The Role of NFκB in T-lymphocyte Development and Function

Elisha de Valle1,2, Laurensius K. Lie1,2, Stuart P. Berzins3 and Raffi Gugasyan1,2,4

1Centre for Immunology, Burnet Institute, Melbourne, Victoria, Australia
2Department of Immunology, Central Clinical School, Monash University, Melbourne, Victoria, Australia
3University of Ballarat, and Ballarat Cancer Research Centre, Victoria, Australia
4Department of Microbiology and Immunology, the University of Melbourne, Melbourne, Victoria, Australia

Abstract

Initially identified as a nuclear factor in B cells, the family of NFκB transcription factors has since been found to operate in almost all cell types, regulating the transcription of a wide range of target genes. The NFκB signalling pathway is of particular importance to T lymphocytes, playing a prominent role in both T cell development and function. This review will focus on the current understanding of the roles of NFκB during thymic T cell development, with an emphasis on some of the emerging roles for NFκB signalling in regulating the development of non-conventional thymocyte lineages. We will also evaluate the function of NFκB signalling in the polarization of T-helper subsets in the periphery, and how NFκB intersects with other T cell-intrinsic pathways through mechanisms of signalling crosstalk. Dysregulated NFκB signalling is implicated in numerous disease states, and a thorough understanding of NFκB function during different phases of T cell development and function will be vital for optimal targeting in a therapeutic setting.

The Family of NFκB Transcription Factors

The mammalian NFκB family of transcription factors consists of five members, (RELA (or p65), c-Rel, Rel-b, NFκB1 (p105/p50) and NFκB2 (p100/p52)), which share a conserved Rel Homology Domain (RHD) at the N-termini. The RHD is vital for NFκB dimerization, DNA binding, nuclear localization, and inhibitor binding [4]. On the basis of their C-terminal domains, NFκB proteins can be classified into two distinct subclasses. The NFκB1 and NFκB2 subunits exist as the precursor proteins p105 and p100, respectively. These proteins contain a high number of inhibitory ankyrin repeat sequences at the C-terminal end. Proteolytic cleavage removes the C-terminal region [5] to produce the DNA-binding proteins; p105 is cleaved to form p50, and p100 to p52. In contrast, the RELA, c-Rel and Rel-b subunits each possess C-terminal transactivation domains (TAD) and their transcriptional activity is not dependent on proteolytic cleavage. The NFκB transcription factors function as dimer proteins, forming either homo or heterodimer complexes. The NFκB dimer combinations each bind distinct 10bp DNA sequences (κB sites) and regulate the transcription of distinct yet overlapping sets of target genes [6]. The p50 and p52 subunits lack TADs, and are transcriptionally active only when dimerized with RELA, c-Rel or Rel-b [7]. Homodimers of p50 and p52 can therefore play an inhibitory role, suppressing the transcription of certain target genes by blocking access to κB sites.

Pre-existing dimers of NFκB transcription factors are found in the cytoplasm of naïve T cells. This allows for rapid transduction of signals through the NFκB pathway, which is tightly controlled by a vast...
number of positive and negative regulatory mechanisms. In the steady state, association with inhibitory proteins known as the Inhibitor of NFκB (IκB) family keeps the transcription factor dimers in an inactive state, sequestered in the cytoplasm [8]. The IκB family includes several members, all containing long ankyrin repeat sequences, which mask the Nuclear Localization Signals (NLS) of NFκB transcription factors [9]. The NFκB precursors p100 and p105 also perform this function, with the ankyrin repeat sequences found at their C terminal ends enabling them to act like IκBs [10,11]. Release of NFκB dimers from IκB inhibitory complexes occurs through the action of a third important player in the NFκB pathway, the IκB Kinase (IKK) complex.

Through the ‘classical’ NFκB activation pathway, signals from a wide range of stimulators including proinflammatory cytokines, bacterial and viral components, UV light and oxidative stress can trigger the activation of IκKs [12]. The IKK complex is composed of two catalytic subunits, IKKa and IKKβ, and the regulatory subunit IKKγ (or NEMO). Following the binding of ligands to various cell surface receptors, the IKK complex is activated, phosphorylating IκB at intact distinct serine residues; 32 and 36 [13], which causes the ubiquitination and proteolytic degradation of IκB proteins [12]. The removal of IκBα releases transcription factor dimers consisting of p50, RELA and c-Rel subunits, which are free to enter the nucleus, bind to κB sites of target genes and initiate transcription [14]. This pathway facilitates the transcription of genes essential for processes including cell growth, survival, inflammation and adhesion. Furthermore, the RELA/p50 dimer promotes transcription of the gene encoding IκBα, which inactivates the DNA bound transcription factor complex, thus limiting the duration of NFκB signalling in a negative-feedback loop [15,16].

In addition to the classical pathway, another distinct mechanism of NFκB activation referred to as the ‘alternate’ pathway has been described [17]. In this pathway, p100 acts as an IκB, sequestering p52/Rel-b dimers in the cytoplasm [10]. Triggered by stimulators such as BAFF, Lymphotoxin-α and RANKL, the NFκB Inducing Kinase (NIK) activates IKKα, which phosphorylates p100, processing it to p52 [14,18]. The released p52/Rel-b dimers then regulate the transcription of target genes that are particularly important in lymphoid development [19,20]. Though the two pathways of NFκB activation are triggered by different stimuli and regulate the transcription of different target genes, the alternate and classical pathways do not operate in isolation, but are highly interconnected [21,22].

The Role of NFκB in the Development of T Lymphocyte Subsets

The expression pattern of NFκB in the thymus

The generation of the T cell repertoire is a tightly regulated process, which takes place within the thymus. Based on the differential expression of CD4 and CD8 co-receptors, thymic T cell development is generally divided into four stages [23] (Figure 1). The NFκB subunits are expressed throughout thymocyte development, with different dimer complexes playing distinct roles during each developmental stage [24]. Despite some functional redundancy between individual subunits, the study of various knockout and transgenic mouse models has provided significant insights into the role of NFκB during T cell development. The predominant expression of RELA within the thymic cortex suggests a role for this subunit during early T cell development [25]. Due to the embryonic lethality of rel-a−/− mice [26], the function of RELA in T cell development remains poorly defined, however a recently generated mouse model with a T cell-specific deletion of RELA [27] should provide further insight into the role of this subunit during key stages of thymocyte development. The elevated expression of Rel-b and c-Rel in the thymic medulla suggests their importance at later stages of T cell development [25]. Along with the p52 subunit, the role of Rel-b in signalling via the alternate pathway is strongly associated with the normal function of the thymic stroma [28,29,30].

The role of NFκB in early T cell development

The primary function of NFκB activation during early thymic T cell development is to promote the survival of developing thymocytes. The activation of NFκB ensures the survival of T cell progenitors from TNF-α-induced apoptosis [31], while a role for the p50/RELA heterodimer has been demonstrated in regulating the expression of Jagged and Delta-like ligands, which are members of the Notch family that are crucial for T lineage commitment [32]. The CD4 CD8 Double Negative (DN) thymocytes can themselves be subdivided into four developmental stages on the basis of CD44 and CD25 cell surface expression: stage I (CD44+CD25-), stage II (CD44+CD25+), stage III (CD44 CD25+) and stage IV (CD44 CD25+) [33]. As DN thymocytes progress through these stages, they gradually lose the capacity to enter other lineages, culminating in the expression of a pre-T-cell receptor (TCR) by DN IV cells [34]. Late stage III and early stage IV DN thymocytes show constitutive NFκB activation [35] to promote survival [36]. For example, signalling through the pre-TCR has been shown to activate NFκB [36,37], promoting survival through the transcription of anti-apoptotic target genes such as Bcl-2 [38].

Within DN thymocytes, the NFκB pathway does not operate in isolation, but has been shown to act in concert with other transcription factor families, such as the basic helix-loop-helix transcription factors E2A and HEB, which are critical for thymic T cell development [39]. Studies in thymocytes and T cell lines have suggested that Id1, the inhibitor of E2A and HEB, may mediate up-regulation of NFκB in response to pre-TCR signals - in particular the c-Rel subunit [40]. As a result, the enhanced expression of Id1 in transgenic mice severely impairs T cell development, which is exacerbated by the aberrant activation of NFκB [39,40]. To date, however, the extent and implications of this crosstalk for thymic T cell development remains poorly defined.

The role of NFκB in T cell selection

Based on the signal strength of the TCR and its interaction with self-peptide-MHC complexes, CD4+CD8+ Double Positive (DP) thymocytes undergo positive and negative selection [41]. Essentially, weak signaling from TCR-peptide/MHC interactions fail to support thymocyte survival (death by neglect), whereas overly strong interactions result in the deletion of potentially autoreactive cells. Only moderate signalling leads to the development of mature CD4+ and CD8+ Single Positive (SP) thymocytes. The precise role of NFκB in mediating thymocyte selection remains controversial. DP thymocytes have been shown to preferentially up-regulate c-Rel and NFκB1-containing dimers [42], but mice lacking individual NFκB subunits have not shown significant defects in T cell selection. An exception are the rel-b−/−, nfkb2−/− and nik−/− mice, in which a defective thymic stroma is responsible for impaired negative selection [29,30,43]. The absence of defects in single knockout mutants most likely reflects functional redundancy amongst individual subunits, as other NFκB-specific mouse models including IκBα super-repressor and IκK knockout mutants display profound defects in T cell development and selection [44].

Studies in mice expressing a transdominant IκBα, which constitutively represses NFκB signalling, have pointed to a role for
Figure 1: The transcriptional control of T cell development and differentiation

Shown is a schematic representation of T cell development along multiple lineages within the thymus (left) and the polarization of distinct T cell subsets in the periphery (right). (A) Highlighted are some of the key transcription factors that direct T cell development along the indicated lineages in the thymus, and promote the phenotype of polarized T helper lineages in the periphery. (B) Shown are the NFκB subunits (and related proteins) for which a cell-intrinsic role in development, differentiation or function has been described. Within the thymus, the CD4+CD8+ DN thymocytes develop into CD4+CD8+ DP thymocytes. Positive selection gives rise to a number of thymocyte lineages, including the CD4+ SP, CD8+ SP and nTreg cells. In response to exogenous IL-4 produced by PLZF+ cells, a further innate memory-like CD8+ T cell lineage arises in some mutant and wild-type mouse models. In the periphery, naïve CD4+ T lymphocytes can adopt a number of functional phenotypes including the TFH, Th1, Th2, Th17 and iTreg cells, each producing a distinct cytokine signature. See text for further details.
NFκB in promoting the survival of DP thymocytes [45-47]. Conversely, others have demonstrated a pro-apoptotic role for NFκB, using TCR transgenic mouse models of negative selection [46]. Furthermore, the expression of a transdominant Iξκα has been shown to promote the apoptosis of DP thymocytes in response to anti-CD3 treatment in vivo [48]. The level of NFκB inhibition in these different Iξκα transgenic mouse strains is likely variable and thus may contribute to the observed differences in these mouse models.

Genetic manipulation of the IKK proteins has primarily demonstrated a pro-survival role for NFκB in thymocyte selection. A T cell-specific dominant-negative IKKβ mutation has been shown to promote anti-CD3 induced apoptosis [49], while the expression of a kinase-deficient IKKβ was found to impair the generation of CD8 SP thymocytes [50]. In further support of an anti-apoptotic role for NFκB, it was shown that negative selection triggers the expression of an NFκB inhibitor [51]. Intriguingly, the conditional deletion of IKKβ in the T cell lineage does not significantly impact the development of conventional T cells, but impairs the generation of both NKT and T regulatory (Treg) cell lineages [50], suggesting that the functions of NFκB during the development of conventional versus non-conventional thymocyte lineages differ significantly.

While the precise role of NFκB in negative selection remains subject for debate, a recent study has suggested that NFκB may contribute to the orchestration of selection thresholds [52]. Low affinity TCR signalling may trigger low levels of NFκB activation to promote survival, while TCR affinity above a defined threshold triggers high levels of NFκB signalling, to provide opposing pro-apoptotic responses [52]. Surprisingly, increased NFκB activity was also shown to skew thymocyte selection in favor of conventional CD8+ T cells, but was dispensable for the development of CD4+ T cells [47,52].

**Lineage commitment of conventional and non-conventional T cells**

The thymic microenvironment promotes the development of DP thymocytes along multiple lineages (see Figure 1). Whilst the role of NFκB in promoting DP thymocyte selection appears to be specific for the CD8+ but not the CD4+ T cell lineage [47,52]. It is also apparent that NFκB signalling is important in regulating the development of CD4+ Treg cells [53,54], NKT cells [55,56] and a unique population of memory-like CD8+ T cells [57].

Although the precise function of NFκB in this context is yet to be fully resolved, the normal development of NKT cells requires the presence of Rel-b in the thymic stroma [55,58], while the NFκB1 subunit has been shown to play a thymocyte-intrinsic role in NKT cell development [55,56]. A critical role for c-Rel has been demonstrated in the thymic development of Treg cells [53,59-62]. The c-Rel subunit is highly expressed within Treg cells [53] and c-rel+/− mice show a T cell intrinsic defect in Treg development [53,54]. This is in contrast with findings from nfκb1−/− mice, which show normal Treg development, suggesting that c-Rel and NFκB1 play distinct roles during lineage commitment [54]. Furthermore, these findings support a role for the classical NFκB pathway in Treg development, initially demonstrated in the IKKβ knockout mutants, which also show defects in the development of Treg cells [50]. The absence of alternate pathway components Rel-B and NFκB2 has been shown to reduce Treg development, though this arises through disruption of the thymic stroma, rather than thymocyte-intrinsic defects. The function of NIK within thymic stromal cells is also required to promote T cell tolerance [30], yet in addition to this developmental role evidence is accumulating that NIK function is required in Treg cells [19,63,64]. Peripheral T cell function is largely normal in the absence of NIK [19], and although NIK-deficient mice have fewer peripheral Treg cells their suppressive function is maintained [63]. Primarily as a result of reduced Treg function, the overexpression of NIK specifically within CD4+ T cells has been reported to induce lethal autoimmunity [64]. These studies suggest a role for NIK in the regulation of Treg cells, through mechanisms that are yet to be defined.

**The development and function of memory-like CD8 SP thymocytes**

Recently, the study of mouse models deficient in critical transcription factors or kinases such as CBP, Id3, Itk, Rlk and Flk2, has revealed a unique T cell lineage termed innate or 'memory-like' CD8+ T cells [65-69]. In contrast to peripheral memory T cells that normally develop in response to foreign antigens, these T cells acquire their memory characteristics during development in the thymus. They are primarily restricted to the CD8 lineage, and posses the classic hallmarks of peripheral memory T cells, which includes the elevated expression of memory markers (CD44, CD122, CXCR3 and Ly6C) [66], and the ability to mount a rapid and robust IFN-γ response when stimulated (Table 1). The T-box transcription factor, Eomesodermin, which promotes a pattern of gene expression that is typical of memory CD8+ T cells [70], is also highly elevated in these memory-like CD8+ T cells.

Aside from their effector/memory properties, memory-like CD8 SP cells are distinct from CD1d-restricted NKT, intraepithelial CD8+ TCRαβ and TCRγδ T cells [71]. Like conventional CD8 SP thymocytes, they express the CD8α co-receptor rather than CD8αα homodimers and utilize a diverse TCR repertoire (Table 1), however the rules of T cell selection appear to vary between conventional and memory-like CD8+ T cells. Unlike conventional CD8+ T cells, which are selected via MHC class I expressing TECs, memory-like CD8+ T cells are positively selected through class Ia or Ib expressing haematopoietic cells [72,73]. The finding that id3+ memory-like CD8 SP thymocytes are positively selected by MHC class I expressing TECs [69] suggests that selection by haematopoietic cells may not be a critical requirement for the acquisition of memory properties by developing thymocytes. While the process of selecting memory-like thymocytes requires further investigation, a recent advance in the field was the finding that developing CD8 SP thymocytes from mouse models, such as the tik−/− and klf2−/− mutants, acquire memory characteristics through exposure to IL-4 produced by NKT or γδ T cells that express the transcription factor PLZF [66].

Our recent finding demonstrated that mice lacking NFκB1 develop a unique population of CD8 SP thymocytes exhibiting memory characteristics [57]. Development of these memory-like thymocytes is independent of the IL-4 producing PLZF+ population. Instead, the acquisition of nfκb1−/− memory-like CD8 SP thymocytes coincided with changes in T cell selection, including reduced efficiency of negative selection and positive selection by either MHC class Ia or Ib molecules presented by haematopoietic cells [57]. Whilst this study highlights the importance of NFκB1 in preventing the development of CD8 SP thymocytes with memory characteristics, a potential role for IL-4 could not be totally excluded. Subsequent work will need to consider the study of CD8+ T cells in il-4− nfkb1−/− double knockout mice, and examine the scenario of altered IL-4 receptor signalling on developing nfkb1−/− thymocytes.

An important issue that remains to be addressed is whether memory-like CD8 SP thymocytes are disease promoting, or have the...
capacity to provide protective immunity, like NKT cells. Firstly, the finding that memory-like CD8+ T cells also arise in the thymi of Balb/c mice [74] supports the physiological relevance of this T cell population and indicates that wild-type mice can harbor these T cells without detrimental effects. Consistent with some mutant mouse models, memory-like CD8+ T cells in Balb/c mice are promoted by IL-4 secreting NKT cells with a critical role for the transcription factor KLF13 [74]. Balb/c mice have a natural resistance to intracellular pathogens such as the adenovirus type 1 [75] and measles virus [76], and it is possible that memory-like T cells may contribute to the resistance to these pathogens [74].

An equivalent population of human memory-like CD8+ T cells is yet to be unequivocally identified, however in some settings these T cells seem to be promoted. For instance, a population of stem cell-like memory CD8+ T cells has been identified in humans [77]. Furthermore, loss of function Itk mutations have been observed [78], and like the murine counterpart [67] Itk deficiency is associated with elevated Eomes+ CD8+ T cells [79]. Itk deficiency has also been linked with EBV-associated lymphoproliferative disease [76], raising the possibility that memory-like T cells are associated with aberrant lymphoproliferative disease. A subpopulation of memory-like CD8+ T cells has been shown to play a critical role in lymphopaenic post-chemotherapy patients heavily susceptible to viral infection [80]. These cells, which resemble the memory-like population in mice, have the capacity to survive exposure to chemotherapy drugs and efflux rapidly to restore immunity in such individuals [80]. Memory-like CD8+ T cells have also been shown to display significant anti-tumour responses in humans [77], suggesting they may be important for the generation of tumour vaccines or T cell therapies. As we gain further insight into mechanisms in T lymphocytes

### Table 1: Distinguishing features of memory-like CD8+ T cells in wild-type and mutant mouse models.

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<thead>
<tr>
<th>Mouse Models</th>
<th>Common features</th>
<th>Unique features</th>
<th>References</th>
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<td><em>itk</em>/-; <em>itk</em>/-; <em>Itk</em>/-; <em>Itk</em>/-</td>
<td>CD8αβ+</td>
<td>Altered disease susceptibility</td>
<td>[164]</td>
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<td></td>
<td>CD8αβ+</td>
<td>Reduced CD5 expression</td>
<td>[165,166]</td>
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<td></td>
<td>CD8αβ+</td>
<td>IL-15 dependent</td>
<td>[167,168]</td>
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<td></td>
<td>CD8αβ+</td>
<td>Selected by MHC class Ia or Ib on haematopoietic cells</td>
<td>[67,72,169]</td>
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<td></td>
<td>CD8αβ+</td>
<td>Development of memory-like T cells is SAP dependent</td>
<td>[170]</td>
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<td></td>
<td>CD8αβ+</td>
<td>Elevated IL-4 producing PLZF+ cell population</td>
<td>[68]</td>
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<tr>
<td><em>id3</em></td>
<td>CD8αβ+</td>
<td>Selection by TECs</td>
<td>[69]</td>
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<td>CD8αβ+</td>
<td>Development of memory-like T cells is SAP-dependent</td>
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<td>CD8αβ+</td>
<td>Elevated CD5 expression</td>
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<td></td>
<td>CD8αβ+</td>
<td>Susceptible to Sjogren’s disease</td>
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<td></td>
<td>CD8αβ+</td>
<td>Elevated IL-4 producing PLZF+ cell population</td>
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<td><em>nfkB1</em></td>
<td>Eomes+</td>
<td>IL-15 independent</td>
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<td>Ly6c hi</td>
<td>No evidence of an elevated PLZF+ cell population</td>
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<td>TCRαβ+</td>
<td>Selected by MHC class Ia or Ib on haematopoietic cells</td>
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<td>CD8αβ+</td>
<td>Elevated CD5 expression</td>
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<td></td>
<td>CD8αβ+</td>
<td>Reduced intrathymic Sirpα+ DCs</td>
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<td>Balb/c</td>
<td>KLF13 dependent</td>
<td>KLF13 dependent</td>
<td>[74]</td>
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<td></td>
<td>Altered disease susceptibility</td>
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<td>Wild-type mouse strain</td>
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* Denotes features to be confirmed in CBP, KLF2 and β-Catenin deficient mice.

### NfκB in Peripheral T cell Function

For optimal T cell immunity, the adaptive immune system maintains a constant pool of naive CD4+ and CD8+ T cells, from which long-lived memory T cells are produced. Naive T cells rarely divide and contain low levels of p50 homodimers in the nucleus [81]. Homodimer binding is believed to repress expression of IL-2, the major cytokine involved in autocrine pro-proliferative signalling [81]. In response to various pathogens including microbes and viruses, TCR and costimulatory signals induce the activation and proliferation of antigen-specific T cells in the periphery. NfκB signalling regulates the activation and proliferation of naive T cells, both in a T cell-intrinsic manner [82-86], and through the control of T cell-extrinsic processes, such as cytokine production by APCs [82,87-90].

As T cells shift from resting to activated, the profile of NfκB transcription factors in the nucleus changes in a biphasic series of events, which are well described in *vivo* [91,92]. Upon T cell receptor engagement, p50/RELA heterodimers accumulate in the nucleus [81] followed by c-Rel containing dimers, which up-regulate the expression of IL-2 [93,94]. This influx of NfκB transcription factors promotes proliferation and survival, with inhibition of NfκB signalling shown to impair proliferation and increase apoptosis of T cells, following mitogenic stimulation [45,48]. However, the outcome of activation is dependent on the type and strength of stimuli, as well as the specific cell type activated. The IKKβ subunit is absolutely required for the generation of optimal antigen-specific T cell responses *in vivo* [95]. The deletion of IKKβ specifically within T lymphocytes was shown to reduce proliferation following antigen-specific restimulation, resulting in sub-optimal T cell dependent B cell help and impaired homeostatic expansion of T lymphocytes [95]. However IKKβ is dispensable for T cell activation and proliferation in response to strong stimuli [50]. This is demonstrated by the ability of IKKβ-deficient T cells to proliferate normally *in response to polyclonal TCR* stimulation, likely the result of residual signalling via remaining IKKα/NEMO complexes [50].

Both the classical and alternate NfκB pathways are important for...
T cell activation and function in the periphery [20], though the former is most heavily implicated in T cell-intrinsic functions. For example, components of the classical pathway are essential for regulating the progression of activated T cells through distinct phases of the cell cycle [93,96]. Both c-Rel and RELA are required to induce the progression of activated T cells through G2 to G1, and promote c-Myc-induced proliferation following TCR stimulation [85]. TCR-induced cell cycle entry and survival is also dependent on the activity of NFκB1 and c-Rel [97]. These subunits serve redundant functions, with defects in T cell proliferation and survival observed in nfκb1−/− c-rel−/− compound mutants, which do not develop in either nfκb1−/− or c-rel−/− mice [97].

**T cell polarization**

The CD4+ T lymphocytes can adopt a number of functional phenotypes, each producing a unique cytokine signature (Figure 1). The key T-helper subsets include the Th1, Th2, Th17 and T-follicular helper (Tfh) cells, and distinct T cell intrinsic and extrinsic factors regulate their polarization and function. The precise function of NFκB in this process is still being elucidated. However, individual NFκB subunits have been shown to preferentially regulate the Th1 versus Th2 response [98]. In particular, c-Rel plays a key role in promoting the polarization of Th1 cells [82], and the loss of this subunit leads to defective Th1 responses via multiple mechanisms [82]. These include the reduced production of IL-12 by APCs [89], which confers disease resistance to Experimental Autoimmune Encephalomyelitis (EAE) in c-rel−/− mice [82]. The c-Rel subunit also plays critical T cell-intrinsic roles during Th1 cell differentiation. When cultured under Th1 polarizing conditions, c-Rel deficient CD4+ T cells show reduced IFN-γ production [83] but normal Tbet expression [82]. In c-rel−/− mice, this defect in the expansion of IFN-γ+ Th1 cells results in enhanced susceptibility to T. gondii infection [86]. Interestingly, a role for c-Rel has recently been described in promoting the polarization of Th17 cells [104]. A number of in vitro studies have suggested a role of NFκB in this particular subset. Interestingly, a role for c-Rel has recently been described in promoting the production of IL-21 by CD4+ T lymphocytes [107], a cytokine which is critical for the expansion and differentiation of Th17 cells [108,109]. As a result, c-rel−/− mice display a defect in Tfh cell development, which is rescued by the administration of exogenous IL-21 [107]. A T cell extrinsic role for the alternate NFκB signalling pathway in Tfh cell differentiation has also been suggested, with NFκB found to regulate the expression of ICOSL in B cells [110]. While Bc6, a viral transcription factor for the differentiation and function of Tfh cells has been shown to act as a direct inhibitor of NFκB1 expression [111]. Considering the emerging association of Tfh cells with autoimmune disease [106], it will be important to further define the role of NFκB in the development and function of this cell subset.

To date, our understanding of NFκB activation during T cell polarization has been complicated by the dual roles of NFκB in mediating both T cell intrinsic and extrinsic processes. Future studies examining mice with T cell specific deletions of NFκB proteins will shed further light on the role of NFκB in regulating T helper cell differentiation. Findings to date suggest that both the classical and alternate pathways of NFκB signalling are important in regulating T cell polarization. However, the classical pathway may be the primary regulator of T cell intrinsic mechanisms, including the direct regulation of critical transcription factors for polarization such as GATA3 [99] and RORγt [103]. Furthermore, little is currently known about the role of NFκB in the more recently identified T helper subsets, the Th17 and Tfh cells. The association of these subsets with human disease and their status as potential therapeutic targets makes this an important area for future research.

**T cell malignancies**

The first suggestion of a link between NFκB and oncogenesis was made with the cloning of nfκb1 in 1990 [112,113]. These initial studies revealed sequence homology with v-Rel, a viral oncogene known to cause aggressive lymphoid malignancies [113]. There are a number of ways in which dysregulated NFκB signalling can contribute to oncogenesis. In the face of excessive or inappropriate activation, the many growth promoting and anti-apoptotic target genes of NFκB can aid the growth and survival of malignant cells [114]. Furthermore, NFκB regulates the expression of adhesion molecules, chemokine receptors, and matrix metalloproteinases; all of which are implicated in the invasion and metastasis of tumour cells [114,115]. Importantly, NFκB signalling is well recognized as a critical link between inflammation and tumour development [3]. Up to 20% of all human malignancies are believed to be the result of this process, wherein chronic inflammation leads to the neoplastic transformation of cells [3,116].
Activating mutations in NFκB subunits occur only rarely in human T cell malignancies however [117], the most common of these being genetic alterations in c-Rel, the cellular counterpart to v-Rel [118]. Amplification of the c-rel gene, which correlates with elevated nuclear c-Rel protein [119], has been identified in a small number of T cell malignancies, in particular T cell lymphomas [118-120]. While mutation of the NFκB2 gene has been identified in approximately 2% of T cell malignancies including Acute Lymphocytic Leukemia (ALL) and cutaneous T cell lymphomas [117]. The majority of these rearrangements involve deletion of the inhibitory C terminal region of p100, producing a truncated protein that confers constitutive p52 activity [121]. Genetic alteration of the RELA, Rel-b or NFκB1 subunits has been identified only in sporadic cases of ALL and NHL.

A much greater proportion of T cell malignancies have been found to display constitutive or enhanced NFκB activation, without genetic alteration [122-124]. This is believed to occur indirectly, as a result of aberrant upstream signalling or a loss of regulatory mechanisms [125]. Studies have identified an important role for constitutive NFκB signalling in promoting the growth, survival and tumourigenicity of many malignant cell types [124,126-128]. Numerous studies have also begun to investigate the types and frequency of NFκB polymorphisms, and evaluate the association with tumourigenesis [129,130]. Although this association remains unclear [130], and the full implications of NFκB polymorphisms in tumourigenesis are yet to be determined, these studies may provide a means for tailored therapies and diagnostics in the future [129,130].

Complex Interactions with Other Signalling Pathways

Recent years have seen our understanding of intracellular signalling grow; from a series of defined pathways to a complex signalling network. It has become clear that signalling ‘crosstalk’ is a critical feature of T cell activation, which allows the integration of multiple stimuli and the precise modulation of intracellular responses. Studies have begun to identify the points at which parallel signalling pathways converge with NFκB. Of these, the most thoroughly investigated is the IKK complex. Once believed to be highly specific for the IKκB proteins, it has since become clear that the IKK complex also targets upstream components of NFκB as well as a number of unrelated pathways.

IKKa

IKKa plays an important role in phosphorylating non-NFκB/IκB substrates, and the IKKa subunit itself has a NLS. As a result, many of IKKa’s non-NFκB targets are nuclear, including histone H3 [131] and other proteins involved in cell growth and metastasis [132,133]. One potential target of IKKa with clear implications for T cell function is IL-17A [134]. It was recently found that during differentiation of Th17 cells, IKKa associated with the Il17a locus to promote transcription and commitment to the Th17 cell lineage [134]. However the identity of nuclear targets and the mechanisms of regulation by IKKa are still poorly defined and little is known about the specific cell types and contexts in which they operate.

The IKKa subunit is also implicated in the extensive crosstalk between NFκB and Notch signalling. The reciprocal regulation of these two pathways involves a complex series of interactions [135]. Stimulation of T cell lines with Notch ligand activates NFκB by promoting the direct interaction of Notch1 with IKKa [136]. In primary murine T cells, direct binding has been demonstrated between Notch1 and p50/c-Rel complexes [137]. Though our understanding of this crosstalk is limited, the activation of NFκB by the Notch pathways has important implications for T cell acute lymphoblastic leukemias (T-ALL), where Notch signalling is commonly activated [138]. Furthermore, crosstalk between IKKa and Notch1 has recently been identified as a critical pathway for inflammatory-mediated tumourigenesis [139].

A further example, which has been a topic of debate for some time, is the crosstalk between NFκB and the tumour-suppressor protein p53. The NFκB and p53 transcription factors are activated in response to many of the same stimuli and present a critical crossroads for cell fate, determining whether the outcome of stimulation will be proliferation or cell death. Competition for binding to the transactivator CBP has long been believed to mediate crosstalk between these two transcription factor pathways [140]. This competition may be regulated by IKKa, which can phosphorylate CBP to induce preferential binding with NFκB over p53, thus enhancing NFκB- and repressing p53-dependent transcription [141]. Another mechanism linking the alternate NFκB pathway and p53 has also been suggested, via direct regulation of p53 transcriptional activity by NFκB2 (p52). The p53 transcription factor may recruit p52 to its target regions, where in turn p52 can either repress or activate p53 [142]. However, more recent studies examining the response of primary murine thymocytes to a range of stress-induced stimuli have failed to demonstrate functional crosstalk between NFκB and p53 [143]. These conflicting findings highlight the difficulties associated with the study of signalling crosstalk. The effect of a given intervention will inherently be obscured by defects in the central signalling pathway, and thus in many cases clear biological relevance of signalling crosstalk is yet to be demonstrated.

These examples are only some of the pathways in which non-NFκB functions for IKKa have been described. Overall, it is clear that the physiological implications of signalling crosstalk with IKKa in a cell type and context specific view are yet to be fully realized. Certainly it may hold implications for various malignancies, where cell fate decisions are critical for oncogenic transformation and progression. Moreover, it will be intriguing to determine how the link between IKKa and Notch signalling impacts on key aspects of normal T cell development and function in vivo, in particular those processes regulated via the alternate NFκB signalling pathway.

IKKβ and Tpl2

Perhaps the best example of signalling crosstalk with the NFκB pathway is the complex relationship between NFκB and Tpl2. Tpl-2 is a Mitogen-Activated Protein Kinase Kinase Kinase (MAP3K), which activates ERK in response to immune and inflammatory signals [144]. In the steady state Tpl2 forms a stable and inactive complex with p105, which is required to prevent the degradation of Tpl2 [145]. Consequently, the levels of Tpl2 found in nfkB1−/− (p105 deficient) cells are severely reduced [145]. Moreover, binding with p105 prevents substrate access to the kinase domain of Tpl2, allowing p105 to function as an inhibitor of MEK kinase activity [146]. An important consequence of IKKβ activation is therefore the release of Tpl2. IKKβ can induce proteolysis of p105, which leads to the release and activation of Tpl2, making IKKβ and p105 crucial regulators of both NFκB and MAPK signalling. Tpl2 is not the sole link between these pathways, as IKKβ can also phosphorylate Dok1, a negative regulator of cell growth, which leads to the inhibition of ERK activation [147]. These opposing effects of IKK activation on the MAPK pathway clearly highlight the stringent regulation required for ERK activation, and the context-dependent nature of signalling crosstalk with NFκB.

With regard to T cell activation, the over-expression of Tpl2 was initially found to induce the production of IL-2 in Jurkat T cells.
This induction is suggested to involve activation of the alternate NFκB pathway via the MAP3K NIK. Similar studies also highlight the possibility thatTpl2 may regulate NFκB signalling by inducing p105 proteolysis [149] and/or by modulating the transactivating potential of RELA [150]. Considering the limitations of over-expression systems, delineating the physiological relevance of NFκB regulation byTpl2 is crucial. In response to TCR stimulation in vitro, CD4+ T cells deficient inTpl2 have been shown to produce normal levels of IL-2 [96,151]. No overt phenotypes have been observed in the tpl2−/− mice unless challenged by antigen [151]. In particular, T cell development in the thymus of these mutants is not impaired [151], which may reflect a redundant role forTpl2 within the ERK signalling pathway, as the importance of ERK1/2 [152] and the fellow MAP3K Raf [153] in thymocyte development and selection are both well recognized.

The study ofTpl2 deficient mice has suggested a critical role forTpl2 in the differentiation of Th1 cells. The expression ofTpl2 mRNA is highest in Th1, as compared with Th2-polarized cells [154], while tpl2−/− CD4+ T cells cultured under Th1 polarizing conditions show reduced IFN-γ production [154], and display marginally reduced Tbet and STAT4 expression in response to TCR stimulation [155]. Furthermore, resistance to the intracutaneous parasite Toxoplasma gondii, which is critically dependent on the optimal production of IFN-γ, is impaired in tpl2−/− mice [154]. The investigation ofTpl2 function in vivo is complicated by the study of an independently generated and phenotypically distinctTpl2-deficient mouse model that showed a heavily skewed Th1 response against Leishmania major infection [156]. To date these discordant results are yet to be resolved and may reflect numerous factors including the background strain, infection model, and method of generation. Furthermore, the role ofTpl2 in the differentiation of other T helper subsets including the Th17, Th1, and Treg cells remains to be determined conclusively.

Like the NFκB proteins,Tpl2 has been identified as a potent experimental oncogene [157]. Early studies described thetpl2 gene as a target of provirus insertion in retrovirus MoMuLV-induced rodent T cell lymphoma [158]. In such cases, viral insertion in the last intron oftpl2 results in a C-terminal truncation of theTpl2 protein. Similarly truncated forms ofTpl2 were subsequently linked with large cell lymphoblastic T cell lymphomas in mice [159]. Intriguingly however, truncated forms ofTpl2 were subsequently linked with large cell T cell lymphoma [158]. In such cases, viral insertion in the last intron ofTpl2 has been shown to produce normal levels of IL-2 [96,151]. No overt phenotypes have been observed in the tpl2−/− mice unless challenged by antigen [151]. In particular, T cell development in the thymus of these mutants is not impaired [151], which may reflect a redundant role forTpl2 within the ERK signalling pathway, as the importance of ERK1/2 [152] and the fellow MAP3K Raf [153] in thymocyte development and selection are both well recognized.

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Despite these murine studies, the screening of human malignancies has to date revealed relatively few cases ofTpl2 mutations, with the exception of a small number of large granular T cell neoplasias [161]. Nonetheless a role for altered expression and/or function ofTpl2 in human malignancies, mediated by factors including inflammation andNFκB activation, cannot be discounted. A detailed examination ofTpl2 expression and function in human disease has not yet been conducted, though there is evidence that tpl2 mRNA levels may be elevated in some forms of malignancy and reduced in others [162].

Importantly, the consequences of linking NFκB and MAPK activation remain largely unknown, and infinitely complex. It is important to note that studies involving the manipulation ofNFκB pathways, in particular the IKKβ/p105 components must be examined with the knowledge thatTpl2 and MAPK activation are likely altered. Furthermore, a key question remains regarding whetherTpl2 functions physiologically only in the regulation ofMAPK signalling, or if it also phosphorylates other proteins and thus other signalling pathways, including NFκB [150]. Taken together, the examples discussed herein highlight a means through which the IKK complex may influence immune function and oncogenesis, independently ofNFκB, by mediating crosstalk with other signalling pathways. And conversely, suggests the possibility that modulation ofNFκB by unrelated signalling pathways may be a critical factor in certain cell types and contexts. Teasing apart such complex interactions, and determining how they function both in physiological immune processes and in the context of disease is a difficult task. What is clear, however, is that determining the functional implications of crosstalk with the NFκB pathways, and identifying the full complement ofIKK targets is an important research goal, particularly in light of the ongoing efforts to develop targeted therapeutics.

**Perspectives**

The complex nature of NFκB signalling presents significant challenges for studying the role of this transcription factor in T lymphocytes. These challenges include the subunit redundancy of individual family members, and the concurrent functions of NFκB in APCs and other cell types, which influence T cell development and function. However, the application of emerging technologies and newer mouse models should help to overcome many of these issues, and provide answers to some of the outstanding questions. The role of NFκB in the development of non-conventional T cell lineages remains unclear, in particular regarding the development ofmemory-like CD8+ T cells. Likewise, our understanding of NFκB function in the differentiation and function of peripheral T helper subsets including the Th17 and Treg cells is limited. Furthermore, there is still much to be learnt about the crosstalk mechanisms that operate between NFκB and other intracellular signalling pathways, and how signalling crosstalk functions physiologically.

Nonetheless, significant insights have been gained over the years into the role of NFκB during various stages of T cell development, and in the function of multiple T cell lineages. It is abundantly clear that NFκB plays vital roles in many physiological processes, and that the consequences of dysregulated NFκB signalling are many and varied. It is for this reason that NFκB remains an attractive therapeutic target. However, its critical role in immune function is also the biggest obstacle facing NFκB-targeted therapies. The inhibition of upstream NFκB components, such as the IKK complex, can have severe and detrimental effects on immune function [163]. To minimize these risks, future research will seek to develop selective inhibitors ofNFκB subunits, as well as methods ofmodulating NFκB function within specific cell types or contexts. A further goal will be to target onlyNFκB-specific functions, while leaving essential mechanisms ofsignalling crosstalk with other intracellular pathways intact. Though a challenging task, the importance ofNFκB in T cell development and function ensures the endeavor to understand it will be worthwhile.

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**References**


