoclast generation is a pathological mechanism of inflammatory bone loss, and aberrant glucagon responses are associated with metabolic diseases[77,161]. BAFF, B cell activating factor; CD40L, CD40 ligand; CREB1, cAMP-responsive element-binding protein; GM-CSF, granulocyte–macrophage colony-stimulating

factor; Ig, immunoglobulin; LTα 1β2, lymphotoxin α 1 β2; RANKL, RANK, receptor activator for NF- κB ligand; TNF, tumour necrosis factor.

Table 1
Immune-stimuli that induce the non-canonical NF-κB pathway

Ligands	Receptors	Refs
TNF superfamily members		
BAFF	BAFFR	30,32
CD40L	CD40	31
CD30L	CD30	35,36
CD70	CD27	34
LTα1β2 and LIGHT	LTβR	29
OX40L	OX40	38
RANKL	RANK	37
TNF (membrane)	TNFR2	39
TWEAK	FN14	33
Other immune stimuli		
MCSF	MCSFR	40
MACs	NA	75
Antibodies directed against CD3 and CD28	TCR and CD28	25

BAFF, B cell activating factor; FN14, fibroblast growth factor-inducible factor 14; L, ligand; LIGHT, also known as TNFSF14; LT, lymphotoxin; MACs, membrane attack complexes; MCSF, macrophage colony-stimulating factor; NA, not applicable; R, receptor; RANK, receptor activator for NF-κB; TCR, T cell receptor; TNF, tumour necrosis factor; TWEAK, TNF-like weak inducer of apoptosis.

Table 2
Pathogens as inducers of the non-canonical NF-κB pathway

Pathogen	Pathogen component	Refs
Virus		
Influenza virus	RNA	45
Vesicular stomatitis virus	RNA	40
Sendai virus	RNA	40
HIV1	RNA	44
Respiratory syncytial virus 1	RNA	43
Epstein–Barr virus	LMP1 protein	47
Herpesvirus saimiri	STP-A11 protein	49
Herpesvirus ateles	Tio protein	50
Human T cell lymphotropic virus 1	Tax protein	46
Kaposi sarcoma-associated herpesvirus	vFLIP protein	48
Bacteria		
Helicobacter pylori	Unknown	51
Legionella pneumophila	LegK1	52

LegK1, *Legionella* kinase 1; LMP1, latent membrane protein 1.