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Review

Regulation of the Immune System by NF-κB and IκB

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NF-κB/Rel transcription factor family participates in diverse biological processes including embryo development, hematopoiesis, immune regulation, as well as neuronal functions. In this review, the NF-κB/Rel signal transduction pathways and their important roles in the regulation of immune system will be discussed. NF-κB/Rel members execute distinct functions in multiple immune cell types via the regulation of target genes essential for cell proliferation, survival, effector functions, cell trafficking and communication, as well as the formation of lymphoid architecture. Consequently, proper activation of NF-κB/Rel during immune responses to allergens, auto-antigens, allo-antigens, and pathogenic infection is crucial for the integrity of host innate and adaptive immunity.

Keywords: IκB, NF-κB

NF-κB/Rel and IκB Signal Transduction Pathways

The discovery of the nuclear factor NF-κB that binds to the κB site of the immunoglobulin kappa light chain gene enhancer in 1986 has since revolutionized the biological aspects of transcriptional regulation (Sen and Baltimore, 1986). NF-κB has since become one of the most widely studied transcription factors with regard to its complex signal transduction pathways (see review (Karin and Delhase, 2000; Ghosh and Karin, 2002)), role in hematopoiesis (Grossmann *et al*., 1999), innate and adaptive immunity, as well as potential involvement in neuro-degeneration. In mammals, there are five members identified thus far for the NF-κB/Rel family: p50 (NF-κB1), p52 (NF-κB2), p65 (RelA), c-Rel, and Rel-B. These members can form 5 kinds of homodimers and at least 7 preferential hetero-dimers. While p50/p65 and their corresponding homodimers are widely expressed in most cells, the c-Rel containing complexes (e.g. p50/c-Rel and the c-Rel homodimers) are predominantly expressed in cells of the hematopoietic lineage (Liou *et al*., 1994). By contrast, RelB preferentially complexes with p52 (or p100) and the p52/RelB complexes have unique roles in splenic and thymic architecture (see below). It has yet to determine the NF-κB/Rel composition in different cell types and which NF-κB/Rel complexes are activated by a given receptor signaling pathway (see below).

In resting cells, NF-κB/Rel dimers are retained in the cytoplasm by complex with either IκBs or with the precursors, p105 and p100 (Liou *et al*., 1992; Naumann *et al*., 1993; Scheinman *et al*., 1993). These various complexes respond differently to a variety of receptor signals (see below). The IκBs contains five members, IκBα, IκBβ, IκBγ, IκBε, and Bcl-3. Except for Bcl-3 that functions as co-activators (Franzoso *et al*., 1992; Fujita *et al*., 1993; Nolan *et al*., 1993), the other IκBs mainly serve inhibitory functions. IκBα contains a nuclear export sequence (NES) and can shuttle NF-κB/Rel complexes in and out of the nucleus (Krappmann and Scheidereit, 1997; Phelps *et al*., 2000; Tam *et al*., 2000, 2001). By contrast, IκBβ does not contain NES and retains NF-κB/Rel complexes exclusively in the cytoplasm via masking both nuclear localization signals (NLS) of the dimers (Malek *et al*., 2001; Tam and Sen, 2001). Hence, distinct NF-κB/Rel complexes would be activated differently depending on whether it associates with IκBα or IκBβ and which IκB is subjected to phosphorylation and degradation by the particular receptor.

Many receptors are capable of transducing signals for NF-κB/Rel activation. Despite signaling components utilized by different receptors may vary, all signaling pathways eventually converge to the activation of IKK complexes, the central regulators of NF-κB/Rel (Karin and Delhase, 2000; Ghosh and Karin, 2002). IKK activation can lead to IκB phosphorylation and degradation by the ubiquitin-proteosome system (Ben-Neriah, 2002). Using gene-targeted deletion of IKK components, it is evident that the IKKβ complexes of one beta, one alpha, and two gamma subunits, are primarily responsible for the activation of canonical NF-κB/Rel complexes (e.g. p50, p65, c-Rel) (Zandi *et al*., 1998; Li *et al*., 1999). By contrast, a distinct IKKα containing complex exhibits a unique function for p100 processing and the

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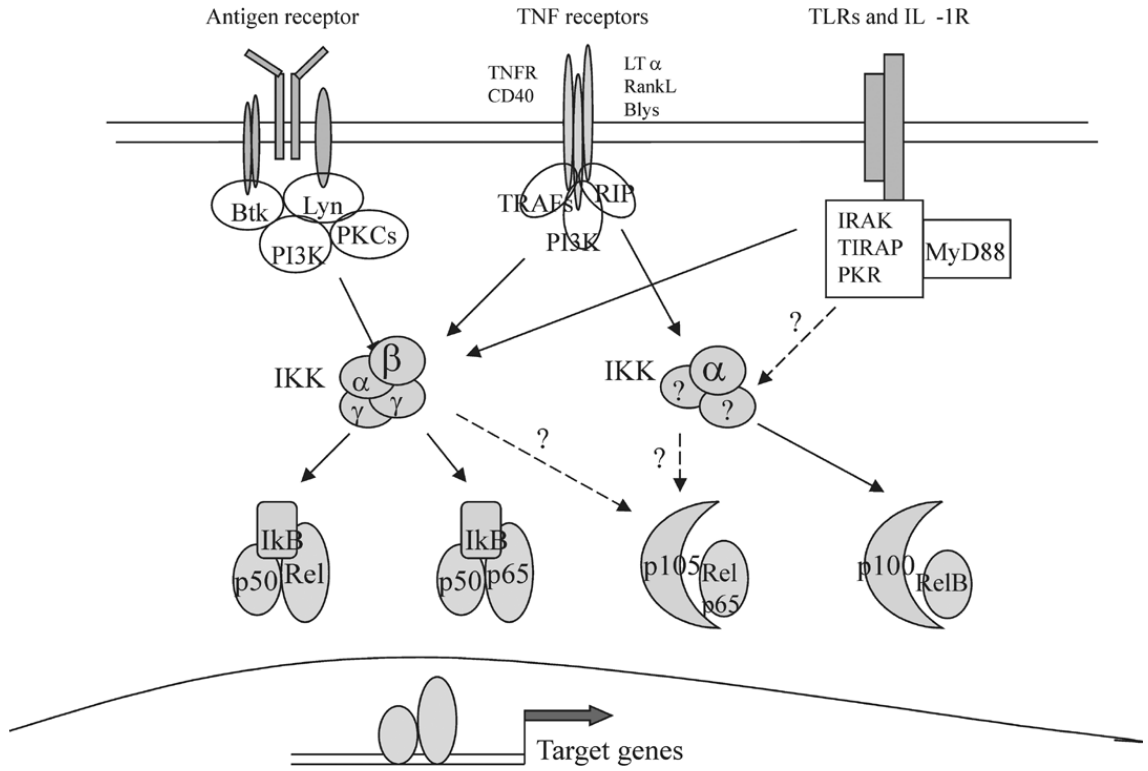

Fig. 1. Distinct receptors and NF-κB/Rel signaling pathways. Summarized here are three classes of receptors that can trigger NF-κB/Rel activation in a variety of immune cell types. Although they each utilize different adaptors, the converging point is the recruitment of IKK complexes to the receptor membrane proximal region, leading to IKK kinase activation. Conventional NF-κB/Rel activation pathway involves the IKK-β containing complexes, which mediates IκB degradation and release of p50/p65, and p50/c-Rel dimers. The alternative pathway is controlled by IKK-α containing complex that has unique role in p100 processing and generation of p52/RelB dimer. It remains unclear whether p105 processing is constitutive or subjected to signaling regulation. In addition, the mechanisms responsible for differential phosphorylation and degradation of IκB-α and IκB-β by distinct receptor signals have yet to be defined.

formation of p52/RelB complex (Senftleben *et al*., 2001).

One of the challenging issues of the field is to understand how different receptor signals engage various IKK and NF-κB/Rel complexes. Here, we summarize the current views of several receptors signaling pathways that are crucial for the regulation of immune responses (Fig. 1).

Antigen receptors Antigen receptors on lymphocytes (TCR and BCR) represent one of the most important receptor signals for the initiation of an immune response. The antigen receptor signals are required for positive selection during early lymphocyte development, for clonal expansion and terminal differentiation of mature lymphocytes upon encountering specific antigens, as well as for negative selection of self-reactive lymphocytes.

In mature lymphocytes, TCR and BCR can trigger several signaling pathways that involve the activation of tyrosine kinases (e.g. Lyn, Syk, Btk), Ca^{+2} influx, production of phospho-inositol derivatives, activation of MAP kinases, and nuclear translocation of several transcription factors (Benschop and Cambier, 1999; Monroe, 2000; Yankee and Clark, 2000). Recent studies on transgenic and knockout mice have helped delineate the signaling pathways for NF-κB/Rel activation. PI3 kinase is central to NF-κB/Rel activation as lymphocytes derived from PI3 kinase knockout mice are unable to activate NF-κB/Rel (Fruman *et al*., 1999; Suzuki *et al*., 1999). Furthermore, the PI3K-NF-κB/Rel pathway is responsible for BCR, CD40, and LPS induced proliferation (Andjelic *et al*., 2000). It is still unknown whether PI3K directly associate with IKK complex or indirectly through Akt or Btk, as Btk deficient B lymphocytes are also defective in NF-κB/Rel activation (Solvason *et al*., 1998; Craxton *et al*., 1999; Marshall *et al*., 2000).

Recently, several PKCs have been implicated in directing NF-κB/Rel activation in response to antigenic signals (Krappmann *et al*., 2001). PKC-θ deficient mature T cells are unable to activate NF-κB/Rel by TCR signals (Sun *et al*., 2000; Villalba *et al*., 2002). PKC-β (-/-) B cells fail to recruit the IKK complex into lipid raft upon BCR ligation (Su *et al*., 2002). In a similar manner, loss of PKC-ζ impairs BCR-induced NF-κB/Rel activation and target gene expression, as well as inhibits cell proliferation and survival (Martin *et al*., 2002). In contrast to the proliferative response of mature B cells, antigenic stimulation of immature B cells leads to

growth arrest and apoptosis (Monroe, 2000). The unresponsive nature of immature B cells has been attributed to their inability to recruit PKC and BCR to the lipid rafts (King *et al*., 1999; Sproul *et al*., 2000; Cheng *et al*., 2001). It has yet to be determined whether PKC is directly involved in IKK activation and which PKC isoforms are selectively suppressed in immature and anergic B lymphocytes. Also, the relationship between PI3K, Btk, and PKCs on the activation of NF-κB/Rel pathway remains to be established.

TNF receptor family Thus far, most of the TNF receptor family members are shown to activate NF-κB/Rel signaling pathway via TRAFs (Fig. 1). TRAFs may help recruit IKK complexes to the proximity of the receptor signaling components and allow auto-phosphorylation or phosphorylation by associated kinases, such as RIP (Devin *et al*., 2001). TNFR1, TNFR2, and CD40 activate NF-κB/Rel via the IKKβ containing complex. In addition to the conventional NF-κB/Rel activation pathway, several TNF receptor members, including LTα, Blys, and RankL, are able to engage the so-called "alternative pathway" that involves the activation of the IKKα containing complex (Ghosh and Karin, 2002). Activation of this IKK complex leads to the processing of p100/RelB into p52/RelB complexes. This has been supported by the observation that IKKα chimeric mice display defective splenic architecture and absence of follicular dendritic cells, similar to the p100 and RelB knockout mice (Senftleben *et al*., 2001).

Collective studies thus suggest that different receptors are wired to activate different IKK complexes through association with unique adaptor molecules that each receptor has affinity for. It will be important to sort out whether different IKK complexes exist in cells and whether they are coupled with distinct IκB-p50/p65 (and IκB-p50/c-Rel) or p100/RelB complexes as they are apparently regulated differently.

Toll-like receptor family The expanding Toll-like receptor (TLR) family members represent the first line of host defense mechanism against invading pathogens via the recognition of pathogen-associated molecular patterns, including LPS, lipoproteins, peptidoglycan, dsRNA, unmethylated DNA with CpG motif (Akira *et al*., 2001; Beutler, 2002). TLRs and IL-1 receptor trigger similar signaling pathways by utilizing signaling molecules, including IRAK, MyD88 (Fig. 1). IRAK is essential for NF-κB/Rel activation by IL-1R and TLR2/6, whereas MyD88 is essential for TLR3 and TLR9 mediated IKK activation (Wagner, 2002). LPS is recognized by TLR4 that mediates IKK activation via TIRAP and PKR (Beutler *et al*., 2001). Lymphocytes and dendritic cells deficient in several NF-κB/Rel components are no longer responsive to LPS/ TLR-4 mediated activation signals. It has yet to determine whether some of the TLR signaling pathways also activate the alternative NF-κB/Rel pathway through the IKKα containing complex.

Proliferation, Survival, and Cellular Responses Mediated by NF-κB/Rel

NF-κB/Rel activation by the aforementioned receptors leads to its nuclear translocation and induction of target genes. The growing list of NF-κB/Rel target genes include cytokines, chemokines, cytokine/chemokine receptors, adhesion molecules, survival genes, cell cycle regulators, acute phase proteins, and inducible effector enzymes. It is important to note that, due to differential tissue expression pattern and DNA binding specificity, each NF-κB/Rel member induces distinct profiles of target gene expression. This is evident from the studies on NF-κB/Rel knockout mice that manifest distinct phenotypes, as summarized below.

Cytokines and immune receptors Cytokines and their corresponding receptors are key mediators of the immune system that are crucial for immune cell communication and effector functions during an active immune response. For example, T helper (Th) cells produce cytokines, such as IL-2, to facilitate the clonal expansion and differentiation of both T helper cells and cytotoxic T lymphocytes (CTL) (Smith, 1986). Cytokines, such as TNF-β and γ-IFN, produced by Th1 cells can lead to activation and maturation of CTLs and macrophages that are specialized for cellular immunity (Glimcher, 2001). By comparison, cytokines produced by Th2 cells, such as IL-4, IL-5, IL-6, can facilitate B cell mediated humoral immunity.

NF-κB/Rel plays key roles in regulating the expression of many cytokine genes. Studies on c-Rel deficient mice have demonstrated that c-Rel is essential for IL-2, IL-3, GM-CSF, γ-IFN expression in T lymphocytes, IL-6 expression in B cells, TNF-α expression in macrophages, and IL-12 expression in dendritic cells (Gerondakis *et al*., 1996; Liou *et al*., 1999; Sanjabi *et al*., 2000; Weinmann *et al*., 2001) (Tumang, J. *et al*., *Cell Immunol*. In press). IL-2 receptor alpha chain is also under the regulation by c-Rel (Tumang *et al*., 1998). It remains to be determined which c-Rel dimers (homo- or hetero-) bind to the promoters of these cytokine genes and why, in some cases, other NF-κB/Rel members fail to compensate for the function of c-Rel.

Using IκBα and p65(-/-) cells, it has been reported that NF-κB/Rel is also involved in the regulation of MHC class I, CD40, CD86 expression (Beg *et al*., 1995a, 1995b; Hinz *et al*., 2002), confirming its role in enhancing interaction between T cells and antigen presenting cells during an immune response.

Survival and apoptotic proteins The role of NF-κB/Rel in cell survival was first demonstrated by the report that inhibition of NF-κB/Rel activity led to decreased expression of TRAF1, TRAF2, c-IAP-1, cIAP-2, X-IAP, and subsequent apoptosis of the tumor cell lines (Wang *et al*., 1998). Further studies on p65 (RelA) knockout mice indicated that deleted of p65 gene in

mice led to death at embryonic stage due to TNF-mediated toxicity of hepatocytes (Beg and Baltimore, 1996). The embryonic lethality can be prevented by crossing the p65(-/-) mice with TNF or TNF receptor knockout mice (Doi *et al*., 1999; Alcamo *et al*., 2001). These studies suggest that p65 is required for protecting liver cells from TNF induced apoptosis.

Subsequently, it has been reported that several survival genes are regulated by NF-κB/Rel. In B lymphocytes, blocking NF-κB/Rel impaired the expression of Bcl-X and Bfl-1 genes (Lee *et al*., 1999; Zong *et al*., 1999; Andjelic *et al*., 2000; Owyang *et al*., 2001). As a result, c-Rel(-/-) B cells are sensitized to several apoptotic stimuli, including gamma irradiation, dexamethasone, and antigenic signals (Owyang *et al*., 2001). Of interest however, c-Rel is dispensable for protection from TNFα and Fas mediated apoptosis. Thus, c-Rel may be specialized in protecting cells from mitochondrial apoptotic pathway through the induction of survival proteins of the Bcl-2 family. By contrast, other NF-κB/Rel members, such as p65, may protect cells from TNF or Fas mediated apoptosis through the induction of distinct anti-apoptotic molecules, such as c-FLIP, TRAFs, and IAPs.

Despite the prevailing data that support the anti-apoptotic roles for NF-κB/Rel, NF-κB/Rel members may play a role in apoptosis under certain circumstances. For example, it has been shown that p65(-/-) MEF cells have reduced Fas expression and are resistant to Fas-mediated apoptosis (Zheng *et al*., 2001), suggesting that p65 induces apoptosis via Fas induction. Studies on T cell lines also suggest that NF-κB/Rel participates in the expression of Fas and Fas ligand genes (Dudley *et al*., 1999; Hsu *et al*., 1999; Kasibhatla *et al*., 1999).

The role of NF-κB/Rel in neuron degeneration has also been investigated. Increased NF-κB/Rel levels are observed in dying neurons exposed to trauma and ischemia, as well as in brains of Alzheimer and Parkinson disease patients. Using mouse models of cerebral ischemia, it was found that deletion of p50 gene in mice alleviated the ischemic damage, suggesting that p50 may facilitate neuronal death in focal ischemia perhaps through TNF production (Botchkina *et al*., 1999; Schneider *et al*., 1999).

Our recent study revealed a dichotomy between p65 and c-Rel in neuronal survival (Pizzi *et al*., 2002). It has been established that glutamate can elicit neural toxicity, whereas IL-1β promotes neuronal survival. While glutamate induces nuclear translocation of p50 and p65, it fails to activate c-Rel. IL-1β, however, is able to activate the nuclear translocation of c-Rel, p50, and p65. Interestingly, c-Rel deletion (using c-Rel knockout mice) abolishes the survival effect of IL-1β, supporting c-Rel's function in conferring neuronal survival. In contrast, p65 was involved in glutamate-mediated cell death. Future studies are required to identify the survival and apoptosis genes in neurons induced c-Rel and p65, respectively.

Cell cycle regulators The role of NF-κB/Rel in cell proliferation is evident from the knockout mouse studies. P50 deficient B lymphocytes have specific impaired proliferation in response to LPS, but the response to anti-IgM and CD40 is normal (Sha *et al*., 1995; Snapper *et al*., 1996; Horwitz *et al*., 1999). By comparison, c-Rel and RelB deficient lymphocytes are unable to proliferate in response to antigenic and mitogenic stimuli (Snapper *et al*., 1996; Grumont *et al*., 1998; Tumang *et al*., 1998; Liou *et al*., 1999). The defect in c-Rel(-/-) T cell proliferation can be attributed to the lack of IL-2 production, as IL-2 is the key growth factor for T cells (Kontgen *et al*., 1995; Liou *et al*., 1999). While the cytokines responsible for B cell proliferation are not yet identified, studies on cell cycle proteins have indicated that c-Rel is necessary for promoting cell cycle progression. Specifically, c-Rel(-/-) B cells have impaired cyclin D3 and E expression, CDK kinase activation, Rb hyper-phosphorylation, and E2F expression (Hsia *et al*., 2002). However, the degradation of CDK inhibitors appears to be normal. This suggests that c-Rel mediates cell cycle progression through the induction of positive regulators, directly or indirectly.

In fibroblasts, myoblasts, and mammary carcinomas, NF-κB/Rel has been shown to regulate cyclin D1 gene transcription (Guttridge *et al*., 1999; Hinz *et al*., 1999). Furthermore, cyclin D2 expression was diminished in Hodgkin tumor cells by overexpressing IκB super-repressor (Hinz *et al*., 2001). It will be important to determine if NF-κB binding sites are functionally active in cyclin D2, D3, E and E2F promoters and which NF-κB/Rel dimers are responsible for the transcriptional regulation of these cell cycle genes.

Chemokines and receptors NF-κB/Rel also participates in leukocyte trafficking through the regulation of chemokines and receptors. For example, in an allergic inflammation model, p50(-/-) airway have reduced expression of eotaxin, MIP-1α, and MIP-1β (Yang *et al*., 1998), whereas c-Rel(-/-) lead to decreased MCP-1 expression (Donovan *et al*., 1999). The reduction of chemokine production may in part explain the absence of eosinophilia and pulmonary inflammation in these NF-κB/Rel knockout mice. Using trans-dominant IκBα mutant, it was shown that IκBα completely inhibited TNF-α mediated induction of eotaxin-1 and CCR3 (Huber *et al*., 2002).

RelB, however, may play a negative role in chemokine gene expression. Stimulation of RelB(-/-) fibroblasts *in vitro* with LPS led to persistent induction of several chemokines, implying a suppressive role of RelB that may be needed for resolving acute inflammation in tissues. This hypothesis has yet to be tested *in vivo*. P100 and RelB knockout mice exhibit impaired splenic and thymic micro-architecture due the defects in medullary epithelial cells, thymic dendritic cells, and follicular dendritic cells (see below). The expression of chemokine, BLC, by stromal cells is reduced, which is in part responsible for the inability to recruit B cells into follicles (Poljak *et al*., 1999).

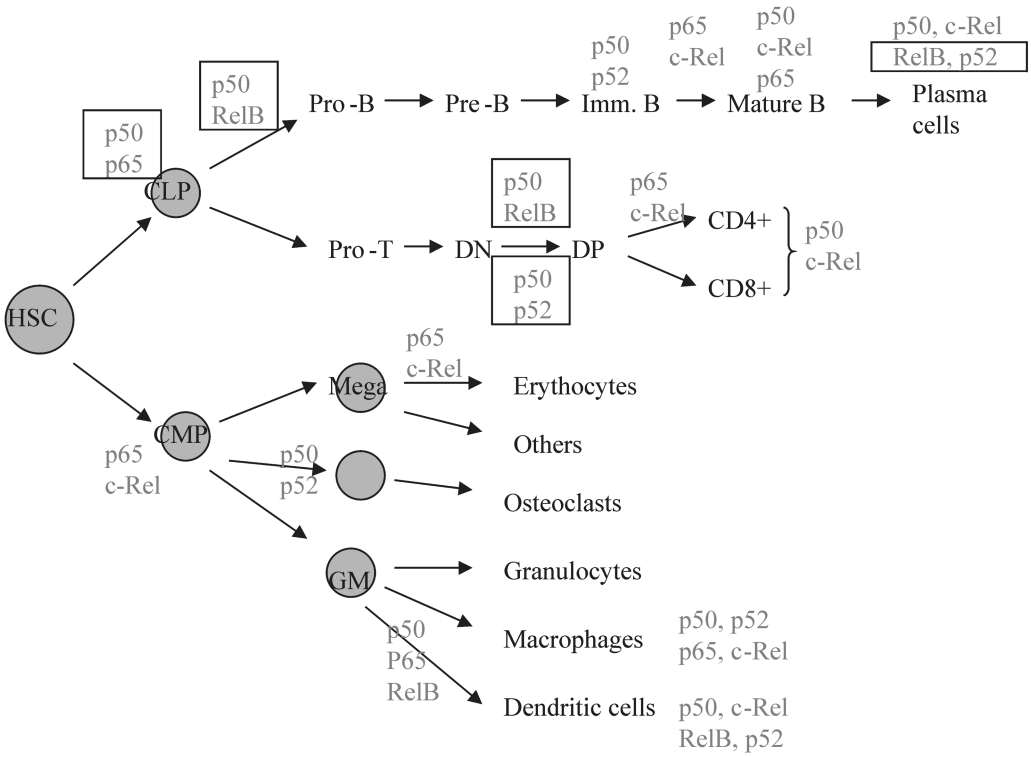

Fig. 2. Regulation of hematopoiesis by NF-κB/Rel members. Distinct NF-κB/Rel dimers are responsible for different stages of hematopoiesis as supported by studies on single and double knockout mice and adoptive transfer chimeras. NF-κB/Rel does not affect the potency of hematopoietic stem cells (HSC) to differentiate into common lymphoid progenitors (CLP) and common myeloid progenitors (CMP). However, p50/p65 is crucial for lymphopoiesis, whereas p65 and c-Rel complexes are complementary for myeloid development. C-Rel expression in lymphoid lineages occurs in later and more mature stages and is mainly responsible for mature lymphocyte activation, clonal expansion, and effector functions. It also plays an important role in the survival and antigen presentation capacity of myeloid cells (e.g. macrophages and dendritic cells). The boxed dimers (mostly containing p52 or RelB) denote that their roles in lymphopoiesis is mostly due to the extrinsic effects on stromal and dendritic cell network that are necessary for lymphoid development in bone marrow, thymus, and follicles in spleen.

Regulation of Hematopoiesis by NF-κB/Rel

The roles of NF-κB/Rel in hematopoiesis have been addressed using either single or double knockout mice and, in some cases, fetal liver cells were utilized for adoptive transfer experiments to overcome the lethality resulted from p65 deletion.

It appears that p50/p65 is crucial for the development of lymphoid lineages that cannot be compensated by other NF-κB/Rel members. In contrast, either p65 or c-Rel containing complexes can compensate for the development of myeloid lineages (Fig. 2). This is supported by the observation that p65(-/-) derived fetal liver cells have severe deficit in lymphopoiesis, but relatively normal development of myeloid and granulocyte lineage. By comparison, deletion of both p65 and c-Rel led to defect in lymphopoiesis, reduced colony-forming unit progenitors for myeloid cells, impaired erythropoiesis, and expansion of granulocytes. Here, I will first review the role of NF-κB/Rel in lymphoid lineage cells followed by their role in the development and function of myeloid cells.

Lymphopoiesis It has been demonstrated that the fetal liver cells derived from p50/p65 double knockout embryos are unable to generate T and B lymphocytes with a block at early precursor stages (Horwitz *et al.*, 1997). These defects, however, are not cell-intrinsic since co-transferring normal fetal liver cells can correct the deficiency. The studies suggest that p50 and p65 regulates the expression of an extracellular factor that is required for lymphopoiesis.

Deletion of both p50 and RelB genes in mice affects lymphoid lineage development, however, this may in part result from defective stromal environment. The p50/RelB double knockout mice have impaired development of B220+ cells in bone marrow, traced as early as pre-B or pro-B stage (Weih *et al.*, 1997). Consequently, there are very few detectable B220+ cells in bone marrow and spleen. For T cell lineage, these mice reveal thymic atrophy and lacking CD4+CD8+ DP thymocyte population. The deficiency may not be intrinsic to lymphoid progenitors. Instead, the phenotype is consistent with the role of RelB in dendritic and stromal cell populations residing in thymus, spleen, and bone marrow that are necessary for supporting lymphopoiesis (see

below).

Deletion of both p50 and p52 affects further maturation of committed progenitors of the lymphoid and osteoclast lineages (Iotsova *et al*., 1997; Franzoso *et al*., 1998). The B cell development is arrested in the discrete immature T1 stage (B220-lo, IgM+, IgD-, CD23-). Naturally, the double knockout mice are unable to form germinal centers in response to antigen challenge. Further experiments are awaited to determine whether the defect is intrinsic to immature B cells or to stromal cells that support the survival of immature B cells in bone marrow. The p50/p52 double knockout mice have no detectable peripheral T cells, however, the defect is not intrinsic to T cell development. Rather, it is due to impaired thymic and splenic architectures resulted from the lack of medullary epithelial cells (MEC) and non-functional thymic dendritic cells (DC). Thus, p50 and p52 containing complexes (which may include RelB) play major roles in the development, differentiation and trafficking of MECs and DCs.

Deletion of both p65 and c-Rel appears to only affect later developmental stages of lymphoid lineages, unlike their essential role in myeloid development (see below). It has been demonstrated that fetal liver cells derived from c-Rel/p65 double knockout mice are blocked at immature B cell stage (CD24-hi, IgM-hi, IgD-hi) that is similar to the defined T1 stage (Grossmann *et al*., 2000). The block is partly due to increased apoptosis in this stage of immature B cells. Overexpression of Bcl-2 can induce further maturation to IgM-lo, IgD-hi phenotype. Similarly, combined deletion of both c-Rel and p65 leads to decreased peripheral CD4+ and CD8+ T cell number but does not seem to affect T cell viability.

Combined deletion of p50 and c-Rel does not affect early hematopoiesis because the double knockout mice have normal levels of erythrocytes, granulocytes, macrophages, and T cells in bone marrow, thymus, and periphery (Pohl *et al*., 2002). Development to the mature B cell stage is normal, except with reduced marginal zone B cells and poor formation of germinal center upon immunization, which is evident in c-Rel single knockout mice (Tumang *et al*., 1998). Like the c-Rel singly knockout mice, the lymphocytes derived from p50/c-Rel double knockout mice have impaired proliferation and survival response to antigenic and mitogenic stimuli (Grumont *et al*., 1998). Together, these studies suggest that the role of p50 and c-Rel is primarily restricted to mature lymphocytes, especially for mediating antigen-induced clonal expansion, cytokine production, effector function, and terminal differentiation that are necessary for germinal center immune responses.

Myelopoiesis As mentioned earlier, deletion of p65 or c-Rel alone does not affect myelopoiesis, presumably due to compensation from the remaining NF-κB/Rel members. However, NF-κB/Rel does play a role in myeloid differentiation, as deletion of both p65 and c-Rel affects early common myeloid progenitors, leading to reduced colony-forming unit progenitors, impaired erythropoiesis, aberrant expansion of granulocytes, and macrophage apoptosis (Grossmann *et al*., 1999) (Fig. 2). These data suggest that p65 and c-Rel serve overlapping roles in regulating differentiation and survival of multiple committed progenitors without affecting the pluripotent stem cells.

As described earlier, p50 and p52 double deletion affects the maturation of committed progenitors of both lymphoid and osteoclast lineages. The most striking defect of the p50/p52 double knockout mice is their failure to generate mature osteoclasts (Iotsova *et al*., 1997; Franzoso *et al.*, 1998). As a result, the mice develop osteopetrosis. The double knockout macrophages also have defects in the production of IL-6 and GM-CSF, suggesting that p50/p52 is also required for macrophage activation.

The p52 and RelB (conjuction with Bcl-3) have a unique role in the formation of thymic and lymphoid microenvironment that is required for supporting lymphopoiesis and germinal center immune response. This is evident from the studies on p52, RelB, and Bcl-3 single knockout mice that they all have impaired B cell follicle formation and are unable to establish proper follicular dendritic cell (FDC) networks upon antigenic challenge (Burkly *et al.*, 1995; Caamano *et al.*, 1998; Franzoso *et al*., 1998; Poljak *et al.*, 1999). The defects in B cell follicles are in part attributed to impaired production of BLC chemokine by FDCs.

Further studies reveal that RelB is crucial for dendritic cell (DC) development (Burkly *et al*., 1995). RelB knockout mice have defective development of thymic medullary epithelial cells and dendritic cells. In addition, the development of myeloid-derived CD11C+CD8- dendritic cells is impaired in RelB deficient mice (Wu *et al*., 1998). Recent reports demonstrate that p50/p65 are required for proper development of both lymphoid and myeloid DCs (Ouaaz *et al.*, 2002). Collectively, these studies suggest that at least RelB, p50, and p65 are required for dendritic cell development. By contrast, loss of p50 and/or c-Rel does not affect DC development, but instead, affecting the maturation and survival of DCs, in part due to reduced Bcl-X expression. Our recent study demonstrate that the loss of c-Rel in DCs compromises their ability to stimulate both allogeneic and antigen-specific autologous T cell response, leading to reduce numbers of cell cycle and Th1/Th2 cytokine production (Boffa, D., Liou, H.-C. *et al*., manuscript submitted).

Conclusion Remark

Significant progress has been made in recent years about NF-κB/Rel signal transduction pathway and the roles of NF-κB/Rel dimers in cell proliferation, survival, and hemapotopiesis. However, fundamental issues regarding the selective activation of distinct NF-κB/Rel dimers by different receptors and unique target gene specificity of each NF-κB/Rel complex

remain to be addressed.

The importance of NF-κB/Rel in innate and adaptive immunity has been evident in various disease and infection models (see review (Caamano and Hunter, 2002)). Deletion of certain NF-κB/Rel components has been shown to compromise immune responses against T-dependent and T-independent antigens (Tumang *et al*., 1998; Cariappa *et al*., 2000), develop resistance to allergic pulmonary inflammation and autoimmunity (Donovan *et al*., 1999; Pahan and Schmid, 2000), and render tolerant to allograft transplantation (Yang *et al*., 2002) (Finn, P. *et al*., *J. Leuk. Biol.* In press). Thus, it is conceivable that manipulating NF-κB/Rel activity may provide potential therapeutic means for the treatment of infection, chronic inflammation, and autoimmune diseases, as well as the basis for tumor immunotherapy and tolerance induction during organ transplantation. The subject will be discussed further in a separate article (Liou, H-C, review in preparation, BioEssays).

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