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REVIEW

NF-κB in immunobiology

Matthew S Hayden[1], Sankar Ghosh[1]

[1]*Department of Microbiology and Immunology, College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA*

NF-κB was first discovered and characterized 25 years ago as a key regulator of inducible gene expression in the immune system. Thus, it is not surprising that the clearest biological role of NF-κB is in the development and function of the immune system. Both innate and adaptive immune responses as well as the development and maintenance of the cells and tissues that comprise the immune system are, at multiple steps, under the control of the NF-κB family of transcription factors. Although this is a well-studied area of NF-κB research, new and significant findings continue to accumulate. This review will focus on these areas of recent progress while also providing a broad overview of the roles of NF-κB in mammalian immunobiology.

***Keywords*:** NF-κB; TLR; TCR; BCR; lymphoid organogenesis; inflammation; hematopoiesis
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Introduction

As discussed in the introduction to the January special issue of *Cell Research*, the discovery and characterization of the NF-κB family of transcription factors resulted from studies in two major areas of research: immunology and cancer biology. Although the role of NF-κB in cancer biology is becoming progressively better established, historically much of our current knowledge of NF-κB resulted from efforts directed at understanding its role in the regulation and function of the immune response. This review will attempt to provide a comprehensive discussion of the diverse functions of NF-κB in immunobiology and update our previous efforts in reviewing the far-reaching role of NF-κB in this area of biology [1-3].

The mammalian immune response comprises various mechanisms by which the physiological and functional integrity of the host is maintained in the face of microbial insult. The bone marrow-derived cells of the immune system are chiefly, though by no means solely, responsible for performing these functions. Thus, we begin by reviewing the role of NF-κB in the development of these hematopoietic cells that mediate both innate and adaptive immune responses. With the relevant cell types and tissues in place, the immune response is triggered by host recognition of foreign pathogens. Our knowledge in this area of the immune response has expanded rapidly in the past decade. Pathogen recognition is followed by pathogen clearance, a highly variable response that may draw upon coordinated responses at the level of cell, tissue, and the organism. Finally, the immune response must be resolved, damage must be repaired and, whenever possible, the triggering insult remembered for future immunity.

In mammals, the NF-κB family is composed of five related transcription factors: p50, p52, p65 (also RelA), c-Rel, and RelB (Figure 1). These transcription factors share an N-terminal DNA-binding/dimerization domain, known as the Rel homology domain, through which they can form homo- and heterodimers. NF-κB dimers can bind to a variety of related target DNA sequences called κB sites to modulate gene expression. RelB, c-Rel, and p65 contain C-terminal transcription activation domains (TADs) that enable co-activator recruitment and target gene expression. As they lack TADs, p50 and p52 can activate transcription by forming heterodimers with p65, c-Rel, or RelB, or by recruiting other TAD-containing proteins. However, as homodimers lacking the ability to drive transcription, they can repress transcription on binding to DNA.

In most cells, NF-κB complexes are inactive, residing predominantly in the cytoplasm in a complex with inhibitory IκB proteins (IκBα, IκBβ, IκBε, IκBζ, p100, p105, Bcl3, IκBns; Figure 1). When signaling pathways

Correspondence: Sankar Ghosh
E-mail: sg2715@columbia.edu

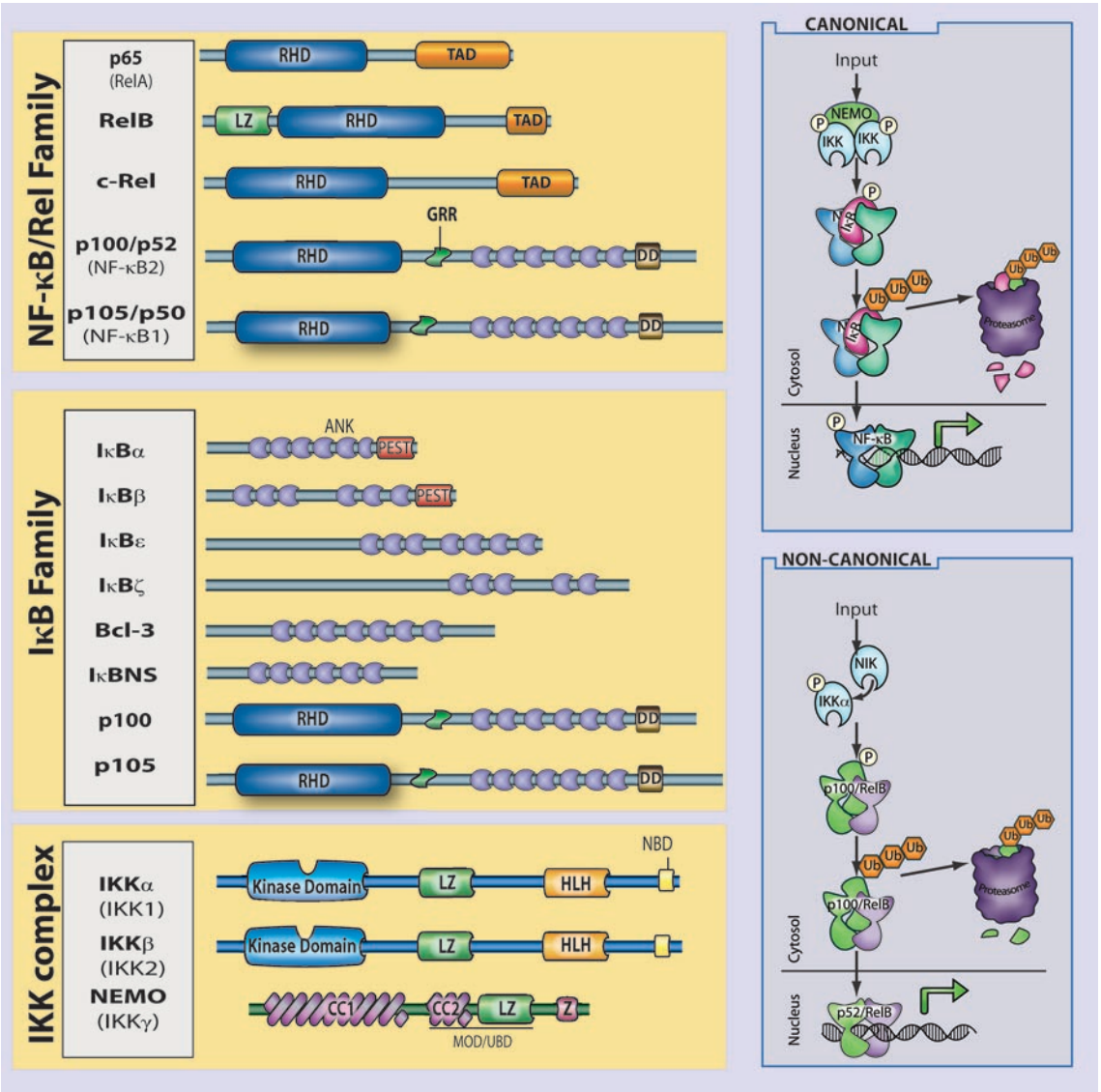

Figure 1 The mammalian NF-κB, IκB and IKK protein families. Relevant domains typifying each protein family are indicated and alternative nomenclatures are provided in parenthesis. The precursor proteins p100 and p105 function as both IκB and, when processed by the proteasome, NF-κB family members. Highly simplified schematics of canonical and non-canonical NF-κB pathways are shown. In the canonical pathway NEMO-containing IKK complexes are activated and induce phosphorylation and degradation of IκBα leading to the release of NF-κB dimers including p65:p50 dimers. In the non-canonical pathway, NEMO-independent activation of IKKα is mediated by the upstream kinase NIK. IKKα, with NIK, induces phosphorylation and processing of p100 to p52 resulting in the activation of predominantly p52:RelB complexes. Although they are illustrated separately here, as discussed in the text, signaling through these two pathways often is initiated by the same receptor – e.g., LTβR or CD40. (ANK, ankyrin-repeat domain; DD, death domain; RHD, REL homology domain; TAD, transactivation domain; LZ, leucine-zipper domain; GRR, glycine-rich region; HLH, helix-loop-helix domain; Z, zinc-finger domain; CC, coiled-coil domain; NBD, NEMO-binding domain; MOD/UBD, minimal oligomerization domain/ubiquitin-binding domain; PEST, proline, glutamic acid, serine, and threonine rich.)

are activated, the IκB protein is degraded and NF-κB dimers enter the nucleus to modulate target gene expression. In almost all cases, the common step in this process is mediated by the IκB kinase (IKK) complex, which phosphorylates IκB and targets it for proteasomal degradation (see Liu and Chen, *Cell Res* 2011; 21:6-21). The IKK complex consists of two catalytically active kinases, IKKα (IKK1) and IKKβ (IKK2) and a regulatory scaf-

folding protein, NEMO (IKKγ; Figure 1). These are the basic players in the NF-κB pathway; however, there is considerable complexity in how the individual proteins function and how they coordinate a nuanced NF-κB response (see Shih *et al.*, *Cell Res* 2011; 21:86-102). The details of individual signaling pathways, the mechanism of action of upstream signaling intermediates, the role of various post-translational modifications, and cross-talk between NF-κB and other signaling pathways are covered in depth elsewhere in this special issue. Here, we focus on the function of NF-κB and the signaling pathways that regulate it in the mammalian immune response.

Development of the immune system

The mammalian immune system consists of a functionally linked group of anatomically disparate tissues and cell types. The dispersed cellular components of the immune system that arise from the bone marrow receive much of the attention in immunology and the study of NF-κB has likewise focused on these cells. However, lymphoid organs that facilitate coordination and dissemination of immune responses carried out by immune cells are also key sites of NF-κB function. Therefore, while this section is largely concerned with the role of NF-κB in hematopoiesis, the role of NF-κB in lymphoid organogenesis is also discussed briefly.

NF-κB and lymphoid organogenesis

NF-κB plays an important role in the development and function of primary (bone marrow, thymus) and secondary (lymph nodes, Peyer's patches, mucosal-associated lymphoid tissue, and the spleen) lymphoid tissues. There is clearly a role for NF-κB in the development and regulation of bone, and we refer the reader to the excellent review on the subject in this special issue (see Novack, *Cell Res* 2011; 21:169-182). The role of NF-κB in thymic architecture, originally thought to be limited to the important role of RelB in thymic architecture [4], has become clearer in terms of the development of medullary thymic epithelial cells [5-7], but still remains to be more fully elucidated [8]. The secondary lymphoid tissues facilitate maintenance and activation of mature lymphocytes by providing an environment within which the interaction of lymphocytes and other leukocytes can be carefully orchestrated [9]. The role of NF-κB in this process is now well appreciated. Multiple NF-κB knockouts exhibit defects in some aspect of secondary lymphoid organ development.

The initial events of lymphoid organogenesis involve the association of lymphotoxin (LT)$\alpha_1\beta_2$-expressing hematopoietic cells and vascular cell adhesion molecule-1 (VCAM-1)-expressing stromal cells [10]. This interaction initiates a positive feedback loop through NF-κB (Figure 2). LT$\alpha_1\beta_2$, receptor activator of NF-κB ligand (RANKL), and TNFα are known to activate NF-κB, and figure prominently in lymphorganogenesis [10] (Figure 2). Also, mediators of lymphoid organogenesis and homeostasis, such as adhesion molecules (e.g., intercellular adhesion molecule, VCAM, peripheral node addressin, glycosylation-dependent cell adhesion molecule-1, and mucosal addressin cellular adhesion molecule (MadCAM)), cytokines (e.g., TNFα and LT$\alpha_1\beta_2$) and organogenic chemokines (e.g., CXCL12 (GRO/MIP-2), CXCL13 (BLC), CCL19 (ELC), and CCL21 (SLC)), are regulated by NF-κB.

Signaling through TNFR1, LTβR, and RANK activates p65-containing complexes and, hence, it is not surprising that *rela*$^{-/-}$/*tnfr1*$^{-/-}$ double-knockout mice lack Peyer's patches and lymph nodes, and exhibit a disorganized spleen [11]. The requirement for p65 in the development of these tissues is attributable to its function in stromal cells, and is likely because of a combination

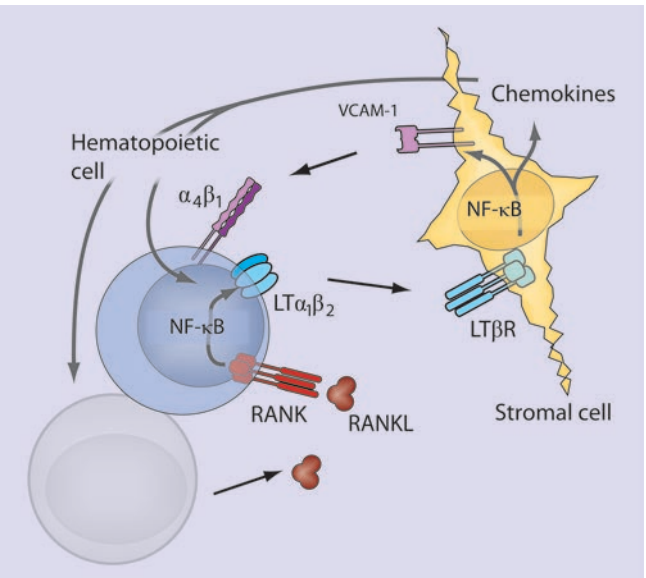

Figure 2 NF-κB in lymphoid organogenesis. NF-κB regulates key components of a positive feedback loop between hematopoietic and stromal cells. LT$\alpha_1\beta_2$-expressing hematopoietic cells induce production of VCAM-1 through the canonical NF-κB pathway and chemokines through the non-canonical pathway in LTβR-expressing stromal cells. Stromal expression of chemokines induces the upregulation of integrins ($\alpha_4\beta_1$) on hematopoietic cells resulting in increased recruitment of LT$\alpha_1\beta_2$-expressing cells, and increased signaling through stromal LTβR. RANKL signaling through RANK on the hematopoietic cell leads to activation of NF-κB, and further upregulation of LT$\alpha_1\beta_2$.

of effects: regulation of apoptosis (e.g., that induced by TNF); regulation of expression of organogenic factors, such as VCAM and $LT\alpha_1\beta_2$; and enhancement of the non-canonical p52/RelB NF-κB pathway. The non-canonical NF-κB pathway has a well-established role in secondary lymphoid organ development. Deletion of components of the non-canonical NF-κB signaling pathway, including NIK, IKKα, LT, and RANK, all lead to severe defects in secondary lymphoid organogenesis [12-17]. The target of the non-canonical pathway, the p52/RelB dimer, is the primary transcriptional regulator of key organogenic factors including CXCL12, CXCL13, CCL19, CCL21, and MadCAM-1 [18]. Thus, the p52 single knockout lacks normal B-cell follicles, germinal centers, and Peyer's patches [19-21], while RelB knockouts lack Peyer's patches and exhibit compromised lymphnode development [18].

Splenic architecture is crucial for B-cell development as well as for the initiation and maturation of B-cell responses. The spleen is divided functionally and histologically into white and red pulp zones. Macrophages in the red pulp are responsible for destroying damaged erythrocytes, while the white pulp is populated by splenic lymphocytes organized into B-cell follicles and T-cell zones. Yet splenic architecture is also dynamic, as exemplified by the formation of germinal centers during the initiation and maturation of B-cell responses. As with other secondary lymphoid organs, NF-κB figures prominently in the development and maintenance of splenic architecture. Mice in which p65 has been deleted exhibit defects in both static and dynamic splenic architecture [11]. Deletion of RelB, NIK, or IKKα leads to defects in splenic architecture, similar to those of *ltbr*$^{-/-}$ spleens [12, 13]. Similar to p65/TNFR1 knockouts, mice with a defective non-canonical pathway fail to segregate B cell/T cell zones and they fail to form germinal centers following immunization. Marginal zone macrophages, which line the border between red and white pulp areas, are also absent or disorganized in RelB, p52, NIK, or IKKα knockouts [21, 22]. Recent work has highlighted the role of $LT\alpha_1\beta_2$ in the development and maintenance of the marginal sinus architecture, suggesting that the role of NF-κB in splenic architecture should perhaps be revisited with an eye toward an analysis of endothelial function. Some splenic defects are also attributable to effects on hematopoietic cells, most notably of the myeloid lineages.

In summary, both the canonical and non-canonical NF-κB pathways are required for the development of most secondary lymphoid organs (Figure 2). However, the non-canonical pathway, as assessed by examining mice deficient for IKKα, p52, NIK, or RelB, is especially important both during organogenesis and for maintenance of splenic architecture. Recent advances suggest that NF-κB signaling in endothelial cells, which are crucial regulators of cellular movement into and out of lymphoid organs, should be better characterized. The functional consequences of defects in these processes are severe and have direct ramifications for the ability of host to mount a robust immune response. As such, the function of NF-κB in the development and regulation of these organs represents an important facet of the role of NF-κB in immunobiology.

NF-κB and hematopoiesis

The immune system includes cells of the lymphoid and myeloid lineages: T cells, B cells, monocytes, macrophages, dendritic cells (DCs), natural killer (NK) cells, basophils, eosinophils, neutrophils, and mast cells (Figure 3). These bone marrow-derived cells are the core constituents of both the innate and adaptive immune responses. Proliferation, differentiation, and apoptosis are the defining characteristic of hematopoiesis, and NF-κB participates in the regulation of each of these processes. While the study of the role of NF-κB in development and homeostasis of hematopoetic cells has focused largely on B cell and T lymphocytes, it is likely that the NF-κB pathway is also important for the development of NK cells, DCs, monocytes, granulocytes, and other cellular components of the immune system. In general, an anti-apoptotic and pro-proliferative role for NF-κB is invoked in the hematopoietic system (Figure 3). Indeed, deletion of the kinase TAK1, an upstream component of both NF-κB and AP1 pathways, results in decreased antiapoptotic gene expression, hematopoietic stem cell apoptosis, and failure of hemtopoiesis [23]. However, in actuality, the situation is much more complex. For example, *ikba*$^{-/-}$*ikbe*$^{-/-}$ cells, which have elevated NF-κB activation are broadly defective in myelopoiesis and exhibit defects in NK cells and lymphoid lineages [24, 25]. Thus, NF-κB function must be interrogated at each point of cellular differentiation in order to fully appreciate its contribution to hematopoiesis. Greater insight into the majority of events that lead to the development of immune cells, awaits both further characterization of the developmental processes and generation of better genetic tools to examine the consequences of perturbing NF-κB function.

NF-κB in non-lymphocyte hematopoiesis

Although most, if not all cells of the body can exhibit some innate immune function, the ability to recognize microbes and initiate an antimicrobial response, we focus here only on the development of certain hematopoietic,

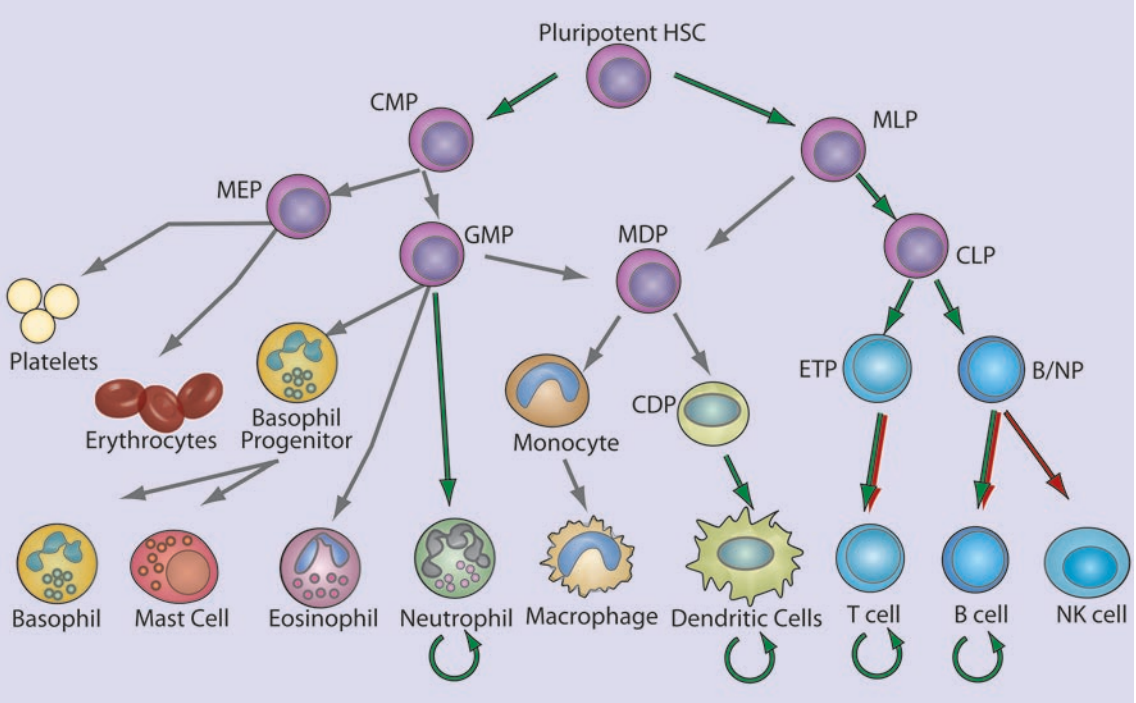

Figure 3 NF-κB in hematopoiesis. Red arrows indicate stages in which NF-κB activation is thought to contribute negatively and green arrows indicate a positive function in the development of the indicated lineages. Curved arrows indicate examples in which NF-κB contributes to the survival of cell population, either in the resting state or during immune responses. Gray arrows indicate developmental events for which NF-κB plays no role or for which the role of NF-κB has not been clearly demonstrated. (HSC, hematopoietic stem cell; CMP, common myeloid progenitor; MLP, myeloid/lymphoid progenitor; MEP, megakaryocyte erythrocyte progenitor; GMP, granulocyte monocyte progenitor; MDP, macrophage dendritic cell progenitor; CDP, common dendritic cell progenitor; CLP, common lymphoid progenitor; ETP, early thymic precursor; and B/NP, B-cell natural killer cell progenitor).

non-lymphoid components of the innate immune system. In general, our understanding of the developmental origin of these innate cells lags far behind that of lymphocytes. Yet recent progress in characterizing the developmental process for several myeloid lineages should provide a better framework within which the contribution of NF-κB can be better assessed in the future.

Significant progress has been made recently in understanding DC development, including the characterization of a common DC progenitor [26-28], yet the contribution of NF-κB to early steps of DC lineage commitment remains poorly understood. The most notable contribution of NF-κB in this area is the requirement for RelB in development of the $CD8\alpha^-$ DCs [4, 29-31]. Although single knockouts of other NF-κB genes do not result in deficiencies in DC development, deletion of both p65 and p50 has a profound effect [32, 33]. Again, however, the limitations of the experimental systems used to examine the contribution of NF-κB, particularly with regard to the need to either delete TNFR1 or utilize fetal liver transfer experiments, should be underscored. In the periphery, NF-κB is clearly required for DC maturation. Loss of IKKβ or inhibition of the IKK complex using a cell permeable peptide prevents DC maturation and antigen-presenting cell (APC) function [34, 35]. In addition, peripheral differentiation and function of myeloid DCs, e.g., monocyte-derived DCs, is significantly impaired by NF-κB inhibition [36]. DCs in the periphery survive for a short period of time following activation, but their survival can be prolonged by CD40L expression on T cells. CD40L activates both the canonical and non-canonical NF-κB pathways, and hence DCs deficient in both p50 and c-Rel, or DCs overexpressing a mutant super-repressor form of IκBα, demonstrate significantly decreased survival [32, 37]. To date, the specific targets of NF-κB in DC development and, indeed, the role of NF-κB at individual stages of DC development, remain quite poorly characterized.

Genetic models of NF-κB deficiency and activation have revealed other roles for NF-κB in the myeloid

lineage. Of the non-lymphocyte lineages, neutrophil development is perhaps best characterized in terms of the role of NF-κB. There remains, however, significant gaps in both our understanding of neutrophil development, and more so the role of NF-κB in this process. IκBα-knockout mice, which have elevated classical NF-κB activity, display robust granulocytosis [38]. RelB-deficient mice exhibit an inflammatory phenotype with a marked increase in peripheral neutrophil numbers. Mice lacking c-Rel and p50, and heterozygous for p65, also exhibit neutrophilia [39]. This increase in peripheral neutrophil numbers resulting from deletion of NF-κB appears to be related to both increased development and increased egress from the bone marrow [39]. In mature neutrophils, the anti-apoptotic signal provided by NF-κB is crucial. In sharp contrast to lymphocytes, which are relatively long-lived in the absence of activation, neutrophils normally exhibit a very short lifespan but require protection from apoptosis during inflammation when they provide effector function. Neutrophils can respond by activating NF-κB in response to numerous pro-inflammatory stimuli, and the activated NF-κB can increase neutrophil survival [40]. Although neutrophils are capable of activating NF-κB in response to many pro-inflammatory stimuli [41] they lack p52 and RelB [42], which are crucial for the maintenance of long-lived lymphocytes. Thus, in neutrophils the role of NF-κB is selective and cell type specific.

NF-κB in lymphopoiesis

Development of T and B cells has, historically, been the subject of much greater scrutiny than the development of cells of the myeloid lineages. NF-κB has been examined in many aspects of lymphopoiesis and found to be vital for the development and function of adaptive immune cells [43] (Figure 4). Despite their potential longevity in the periphery, lymphocyte development is characterized by abundant apoptosis. As a consequence, the anti-apoptotic properties of NF-κB play a key role in lymphopoeisis. Indeed, in many instances the requirement for NF-κB can be overcome by transgenic expression of the anti-apoptotic factor Bcl-2 [44]. Indeed, the role of NF-κB in B cell development was recently re-examined [45]. Although NF-κB was expendable in cells undergoing rearrangement of the eponymous *Igκ* loci, NF-κB was required to protect the resulting cells from apoptosis, and for these cells to progress to rearrangement of the *Igλ* locus. This deficit in NF-κB function could be circumvented through overexpression of Bcl-2 [45]. The necessity of NF-κB for lymphopoesis is strikingly illustrated in human genetic diseases in which the gene encoding NEMO is inactivated by mutation. Because the *NEMO* gene is located on the X chromosome, it is usually subject to random inactivation in individual cells in females. However, in female patients who are heterozygous for a mutant version of *NEMO*, all peripheral lymphocytes possess an intact *NEMO* gene, rather than the 50% predicted by random inactivation, suggesting that in the absence of NEMO-dependent NF-κB signaling, B and T cells fail to develop.

The effects of NEMO inactivation in both mice and humans solidify the role of NF-κB in lymphopoiesis, although the details by which NF-κB functions in this process remain obscure. NF-κB plays diverse roles in lymphocyte development that can be grouped according to timing – that is, before, during, or after pre-antigen receptor signaling. Although no single NF-κB-subunit-knockout mouse has as severe a phenotype as NEMO knockouts with regard to the generation of mature lymphocytes, double knockouts of Rel proteins confirm the essential anti-apoptotic function of NF-κB. For example, loss of both p50 and p65, or both p65 and c-Rel, terminates lymphopoiesis before expression of the pre-antigen receptors [46, 47], suggesting that NF-κB regulates anti-apoptotic factors required for early lymphoid cell survival in response to pro-apoptotic stimuli (Figure 4). In fact, hematopoietic stem cells can activate NF-κB in response to TNFα, and in these cells NF-κB acts as a pro-survival factor [48]. The role of NF-κB in early lymphocyte development seems clear – expression of either pre-T-cell receptor (pre-TCR) [49] or pre-BCR (pre-B-cell receptor) coincides with increasing NF-κB activity and induction of anti-apoptotic signals through NF-κB [50, 51]. However, it remains unclear how NF-κB is activated downstream of the pre-AgR as the nature of the signaling pathway at play remains almost entirely uncharacterized. Whether NF-κB contributes to lineage choice made at these early stages or merely promotes survival is also unknown.

The extent of NF-κB activation serves as a rheostat in the selection of DP (double positive; $CD4^+CD8^+$) thymocytes (Figure 4). TCR-mediated NF-κB activation follows binding to peptide:major histocompatibility complex (MHC). A thymocyte that expresses a TCR that cannot bind MHC succumbs to "death by neglect", whereas those that bind peptide:MHC are either positively or negatively selected depending on the strength of signaling. Thymocytes that bind self-peptide:MHC with very high affinity, are likely to be self-reactive, and hence are deleted through negative selection. Thus, only DP thymocytes that recognize self-peptide:MHC and signal within a defined range are positively selected and become single-positive (SP) T cells. During negative selection, NF-κB facilitates the induction of apoptosis following high affinity TCR ligation [52], perhaps by facilitating

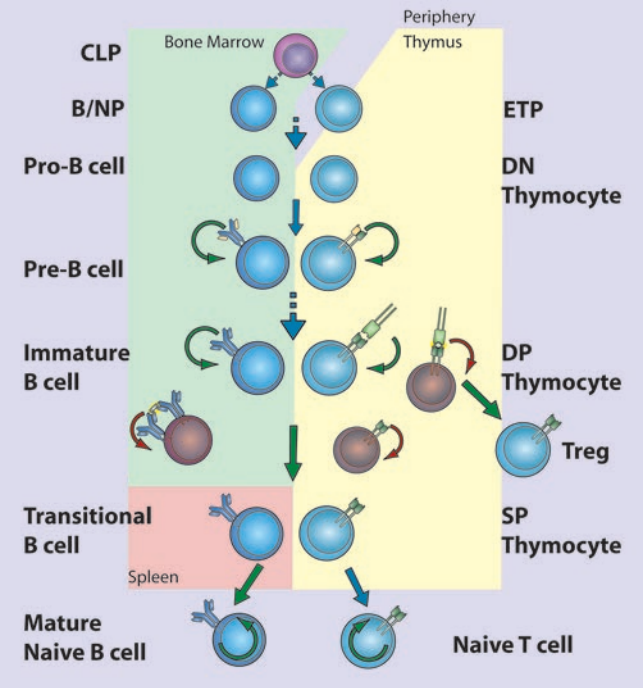

Figure 4 NF-κB in lymphopoiesis. NF-κB plays a pro-survival role in common lymphoid precursor (CLP) cells which give rise to B- and T-cell lineages. B-cell development occurs in the bone marrow, where NF-κB protects pre-B cells from pro-apoptotic stimuli including TNFα. Signaling to NF-κB through the pre-B cell receptor mediates survival of Pre-B cells, which then undergo light chain recombination to produce a functional B cell receptor. NF-κB provides a necessary pro-survival signal during Igλ but not Igκ rearrangement. Expression of BCR leads to NF-κB-dependent differentiation into immature B cells. High levels of BCR signaling, i.e., through recognition of self-antigen, results in negative selection through the loss of NF-κB activity. Transitional B cells exit the bone marrow and migrate to the spleen, where they mature and differentiate, a process that also requires NF-κB. T-cell development occurs following migration of precursor cells into the thymus. Stimulation of NF-κB through pre-TCRα provides a pro-survival signal allowing recombination of the TCR α chain and maturation to the double-positive (DP) stage. Optimal signaling through the TCRα/β complex induces NF-κB-dependent survival pathways, while a failure to signal or high level signaling results in death by neglect or negative selection, respectively. Intermediate high NF-κB activation facilitates intrathymic regulatory T cell (Treg) development. NF-κB activity is required for the maintenance of long-lived B and T cells. (CLP, common lymphoid progenitor; ETP, early thymic progenitor; DN, double negative – $CD4^-CD8^-$; DP, double positive – $CD4^+CD8^+$; SP, single positive – either $CD4^+CD8^-$ or $CD4^-CD8^+$.

expression of pro-apoptotic genes and consequent sensitization to pro-apoptotic signals [53]. The role of NF-κB in positive selection of thymocytes is more in keeping with the better-established role of NF-κB as an inducer of anti-apoptotic genes. Unlike in thymocytes, however, NF-κB functions as a pro-survival factor during negative selection of B cells. Immature B cells display constitutive NF-κB activity that is downregulated following BCR ligation [54] (Figure 4). Decreased NF-κB activity might then sensitize these cells to pro-apoptotic signals. Interestingly, some signaling components required for NF-κB activation in mature B and T cells can be genetically disrupted without affecting their development, suggesting that pathways leading to activation of NF-κB in developing B or T cells differ significantly from the pathways engaged following AgR ligation in mature lymphocytes.

Following positive and negative selection, DP thymocytes must make a lineage commitment as SP thymocytes ($CD4^+CD8^-$ or $CD4^-CD8^+$) and thereafter emigrate from the thymus. This process requires NF-κB as deletion of *NEMO* in *cd4*-Cre mice results in loss of mature peripheral T cells [55]. Equivalent deletion of the upstream kinase TAK1 has a similar outcome [56]. The exact nature of NF-κB pathway requirement is somewhat unclear, as *ikkb*$^{-/-}$ chimeras, or *cd4*-Cre IKKβ conditional knockouts, are not defective in the production of naive T cells [55, 57]. Further, the contribution of NF-κB to CD8 and CD4 lineages is not equivalent. CD8 SP cells have significantly higher levels of NF-κB activity than CD4 SP thymocytes [49, 58], yet the anti-apoptotic factor Bcl-2 is more highly expressed in CD4 than CD8 cells. Therefore, CD8 SP thymocytes are dependent on NF-κB for survival, while CD4 SP thymocytes are not [58]. NF-κB is, however, clearly important in CD4 SP cell development, and forced activation of NF-κB in CD4 SP cells results in negative selection [58].

In addition to peripheral maturation and differentiation of T cells into various subsets, thymic differentiation into both T_H17 cells and regulatory T cells (Tregs) is known to occur. The transcriptional control of T_H17 cell differentiation and the role of thymic T_H17 remain relatively obscure. One recent report has described an important role for IκBζ in T_H17 differentiation [59]. In this capacity, IκBζ acts with the nuclear orphan receptors RORγ and RORα to promote IL17A gene expression. Beyond this tangential role in T_H17 development, a T_H17-intrinsic role for NF-κB has been minimally interrogated. Instead the role of NF-κB in Treg development [60] is somewhat better understood. The differentiation of thymocytes into Foxp3/CD25-positive Tregs occurs in the thymus and depends on NF-κB. It is thought that NF-κB acts as a pioneer transcription factor enhancing accessibility of the *foxp3* locus [61-64].

Immature B cells complete development into either follicular or marginal zone B cells after exiting the bone marrow as transitional B cells. NF-κB-regulated expres-

sion of pro-survival factors is important to these final steps of B-cell development [65]. Signaling through the TNFR superfamily member BAFFR is crucial for transitional B-cell survival through induction of anti-apoptotic Bcl-2 family members [66-68]. Thus, BAFF-knockout mice exhibit a complete failure of transitional B-cell maturation, which mirrors that seen in Bcl-X_L-knockout mice [66, 69, 70]. BAFF activates both non-canonical and canonical NF-κB pathways, though the former is principally responsible for the anti-apoptotic function in transitional B cells. Nevertheless, deficiency in NEMO, IKKα, or IKKβ decreases the numbers of mature B cells [57, 71, 72]. Likewise, p50/p52 or p65/c-Rel double-knockout progenitor cells are defective in their ability to mature beyond the transitional B-cell stage [47, 73]. A requirement for both the canonical and non-canonical NF-κB pathways may explain why deletion of p50 and p52 produces a more complete block in B-cell development than loss of p65 and c-Rel. Thus, only those knockouts that target both the canonical and non-canonical NF-κB pathways have an effect that approximates the phenotype seen in BAFF or Bcl-X_L deficiency. Constitutive hyperactivation of the canonical pathway can circumvent BAFF-R deletion [74], yet it remains unclear whether the canonical pathway merely supports the non-canonical pathway [75], or has an independent function.

NF-κB in innate immunity

Pattern recognition receptors

Recognizing pathogens is an absolute requirement for the initiation of effector functions. Mammals express several diverse classes of pattern recognition receptors (PRRs) that are dedicated to this purpose. These PPRs include transmembrane Toll-like receptors (TLRs), cytoplasmic Nod-like receptors (NLR) and RIG-I like receptors (RLR), scavenger receptors, C-type lectins, and the complement system. Although epithelial cells are frequently the first to encounter pathogens, they are also constantly exposed to non-pathogenic microbes. Therefore, while a variety of PRRs are differentially expressed in epidermis, gut, pulmonary, urinary, and reproductive epithelium, their expression and responsiveness is tightly controlled. Instead sentinel cells of the innate immune system, particularly tissue resident DCs and macrophages, express a more complete complement of PRRs, and exhibit a higher propensity to signal on exposure to pathogens.

TLRs are evolutionarily conserved pattern recognition receptors that recognize unique, essential molecules characteristic of various classes of microbes [76]. The 11 characterized mammalian TLRs have varied tissue distribution and serve as recognition receptors for pathogen-associated molecular patterns (PAMPs) present on bacteria, viruses, fungi, and parasites. Perhaps because of the modular nature of the TLR extracellular domain, which consists of multiple leucine-rich repeats (LRRs), these receptors are capable of recognizing more than one microbial molecule (Figure 5). Heterodimerization of TLRs further expands the repertoire of recognized molecules. In general, the ability of TLRs to distinguish between pathogen types is translated into appropriate innate and adaptive responses through the selective activation of NF-κB, IRFs, and other inducible transcription factors. TLR signaling pathways are complex and have been extensively reviewed elsewhere [2, 77, 78]. In general, TLR signaling is often divided into MyD88-dependent and TRIF-dependent pathways: MyD88 signaling predominantly leads to the activation of NF-κB, while TRIF signaling leads to both IRF3 and, to a lesser extent, NF-κB activation.

In recent years, there has been significant activity in characterization of cytoplasmic PRRs, particularly those recognizing viral nucleic acids. These efforts followed the initial description of nucleotide oligomerization domain proteins (NOD), NOD1 and NOD2. NLRs, of which NOD1 and NOD2 are the best-known examples, are characterized by LRRs and NODs. NOD1, NOD2, and IPAF have CARD domains and can signal to NF-κB (see Blonska and Lin, *Cell Res* 2011; 21:55-70). NOD1 recognizes a peptidoglycan containing *meso*-diaminopimelic acid and induces NF-κB through a canonical pathway that includes activation of IKKβ. NOD2 recognizes muramyl dipeptide (MDP), a ubiquitous component of nearly all bacterial cell walls. The CARD-containing kinase RIP2 is required for NF-κB activation. The function of RIP2 tyrosine kinase activity is unclear, although it was recently shown that kinase inhibitors could prevent MDP-induced cytokine responses [79]. Although RIP2 binds to NEMO, it is not thought that RIP2 tyrosine kinase activity mediates IKK phosphorylation directly. Instead it has been proposed that RIP2 directly mediates activation of the IKK complex through NEMO binding and induced proximity [80].

Retinoic acid inducible gene I (*RIG-I*) and melanoma differentiation-associated gene 5 (*MDA5*) are DExD/H-box RNA helicase-containing cytoplasmic proteins that bind directly to dsRNA [81-83]. The details of RNA binding to RIG-I and MDA5 continue to be the subject of intense research. Thus, it was thought that RIG-I recognizes dsRNA replicative intermediates of negative stranded single-stranded RNA (ssRNA) viruses, while MDA5 responds to long dsRNA replicative intermediates of plus strand ssRNA viruses [84-87]. Indeed, the struc-

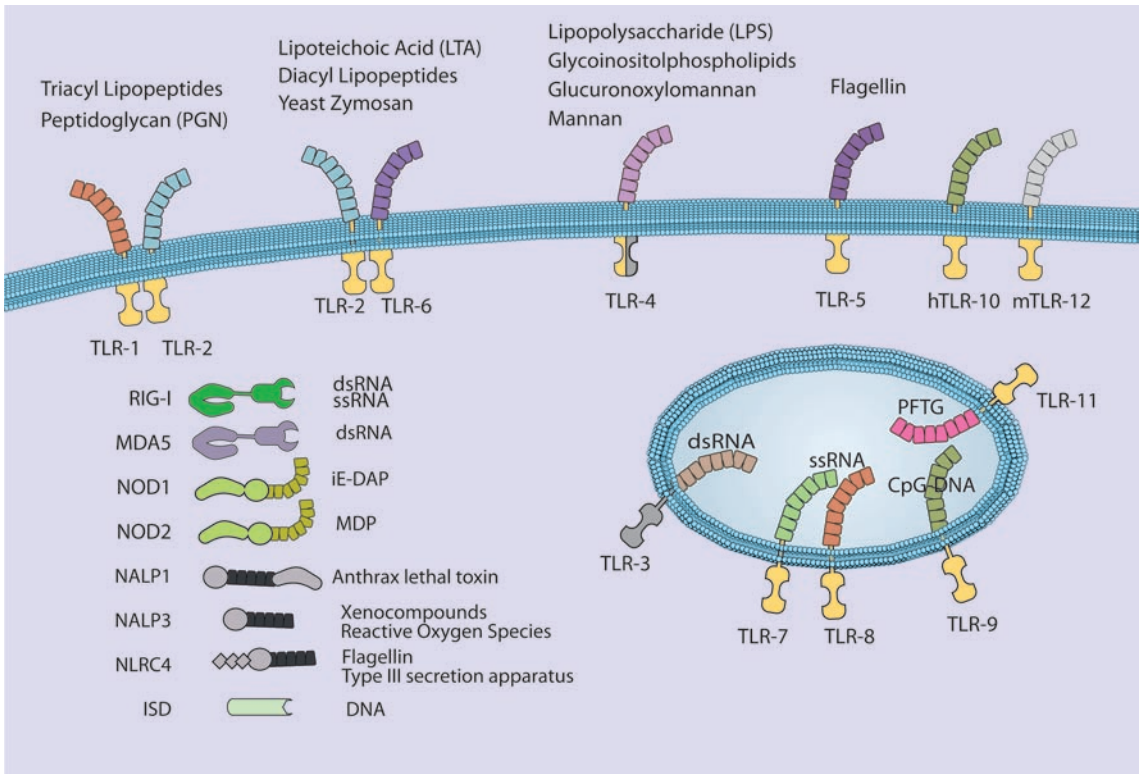

Figure 5 Pattern recognition receptors and their cognate ligands. TLRs 3, 7, 8, 9 and 11 have been reported to exhibit endosomal or intracellular localization while NOD1, NOD2, RIG-I, MDA-5, NALP1, NALP3, NLRC4, and the intracellular DNA sensor (ISD) function in the cytoplasm. Only a partial list of ligands or classes of ligands for each receptor is given.

ture of RIG-I bound to short dsRNA products has recently been solved [88, 89]. RIG-I has also recently been implicated in the recognition of DNA viruses by binding short dsRNAs generated by RNA polIII [90]. However, it has been shown that RIG-I can directly recognize ssRNA genomes containing 5′ triphosphates, and suggested that for negative stranded RNA viruses, this may be the relevant PAMP [91]. On binding to either the viral genome, viral replication intermediate, or RNA product, RIG-I, and MDA5 induce the activation of IRF3 and NF-κB. The CARD-containing protein MAVS (also known as CARDIF, IPS1, and VISA) mediates signaling from RIG-I [92-95]. RIG-I and MDA5 differentially recognize various groups of RNA viruses and are independently required for robust antiviral responses [86]. Therefore, together with TLRs, RLRs provide an additional mechanism of sensing viral infection and mediating type-I IFN (IFN-α/β) responses.

Pathogen recognition in innate immunity

Pathogens recognized by PRRs can be categorized as bacterial, viral, or eukaryotic, and each of these categories has several well-described PAMPs [96]. Both in terms of accessibility and uniqueness to prokaryotes, the bacterial cell well is the most abundant source of PAMPs for TLRs and other PRRs. Lipoproteins, glycolipids, and protein components of bacterial cell wells have all been shown to function as PAMPs. The TLR4 ligand LPS, a glycolipid component of the outer membrane of Gram-negative bacteria, is the most thoroughly studied PAMP and the most potent TLR ligand known. Trace amounts of LPS activates the innate immune system via TLR4, leading to the production of numerous proinflammatory mediators, such as TNFα, IL-1, and IL-6. TLR4-mediated responses to LPS require CD14 and MD-2.

Conserved differences in bacterial nucleic acid structures can also be recognized by the innate immune system. TLR9 recognizes bacterial DNA containing unmethylated CpG motifs, and TLR9-deficient mice are not responsive to CpG DNA challenge [97]. The low frequency and high rate of methylation of CpG motifs prevent recognition of mammalian DNA by TLR9 under physiological circumstances. A recent report indicated that the intracellular, endosomal restriction of TLR9 is critical for discriminating between self and non-self DNA, as host DNA, unlike microbial DNA, does not

usually enter the endosomal compartment [98].

Nucleic acids are also key viral PAMPs, and are recognized by TLRs 3, 7, 8, and 9, as well as by RLRs. Signaling from viral PAMPs results in the activation of NF-κB and IRFs, which cooperatively mediate the production of IFN-α/β [99, 100]. TLR3 recognizes dsRNA, a common viral replicative intermediate [101]. TLR7 and TLR8, initially found to recognize synthetic antiviral compounds [102, 103], recognize guanosine- or uridine-rich ssRNA derived from RNA viruses [104-106]. Interestingly, mammalian RNA is significantly less stimulatory than bacterial RNA, suggesting that nucleoside modifications facilitate discrimination between endogenous and pathogen RNA [107]. In addition, subcellular localization of TLR7 and TLR8 to endocytic compartments, and limited constitutive cell type expression, may also facilitate self/non-self discrimination by minimizing exposure to host RNA. TLR9 recognizes viral CpG sequences and induces the induction of IFN-α [108-110]. However, as membrane restriction prevents TLRs from sampling the cytosol where much of the viral lifecycle occurs, cytosolic PRRs provide comprehensive innate immune recognition. Thus, TLR3 and the adaptor TRIF are not required for viral induction of type I IFN in many tissues and cell types [111], although specialized innate antiviral cells, such as plasmacytoid DCs, rely more heavily on TLR mediated recognition of viral nucleic acids [112]. Recognition of cytoplasmic dsDNA leading to NF-κB activation and type I interferon production has also been reported [113, 114]. Although several candidates have been found, the relevant receptor has not yet been conclusively identified [115]. This receptor(s) is predicted to be important for type I IFN production in response to viruses and intracellular pathogens, such as *Listeria sp*.

Although research on the recognition of PAMP/PRR pairs is an ongoing process of discovery, defective responses of MyD88-deficient cells indicate that many fungal and parasite species are capable of activating TLR pathways. TLR4 has been shown to recognize *Aspergillus hyphae* [116], and *Cryptococcus neoformans* capsular polysaccharide [117]. TLR2 and TLR6 are required for recognition of yeast zymosan, while TLR4 is thought to recognize certain yeast mannans. The identification of parasite PAMPs has been more elusive, and their existence is somewhat controversial. Nevertheless, TLR2 heterodimers recognize various parasite GPI-anchored proteins and glycoinositolphospholipids from the parasitic protozoa *Trypanosoma cruzi* [118]. TLR9 was initially reported to recognize the malarial pigment hemozoin, a byproduct of heme metabolism in infected erythrocytes [119], although subsequent studies suggest that DNA associated with hemozoin is the relevant TLR9 ligand [120]. Instead, hemozoin may be recognized by the NLRP3 inflammasome [121]. TLR11 recognizes a profilin-like protein that is conserved in apicomplexan parasites including *Toxoplasma gondii* [122]. Recognition of helminths remains an area of controversy [123]. In particular, it remains unclear how helminth PAMPs might preferentially induce T_H2 responses that are characteristic of the immune responses to this class of pathogen. Direct skewing of adaptive responses by the mechanism of innate recognition is suggested to occur in other scenerios as well [124]. For example, C-type lectins can directly affect T_H activation and differentiantion [125], thus recognition of *C. albicans* α-mannans by dectin-2 induces a protective T_H17-predominant response [126]. Therefore, whether through cell type or PRR specificity, the recognition of specific PAMPS can shape the nature of the subsequent innate and adaptive immune responses.

Immediate anti-microbial responses

PAMP recognition initiates a complex series of events: the first is the mounting of immediate antimicrobial responses at the cellular level. This effective and evolutionarily conserved function of PRRs is dependent on activation of *NF-κB* gene programs. In particular, much of the early innate response has been demonstrated to depend on the canonical NF-κB pathway. Thus, *rela*$^{-/-}$/*tnfr1*$^{-/-}$ and *ikbkb*$^{-/-}$/*tnfr1*$^{-/-}$ double-knockout mice have increased susceptibility to bacterial infection [57, 127-129]. MEFs from *Nemo*-deficent mice do not exhibit NF-κB activation by LPS or IL-1 [130]. Therefore, activation of NF-κB responsive genes by the innate immune system depends on NEMO and likely progresses through the canonical NF-κB signaling pathway. Early antimicrobial effectors include NF-κB-dependent expression of defensins, which are cationic peptides that exert direct bactericidal activity by inducing membrane permeabilization. The classic mediators of defensin release are small intestinal Paneth cells, which secrete α-defensins into the intestinal lumen following exposure to bacterial PAMPs [131]. The production of anti-microbial nitrogen and oxygen species, which are acutely toxic to a variety of microbes, augments the activity of anti-microbial peptides. Production of nitric oxide (NO) is mediated in part by inducible NO synthase (iNOS), which is coordinately regulated by NF-κB and STAT transcription factors [132].

Inflammation

Inflammation begins with epithelial or stromal cells of the infected tissue or tissue resident hematopoietic cells such as mast cells or DCs recognizing an inflammatory stimulus and propagating pro-inflammatory signals.

These signals lead to the recruitment and activation of effector cells, initially neutrophils and later macrophages and other leukocytes, resulting in the tissue changes characteristic of inflammation – *rubor*, *calor*, *dolor*, and *tumor* (redness, heat, pain, and swelling, respectively). There is a vast amount of literature that correlates NF-κB activation with inflammation in a wide array of diseases and animal models. There are, likewise, numerous studies using gene targeting and inhibitors of NF-κB that have established the causative role of NF-κB in inflammatory processes.

NF-κB is responsible for the transcription of the genes encoding many pro-inflammatory cytokines and chemokines. The pathway from pathogen recognition to pro-inflammatory cytokine production demonstrates a particular reliance on NF-κB. The immediate targets of NF-κB-dependent pro-inflammatory cytokines, such as TNFα, tend to be receptors that, in turn, activate NF-κB. Therefore, NF-κB is crucial to the propagation and elaboration of cytokine responses. TNFα is particularly important for both local and systemic inflammation, and it is a potent and well-studied inducer of NF-κB. One important early target of these effectors is the vascular endothelium. Changes in vascular endothelial cells both recruit circulating leukocytes and provide them with a means of exiting the vasculature into the infected tissue. NF-κB regulates the expression of adhesion molecules, both on leukocytes and on endothelial cells, which allow the extravasation of leukocytes from the circulation to the site of infection [133]. Thus, in the absence of p65, the recruitment of circulating leukocytes to sites of inflammation is impaired [127].

Recruited neutrophils are the key mediators of local inflammation and, as mentioned above, NF-κB is important for their survival and function under relatively toxic conditions [134]. NF-κB is important for the production of the enzymes that generate prostaglandins and reactive oxygen species (e.g., iNOS and Cox, both NF-κB target genes) and may, furthermore, be involved in the signaling induced by prostaglandins [135, 136]. NF-κB has also been implicated in the response to leukotrienes, which similar to prostaglandins are short-lived paracrine effectors. For example, leukotriene-induced IL-8 production appears to be dependent on rapid activation of NF-κB [137]. Finally, matrix metalloporteinases are also crucial mediators of local inflammation and leukocyte chemotaxtis, and their expression is also regulated by NF-κB [138-140].

Resolution of inflammation and subsequent tissue repair is a crucial event, and its failure is a common source of pathology. Resolution of inflammation is an active process that is as complex as the inflammatory response itself, and involves numerous pathways that are not all directly relevant to NF-κB [141]. Macrophages are key regulators of both inflammation and its resolution, and NF-κB plays a key role in the instruction of macrophage responses. Macrophage differentiation into pro-inflammatory (M1) or anti-inflammatory (M2) cells depends both on cell intrinsic NF-κB pathway activation as well as the local cytokine milieu, which is strongly influenced by NF-κB.

During acute inflammation, there are multiple negative feedback pathways that help to rein in inflammatory responses [3]. It has long been known that cells, such as macrophages, become resistant to repeated pro-inflammatory stimuli [142]. The IκB family protein Bcl-3 can function both as an inhibitor and mediator of NF-κB transcriptional programs [2]. Following LPS stimulation, Bcl-3 is induced and binds p50 dimers, with which it can repress the expression of a subset of NF-κB target genes. In this manner, Bcl-3 mediates the selective inhibition of repeated LPS responses in macrophages [143]. Although clearly not the only mechanism, induced transcriptional repression by Bcl-3/p50 likely contributes to LPS-tolerance. Furthermore by selectively affecting chromatin remodeling, Bcl-3 mediates repression of pro-inflammatory genes, and also facilitates the expression of the anti-inflammatory gene *IL-10*. Thus, it appears that Bcl-3 acts appropriately in the regulation of genes that are categorized as either "tolerizable or "non-tolerizable" [144]. NF-κB p50 appears to be capable of mediating anti-inflammatory responses under several additional circumstances as well. For example, p50 negatively regulates IFNγ production by and proliferation of NK cells [145]. Also, expression levels of p50 in M2-like tumor associated macrophages (TAMs) regulate the balance between anti- and pro-inflammatory functions. Thus, it was shown that TAMs overexpress p50 and that deletion of p50 renders these macrophages pro-inflammatory [146].

Inhibition of NF-κB during the resolution phase of inflammation can prolong the inflammatory process and prevent proper tissue repair [147]. Furthermore, IKKα-deficient mice display increased inflammatory responses in models of local and systemic inflammation [148], and show increased production of pro-inflammatory chemokines and cytokines [148, 149]. Rather unexpectedly, IKKα-deficient mice and embryonic-liver-derived macrophages from IKKα-deficient mice exhibit augmented inflammatory responses compared with wild-type mice [149]. The mechanisms of IKKα-mediated repression of transcriptional responses seem to be through effects on the level of nuclear p65 and c-Rel [148]. Although there are significant differences between these two reports with regard to the proposed mechanism of action, both,

nevertheless, observe the same biological consequence of IKKα deficiency in macrophages. Furthermore, recent work has suggested that IKKβ also exhibits some anti-inflammatory capacity in macrophages. First, IKKβ suppresses the secretion of the potent pro-inflammatory cytokine IL-1β [150]. Second, IKKβ is associated with the maintenance of an anti-inflammatory M2-like phenotype in TAMs [151]. Third, IKKβ is thought to inhibit M1 macrophage function by interfering with the STAT pathway during infection [152].

Initiation of adaptive responses

The innate immune system invokes potent anti-microbial activities and maintains host protection under many settings. Nevertheless, initiating the adaptive immune response remains a crucial step for robust and durable pathogen clearance. The process of information transfer from the innate to adaptive immune system is mediated by activation and maturation of APCs. The nature of the PAMP and the signaling pathways activated by the cognate PRR, as well as the context within which pathogen recognition occurs, all influence the manner in which the APC instructs T and B cell responses.

DC maturation mediated by pathogen recognition is crucial for the initiation of the adaptive immune response. To activate naive T cells, DCs must alter their chemokine receptor expression to enable migration into lymphoid tissues. During activation DCs fine-tune their antigen processing machinery such that the presentation of pathogen epitopes is favored. Finally, the expression of co-stimulatory molecules including B7.1/B7.2 (or CD80/CD86) is upregulated. These co-stimulatory molecules ligate the co-stimulatory molecule CD28, thus providing the second signal necessary to induce full T-cell activation. The response to pathogens is tailored on the basis of the distribution of PRRs in different cell types, and the ability of different cell types to, in turn, interact with T cells in a biasing manner [153].

There continues to be debate surrounding how PRRs differentially induce T_H1 versus T_H2 responses. Maturation of DCs following viral infection depends on nucleic acid-binding PRRs [154, 155]. Plasmacytoid DCs, which are robust inducers of interferon during viral infection, express viral PRRs including elevated expression of TLR7 and TLR9. Cytoplasmic nucleic acid-sensing PRRs, RLRs, are expressed more broadly, although they are further induced by type-I interferons, and they may, therefore, provide crucial amplification of antiviral responses following virus recognition by TLRs. While studies of anti-viral responses suggest that both cell type-specific distribution of PRRs, and selective activation of signaling pathways by certain PAMPs are key determinants of T_H1 versus T_H2 responses, the situation is less clear for other pathogens. It appears that multiple mechanisms converge in shaping how APCs interact with the adaptive immune system.

Role of NF-κB in the adaptive response

T-cell responses mediated by NF-κB

T-cell activation, differentiation, proliferation, and effector function are all influenced by gene programs regulated by NF-κB. The majority of effort directed at understanding the role of NF-κB in T cell activation has focused on $CD4^+$ T cells (Figures 6 and 7). On the whole, the historical lack of $CD8^+$ T-cell conditional knockouts and the selective loss of CD8 cells in the absence of functional NF-κB have impeded thorough characterization of the role of NF-κB in these cells. Nevertheless, there continues to be progress in this area and, in fact,

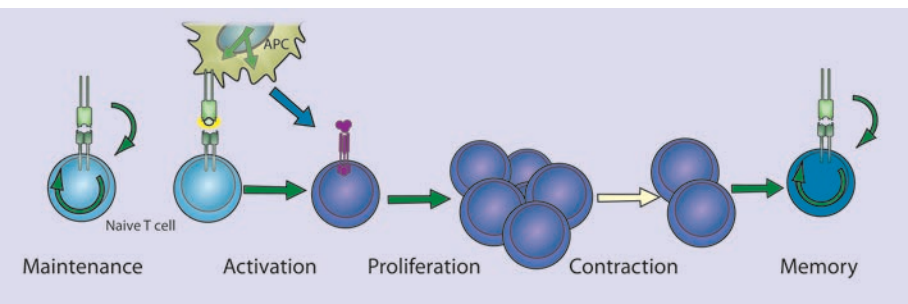

Figure 6 NF-κB in T cell activation. NF-κB participates in the maintenance, activation, differentiation and proliferation of naive T cells. Tonic TCR stimulation promotes T cell maintenance, of both memory and naive cells, through NF-κB activation. Activation occurs when the naive T cell recognizes its cognate antigen presented by an activated APC expressing both peptide:MHC and B7 family co-stimulatory molecules. NF-κB-dependent proliferation and differentiation ensue and are influenced by the local cytokine milieu. NF-κB supports proliferation, differentiation and survival as indicated (green arrows).

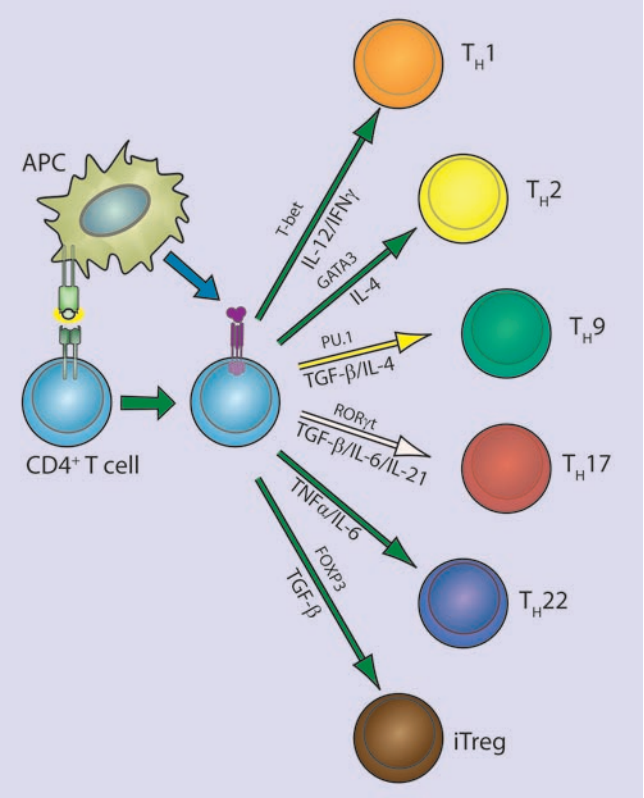

Figure 7 T_H cell differentiation. NF-κB participates in differentiation of several T_H cell types following activation of naive $CD4^+$ T_H cells. Differentiation pathways in which NF-κB is implicated are indicated with a green arrow. Key transcription factors in each differentiation pathway are indicated above each arrow, while cytokine responsible for skewing T_H cells toward a given pathway are indicated below each arrow. Additional cytokines and transcription factors implicated in several of the pathways are not depicted.

there has been a resurgence of interest in recent years. $CD4^+$ T cells have long been known to undergo differentiation into either T_H1 or T_H2 subsets depending on the cytokines present during activation [156]. However, an appreciation of the ability of $CD4^+$ helper T cells to differentiate into additional effector cell types, induced regulatory T cells, pro-inflammatory T_H17 cells, T_H22 cells T_H9 cells, has prompted a fresh look at the contribution of NF-κB to T cell differentiation and effector function (Figure 7).

Activation of naive T cells requires antigen-specific and co-stimulatory signaling provided by activated APCs. Binding of the TCR to its cognate peptide presented in the binding cleft of MHC supplies the antigen-specific signal, while co-stimulatory signaling results from ligation of CD28 by B7 molecules expressed on activated APCs. Activated naive T-cells proliferate rapidly while simultaneously differentiating into effector cells (Figure 6). In the case of $CD4^+$ T cells, proliferation leads to differentiation into immature effector cells, T_H0, which subsequently differentiate into T_H1, T_H2, T_H9, T_H17, T_H22, or inducible Treg (iTreg) cells depending on the cytokine milieu.

Stimulation of T cells through TCR and CD28 results, initially, in activation of p65-containing NF-κB complexes followed by delayed and sustained activation of c-Rel complexes [157-160]. Rapidly proliferating activated T cells rely on NF-κB activity for protection from apoptosis as well as for the production of cytokines, especially IL-2, supporting proliferation and differentiation (Figure 6). As expected, inhibition of NF-κB in activated T cells facilitates progression toward activation-induced cell death (AICD) or apoptosis [161, 162], and stimulation of p65-deficient naive T cells induces cell death [163]. In contrast, T cells lacking c-Rel do not undergo apoptosis, but nevertheless, fail to proliferate in response to typical mitogenic stimuli [164]. T cells, in which p105 cannot undergo inducible processing to p50 also fail to proliferate normally [165]. *In vitro*, c-Rel is needed for production of IL-2 and therefore, for proliferation, but this need is not recapitulated *in vivo* [166]. Interestingly, c-Rel-deficient T cells appear to have a defect in T_H1 proliferation and production of IFNγ, indicating a selective role for NF-κB family members in T_H1/T_H2 differentiation, independent of that mediated by the innate response.

Multiple transcriptional activators and repressors regulate expression of IL-2. Amongst these, members of the NF-κB family play multiple roles. In naive T cells, which do not express IL-2, repressive p50 homodimers are found associated with the IL-2 promoter [167]. Failure of T-cell proliferative responses in c-Rel-knockout mice is attributable to a failure to produce IL-2 [164]. In naive T cells, c-Rel is responsible for mediating chromatin remodeling across the IL-2 locus following CD3/CD28 co-stimulation [158]. Thus, naive T cells can be primed by activation of c-Rel by pro-inflammatory cytokines, such as those elicited following stimulation with TLR ligands [157]. Priming, which requires NF-κB activation, results in a more robust response to CD3/CD28 co-stimulation [168]. Conversely, overexpression of p65 with c-Jun can overcome the requirement for co-stimulation in naive T cells [169].

T_H0 differentiation into T_H1, T_H2, T_H9, T_H17, Treg effector cells depends on the induction of specific transcription factors, namely T-bet, GATA3, PU.1, RORγt, and Foxp3, respectively (Figure 7). There is increasing evidence that NF-κB family members are key arbitrators of the decision. For example, mice lacking p50 are

unable to mount an asthma-like, airway T_H2 response [170]. Indeed, p50-deficient T cells fail to induce GATA3 expression during T-cell stimulation under T_H2 differentiating conditions. These results suggest a transcriptional role for p50, and it was subsequently found that Bcl-3-deficient T cells also fail to undergo T_H2 differentiation, suggesting that p50/Bcl-3 complexes are crucial for T_H2 differentiation [171]. Conversely, the same authors found that RelB-deficient T cells are deficient in T_H1 differentiation due to decreased expression of T-bet. RelB may mediate upregulation of STAT4, which is responsible for T-bet induction downstream of IFNγ. Therefore, it appears that NF-κB activation during TCR stimulation may render cells competent for both proliferative and differentiating stimuli. As a corollary, NF-κB transactivation of the *IL-2* gene is repressed as T_H cells differentiate into T_H1 or T_H2 and become dependent on T_H1 and T_H2 cytokines (e.g., IFNγ and IL-4). It is thought that direct binding of T-bet to p65 that is associated with the *IL-2* gene enhancer, may mediate the repression of IL-2 production in T_H1 cells [172]. In T_H2 cells the lack of IL-2 transcription may be because of the decreased levels of p65 activation [173]. As discussed above, several groups recently implicated NF-κB, and specifically c-Rel, in the regulation of Foxp3 expression and Treg development [61-63, 174]. The relative contribution of c-Rel to iTreg development still requires further clarification. While a recent report demonstrated that IκBζ cooperates with ROR and is required for T_H17 differentiation [59], the more general contribution of NF-κB in this process, either in the thymus or periphery, remains unclear. While the role of NF-κB in T_H9 cells has yet to be interrogated, there are interesting clues suggesting an important role for NF-κB. Recently, PU.1 was reported to be the transcription factor responsible for T_H9 differentiation [175]. In unrelated recent work on acute myelogenous leukemia, it was noted that NF-κB regulates the transcription of PU.1 [176]. Furthermore, it has been suggested that IL-9 expression in T cells is regulated by NF-κB [177]. Thus, it would seem reasonable to speculate that NF-κB may play an important role in T_H9 differentiation (Figure 7). The transcription factors responsible for the differentiation of T_H22 cells have not yet been identified. However, the possible role of TNFα as a T_H22 differentiating cytokine is suggestive that NF-κB may be an important mediator of this differentiation process as well. In summary, NF-κB participates in both the initial T cell responses by supporting proliferation and regulating apoptosis, and is increasingly being shown to have an important role in the regulation of T_H differentiation.

B-cell responses mediated by NF-κB

There are many parallels in the functions of NF-κB in T and B cells. NF-κB acts to support proliferation, regulate apoptosis, and control the processes of differentiation and maturation in B cells (Figure 8). B-cell responses are classified into two groups: thymus-dependent (TD) or thymus-independent (TI). In TD response follicular B cells require co-stimulatory signaling from T_H cells expressing CD40L and cytokines, such as IL-4. These

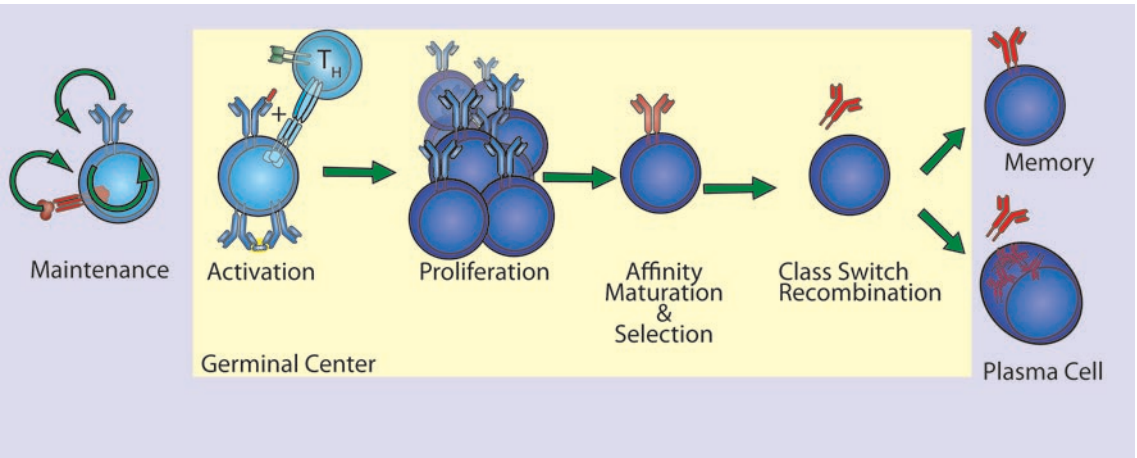

Figure 8 NF-κB in B cell activation. NF-κB participates in the maintenance, activation, differentiation and proliferation of naive B cells. Tonic BCR and cytokine, BAFF, stimulation promotes naive B cell maintenance through NF-κB activation. Activation occurs when the naïve B cell recognizes its cognate antigen and receives co-stimulatory signaling (CD40L) from an activated T_H cell within the germinal center. NF-κB-dependent proliferation and differentiation ensue and are coupled to BCR affinity maturation. NF-κB signaling in B cells expressing selected BCRs results in class switch recombination and differentiation into either memory B cells or plasma cells. NF-κB supports proliferation, differentiation and survival as indicated (green arrows).

initial steps trigger germinal center formation, in which somatic hypermutation, isotype switching, and plasma cell differentiation occur. Thus, in individuals with a mutation in CD40L B cells fail to undergo class switch recombination in response to T-dependent antigens [178]. Signaling through CD40 activates both canonical and non-canonical NF-κB pathways, although several lines of evidence suggest that the non-canonical pathway is not required in the response to TD antigens. Thus, B cells lacking p52 are fully capable of appropriate TD antigen responses when transferred [21], and B cells from *relB*$^{-/-}$ mice, although crippled in their proliferative response, undergo normal IgM secretion and class switching [179]. In contrast to these results, B cells lacking either functional NIK or IKKα, exhibit defective responses [180, 181]. It remains unclear whether the role of NIK and IKKα in this context is to induce p100 processing (non-canonical pathway) or to contribute to activation of the canonical pathway. Although both pathways are triggered by CD40 ligation, the current data suggest that canonical pathway activation is of great importance.

Evidence also supports a role for the canonical pathway in class switch recombination. In contrast to B cells lacking RelB or p52, those from *rela*$^{-/-}$ mice exhibit markedly diminished class switching, despite a modest loss of lymphocyte proliferation following various stimuli [182]. Likewise, c-Rel-deficient mice fail to generate protective humoral immune responses demonstrating the requirement for c-Rel in class switch recombination [164, 183, 184]. B cells from *nfbk1*$^{-/-}$ mice exhibit decreased proliferation in response to mitogenic stimulation, and p50/p65 double-knockout B cells exhibit greater defects in proliferation and class switching [185, 186]. Therefore, B cell proliferation and the TD B-cell maturation response are dependent on the canonical NF-κB pathway.

TI antigens are those that have an intrinsic ability to initiate B-cell responses in the absence of T-cell help. TI responses are carried out by marginal zone and CD5^{+} B cells. TI antigens function by acting as both antigen and B-cell mitogens, for example antigens that are PRR ligands or antigens with high avidity that are capable of crosslinking the BCR through repetitive structural features. In such cases it is expected that B-cell responses are more dependent on members of the canonical pathway that have well-documented roles in TLR and BCR signaling. For example, c-Rel-deficient B cells are highly sensitive to apoptosis following BCR cross-linking [187-189]. As mentioned above, p50 and p50/RelA double-knockout B cells are deficient in responses to TI stimulation. Likewise, IKKβ-deficient B cells fail to mount TI or TD responses [190]. These IKKβ-deficient B cells also exhibit increased spontaneous apoptosis, supporting that NF-κB is important in survival of B cells.

NF-κB and lymphocyte longevity

Lymphocyte homeostasis reflects balanced lymphocyte turnover and lymphopoiesis. Turnover of mature lymphocytes is complex as it depends upon both activation induced and homeostatic proliferation, and cell death. Lymphocyte survival is mediated through tonic stimulation provided by both the antigen receptor and certain cytokine receptors. As such, NF-κB can be assumed to play an important role in lymphocyte homeostasis and this assumption is supported by various genetic models.

T cells can be quite long-lived and naive T cells require continued contact with MHC:self-peptides for survival. These self-peptide complexes are most likely expressed on lymphoid DCs and generate a tonic low grade TCR signal. Survival of memory cells, on the other hand, is independent of continued contact with self-peptide:MHC complexes. B cells are formed at a far higher rate than T cells and therefore, B cells also undergo a significantly higher rate of turnover. Nonetheless, B cells too require maintenance signals to achieve peripheral homeostasis. The antigen receptor on B cells provides a basal level of signaling, albeit independent of the presence of antigen, which is required for maintenance of mature B cells. Thus, deletion of the BCR from mature B cells results in a complete loss of the peripheral B cell pool [191]. The assumption is that BCR provides tonic activation of the NF-κB pathway resulting in the expression of anti-apoptotic proteins. Consistent with this assumption, loss of IKKβ, NEMO, or components of the CBM complex in mature B cells also results in complete loss of peripheral B cells [71, 190, 192]. Furthermore, B cells from *rela*$^{-/-}$, *nfkb1*$^{-/-}$, *nfkb2*$^{-/-}$, and *c-rel*$^{-/-}$ mice all have increased sensitivity to apoptosis and/or decreased survival *ex vivo* [67, 188, 193]. In addition to contributing directly, it is thought that low level NF-κB activation in B cells contributes to survival through upregulation of p100, which is necessary for BAFFR-induced activation of the alternative pathway [67, 75, 166].

Several studies have implicated BAFFR in this aspect of the B lymphocyte survival [194], suggesting that the non-canonical NF-κB pathway is also relevant to B-cell survival. Consistent with this idea, constitutive activation of NIK or IKKα can circumvent the requirement for B cell BAFFR [74, 195]. Conversely, B cells lacking IKKα exhibit defective survival [72, 196]. The relevant targets of the non-canonical pathway downstream of BAFFR are thought to be Bcl-2 family members, e.g., the anti-apoptotic factor A1 [187] or Bcl-X_L, which are known to be regulated by p52/RelB-containing complexes, and are required for the maintenance of mature B cells. Indeed,

Bcl-X_L can complement B cells with a mutant BAFFR [197]. Thus, tonic BCR stimulation, through upregulation of p100, may cooperate with BAFFR ligation and alternative pathway activation, to upregulate anti-apoptotic Bcl2 family members and mediate maintenance and survival of mature B cells.

Concluding remarks

The areas covered in the current review necessarily represent a subset of those in which NF-κB is a crucial regulator of immune responses. Recent work has highlighted the importance of NF-κB in the maintenance of epithelium barriers [198]; as a regulator of inflammation associated with obesity and hyperlipidemia; and as a key factor in the development of inflammatory diseases and cancer. Our understanding of the immune system continues to improve, and coordinately our recognition of the many roles played by the NF-κB family of transcription factors also grows. As one example, the expanding universe of T-cell subsets opens up a new area for the analysis of the role of NF-κB in T-cell differentiation and effector function. It will be intriguing to see how NF-κB contributes to the development of T_H9, T_H17, T_H22, and iTreg/T_H3 cells. Deciphering the role of NF-κB in the effector function of these recently described cell types should also prove to be an area of great interest, particularly given the growing evidence for dysregulation of these cell types in autoimmune and inflammatory disease. There is much that remains unknown about the contributions of NF-κB in early and non-lymphoid hematopoiesis; in responses to danger signaling and allergens; in the coordination of pathogen-specific adaptive immune responses by the innate immune system; and in the differentiation and effector responses of both lymphoid and non-lymphoid innate immune cells. Thus moving forward, as in the past, much of the work done to understand the physiological and pathophysiological role of NF-κB will continue to focus on the role of this transcription factor family in Immunobiology.

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