Review

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Role of nuclear IκBs in inflammation regulation

Abstract: A wide variety of environmental cues, including inflammatory cytokines, ligands for pattern recognition receptors and endogenous danger signals, activate the inducible transcription factor nuclear factor-κB (NF-κB), which is a central regulator of inflammatory and immune responses. Excessive activation of NF-κB results in the development of severe diseases, such as chronic inflammatory disorders, autoimmune diseases and cancer. Therefore, the transcriptional activity of NF-κB is tightly regulated at multiple steps. One mechanism is mediated by the inhibitor of κB (IκB), a well-defined regulator of NF-κB that resides in the cytoplasm and prevents NF-κB from nuclear entry by sequestration. Recently, several atypical IκBs that reside in the nucleus were identified: Bcl-3, IκBζ, IκBNS and IκBη. In contrast to conventional IκBs, these atypical IκBs positively and negatively modulate NF-κB-mediated transcription. The function of atypical IκBs is independent of the prevention of NF-κB nuclear entry. Therefore, atypical IκBs are considered distinct from conventional IκBs and have been termed 'nuclear IκBs.' In addition to these members, our recent study indicated that IκBL, originally reported as a susceptibility gene for rheumatoid arthritis, also serves as a nuclear IκB. Biological and genetic studies strongly suggest that nuclear IκBs play important roles in the pathogenesis of inflammatory and autoimmune diseases via the regulation of both innate and adaptive immunity. In this review, we discuss the recent advances in our understanding of nuclear IκBs in the context of NF-κB-mediated transcriptional regulation and inflammatory responses.

Keywords: inflammatory cytokine; NF-κB; nuclear IκB; transcriptional regulation.

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Introduction

Nuclear factor-κB (NF-κB) plays important roles in various biological processes, such as development, immunity, inflammation and cancer. NF-κB activity is attributed to homodimers or heterodimers of the Rel transcription factor family: RelA (p65), RelB, c-Rel, p50 (p105, NF-κB1) and p52 (p100, NF-κB2) (1). Under homeostatic conditions, these dimers are sequestered in the cytoplasm as inactive complexes in association with inhibitors of κB (IκB) proteins. The IκB family of proteins, in which each protein contains multiple ankyrin repeat domains (ANKs), includes IκBα, IκBβ, IκBε and IκBδ (Figure 1) (2). Environmental stimuli elicit two distinct NF-κB pathways (3). In the canonical pathway, inflammatory cytokines and ligands for Toll-like receptors (TLRs), including lipopolysaccharide (LPS), induce phosphorylation and subsequent degradation of IκB proteins, such as IκBα, resulting in the release of RelA- or c-Rel-containing dimers, which were sequestered as an inactive complex. The released dimers move from the cytoplasm into the nucleus and activate target genes. The second NF-κB pathway is activated by a non-canonical (alternative) mechanism. The engagement of certain tumor necrosis factor superfamily receptors proteolytically induces processing of the RelB/p100 dimer into the RelB/p52 dimer, which then translocates into the nucleus and activates target genes. Both the canonical and non-canonical NF-κB pathways are largely involved in inflammation and lymphoid organogenesis, respectively, and are regulated by each other (4).

Several coordinately regulated genes that are activated by NF-κB upon inflammatory stimulation have been categorized into two groups (5). One group, which includes the *Tnfa* (TNFα) gene, is characterized by the rapid induction (within 1 h) of transcription and a CpG-rich region in its promoter. Another group is defined by delayed induction (after a few hours), which requires *de novo* protein synthesis and chromatin remodeling. This group of genes includes *IL-1β*, *IL-6* and *IL-12p40*. These rapid and delayed-induction genes are classified as primary and secondary response genes, respectively (5).

Once the inflammatory response is triggered, NF-κB promotes the transcription of inflammatory cytokines as

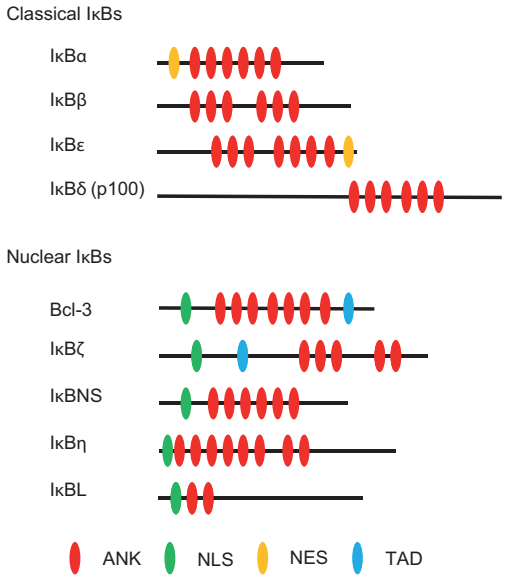

Figure 1 The IκB family.
Schematic representation of the IκB family of proteins. Ankyrin repeat domain (ANK), nuclear localization signal sequence (NLS), nuclear export signal sequence (NES) and transactivation domain (TAD) are shown as red, green, yellow and blue ovals, respectively.

well as their regulatory molecules that are involved in feedforward and feedback regulation of NF-κB activity (6). Recently, it has been reported that some unique IκB family proteins are induced by NF-κB. These proteins, including Bcl-3, IκBζ, IκBNS and IκBη, contain multiple ANKs with homology to those of classical IκBs. In contrast to the classical IκBs, which are located in the cytoplasm, these proteins reside in the nucleus, are dependent on their nuclear localization signal sequence (NLS) and are designated as 'nuclear IκB' proteins (Figure 1) (7, 8). Whereas classical IκBs bind to Rel family proteins within the cytoplasm and mask the NLS of Rel proteins to prevent their nuclear entry, nuclear IκBs bind to Rel family proteins in the nucleus and thereby modulate the transcriptional activity of NF-κB (2).

Recently, we identified a novel member of the nuclear IκB proteins, IκBL (9). IκBL was originally reported as a susceptibility gene for rheumatoid arthritis (10, 11). We found that IκBL prevents collagen-induced arthritis (CIA) via the regulation of inflammatory cytokine production in antigen-presenting cells (12). In contrast to other nuclear IκBs, IκBL is gradually induced upon LPS treatment and inhibits inflammatory cytokine transcription. Therefore, we assume that IκBL is distinct from the other previously reported nuclear IκBs in the context of inflammatory settings.

In this review, we highlight recent research on the nuclear IκBs in the regulation of inflammatory response and discuss their role in the pathogenesis of autoimmune and inflammatory disorders.

Bcl-3

B cell lymphoma-3 (Bcl-3) was originally identified as a putative proto-oncogene in chronic lymphocytic leukemia, and elevated expression of Bcl-3 has been detected in a wide variety of cancer types (13). Bcl-3 contains seven ANKs with similarities to those of the Rel family members p105 and p100 and preferentially associates with p50/p52 homodimers, which suggests that Bcl-3 acts as an IκB (14, 15). Unlike classical IκBs, Bcl-3 is mainly localized in the nucleus and has a well-defined transcriptional activation domain. The oncogenic function of Bcl-3 relies, at least in part, on the transcriptional activation of *cyclin D1* and *MDM2*, both of which are involved in cell cycle progression (16, 17). It is known that Bcl-3 is an inhibitor of NF-κB. NF-κB-mediated TNFα transcription is suppressed by the recruitment of Bcl-3, which forms complexes with p50 homodimers on the TNFα gene promoter (18). In addition, Bcl-3 prevents p50 ubiquitination and proteasomal degradation. In Bcl-3-deleted macrophages, RelA and c-Rel binding to the TNFα promoter is stable both in homeostatic and LPS-activated conditions (19). Indeed, mice that were injected with Bcl-3-deficient bone marrow cells died of severe septic shock following repetitive intraperitoneal injections of LPS, whereas control mice that received normal bone marrow cells did not (19). These results suggest that Bcl-3 plays key roles in the transcriptional control of *TNFα* throughout the inflammatory response.

The physiological functions of Bcl-3 in the immune system have also been examined using Bcl-3-deficient mice. The formation of germinal centers and splenic structures is severely impaired in Bcl-3-deficient mice (20, 21). Furthermore, Bcl-3-deficient mice fail to produce antigen-specific antibodies after infection with *Streptococcus pneumonia*, although total immunoglobulin levels are not altered (21). Expression of GATA-3, a transcription factor indispensable for Th2 cell differentiation, is decreased in Bcl-3-deficient T cells. These findings suggest that Bcl-3 may play a crucial role in the induction of the Th2-type response *in vivo* (22).

Elimination of self-reactive T cells in the thymus, which is known as negative selection, is important for establishing immunological tolerance to self (23). The generation and development of medullary thymic epithelial cells (mTECs) are considered central to this process. Loss of both Bcl-3 and its partner p52 results in impaired differentiation of mTEC and expression of AIRE, a crucial factor for ectopic

expression of tissue-restricted self-antigens in mTECs (24). Therefore, mutant animals show a profound breakdown of central tolerance and die earlier due to multi-organ inflammation (24). Bcl-3, expressed in cells of both hematopoietic and non-hematopoietic origin, is indispensable for control of the immune and inflammatory responses.

IκBζ

IκBζ (also known as MAIL and INAP) was first described in three independent investigations as an LPS-induced, nuclear-resident protein with high homology to Bcl-3 (25–27). IκBζ expression is rapidly induced by IL-1/TLR within 1 h in a MyD88-dependent manner; however, expression is not induced by TNFα signaling. The *IκBζ* promoter contains an NF-κB-binding element, which is crucial for its expression (28). It has been reported that IκBζ has 3 different isoforms: IκBζ(L), IκBζ(S) and IκBζ(D). IκBζ(D) lacks an N-terminal transactivation domain, and thus may act as a dominant negative form of IκBζ (29).

IκBζ-deficient cells have altered expression of a set of genes, especially secondary response genes. The expression of secondary response genes, such as *IL-6*, *IL-12p40* and *GM-CSF*, are severely impaired in IκBζ-deficient cells after exposure to LPS, although the expression of primary response genes, such as *TNFα* and *Cxcl2*, is not impaired, but in fact is slightly increased (30). IκBζ forms a complex with the p50 homodimer and is recruited to the promoters of secondary response genes. IκBζ has been shown to be required for the trimethylation of histone 3 Lys-4 (H3K4), a histone marker for the transcriptionally active state, and the subsequent recruitment of the pre-initiation complex containing RNA polII, TATA-binding protein (TBP) and RelA (31). However, the ATP-dependent nucleosome remodeling factor Brg1 is normally associated with promoters in the absence of IκBζ. Therefore, IκBζ acts independently of nucleosome remodeling through SWI/SNF family proteins such as Brg1. It should be noted that the binding sites for CCAAT/enhancer binding protein (C/EBP)-β and NF-κB are crucial for the transcriptional control of some genes by IκBζ (32).

IL-17A in combination with TNFα, but neither alone, induces expression of IκBζ (28). In contrast, it has been reported that IκBζ is regulated at the posttranscriptional and transcriptional levels (28). IL-17A stimulation leads to the stabilization and sustained expression of IκBζ mRNA after exposure to LPS (28). Therefore, it is assumed that IL-17A modulates IκBζ expression at the transcriptional and posttranscriptional levels. Recently, it has been shown that IκBζ is highly expressed in IL-17A-producing $CD4^+$ T cells (Th17 cells) and is indispensable for the differentiation and function of these cells (33). However, it is not clear whether the high level of IκBζ expression in Th17 cells reflects its mRNA stabilization via autocrine IL-17A stimulation or direct transcriptional activation by IL-17A.

To evaluate the physiological role of IκBζ, IκBζ-deficient mice were generated. The IκBζ-deficient mice grew normally after birth, but manifested atopic dermatitis-like lesions from 4–5 weeks of age and have higher concentrations of serum IgE (34). Elevated expression of TARC and eotaxin, both of which are known chemoattractants for Th2 cells and eosinophils, were observed in the skin of IκBζ-deficient mice. In contrast, myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis (EAE), a mouse model of human multiple sclerosis, is completely prevented by IκBζ deficiency (33). As Th17 cells play a central role in the pathogenesis of EAE (35), it is plausible that IκBζ is, at least in part, involved in the other Th17-mediated immunopathologies, such as rheumatoid arthritis and psoriasis.

IκBNS

IκBNS was originally identified as a protein that was expressed in T cells undergoing negative selection in the thymus (36). However, mice deficient for *Nfkbid*, which encodes IκBNS, exhibit no significant differences in the development of T cells, both in the thymus and the periphery, except for decreased IL-2 production and reduced proliferative capacity (37). The lower proliferative capacity of IκBNS-deficient T cells is rescued by exogenously supplied IL-2, which indicates that IκBNS modulates expansion of T cells through the regulation of IL-2 production (38). In contrast, developmental defects are observed in B1, germinal centers and marginal zone B cells of IκBNS-deficient mice (39). Furthermore, plasma cell differentiation is also impaired. The total amounts of IgM and IgG3 in the serum and T cell-dependent/-independent antigen-specific immunoglobulins are reduced in IκBNS-deficient mice. Delayed, but equivalent production of antigen-specific IgG1 was observed in IκBNS-deficient mice. However, normal IgG1 production is restored in the presence of WT T cells, which suggests that IκBNS expressed in T cells is required for optimal antigen-specific IgG1 production (39).

It has been shown that IκBNS plays important roles in both innate and acquired immunity. TLR ligands strongly and rapidly induce IκBNS mRNA expression in macrophages (38). Increased production of some secondary response genes, but no primary response genes, is

observed in IκBNS-deficient macrophages. IκBNS binds to the p50 subunit of NF-κB and to the stabilized p50 homodimer, which is a transcriptionally inactive dimer, on the IL-6 promoter (40). Prolonged binding of the RelA/p50 dimer to the promoter is observed in IκBNS-deficient macrophages (38). Thus, IκBNS is a transcriptional repressor of delayed-expressing cytokines.

It was reported that the anti-inflammatory cytokine IL-10, as well as LPS, induces IκBNS and that IκBNS is crucial for the prevention of LPS-induced endotoxin shock. IκBNS is constitutively expressed in colonic macrophages, which are constantly exposed to microbiota, which suggests that IκBNS plays a role in regulating the mucosal immune system. Indeed, IκBNS-deficient mice exhibit higher sensitivity to DSS-induced colitis and increased IFNγ production in $CD4^+$ T cells (38). Furthermore, IκBNS deficiency leads to increased production of secondary response genes such as IL-12p40, which indicates that the loss of IκBNS promotes a Th1-skewed environment in the context of inflammation.

IκBη

Ankrd42 encodes an 8 ANK-containing protein with significant homology to IκBβ. The *Ankrd42* gene product resides in the nucleus and binds to the p50 subunit of NF-κB. A small interfering RNA (siRNA)-mediated knockdown of *Ankrd42* exhibited reduced expression of both primary and secondary response genes, such as *TNFα* and *IL-6*, respectively. Thus, the *Ankrd42* gene product is a nuclear IκB family member and is designated IκBη (8). IκBη is ubiquitously expressed in a wide variety of tissues, including immune cells. Although nuclear IκB is inflammation inducible, IκBη is only marginally induced by TLR agonists and is constitutively expressed at the basal level. It has been suggested that constitutive expression of IκBη enables it to control both primary and secondary response genes. As the silencing of IκBη in macrophages by siRNA does not affect the induction of IκBζ expression, IκBη may act independently of IκBζ in NF-κB-mediated gene activation.

IκBL

NFKBIL1, which encodes the inhibitor of κB-Like (IκBL), is located in the MHC class III region and was originally reported as a susceptibility gene for rheumatoid arthritis (10, 11). We found that forced expression of IκBL in mice reduced the severity of CIA and collagen antibody-induced arthritis via the regulation of antigen-presenting cell function (12). Given that SNPs within the *NFKBIL1* gene locus are also associated with chronic thromboembolic pulmonary hypertension, Takayasu's arteritis and type I diabetes among Japanese and ulcerative colitis, SLE and Sjogren's syndrome in Caucasians, IκBL may play a role in the pathogenesis of these disorders (10, 11, 41, 42).

In human cells, the *NFKBIL1* gene generates four different isoforms by alternative splicing that are designated IκBL-α(L), -α(S), -β(L) and -β(S). In rodents, only two isoforms, α(L) and α(S), are generated (9, 43). Two ANKs reside in the N-terminus of the IκBL protein and show significant homology to those of IκBs. Although an early study suggested that IκBL did not modulate NF-κB activity (44), we have shown that IκBL suppresses LPS-induced NF-κB-driven luciferase activity and reduces the expression of some inflammatory cytokines in macrophages. However, the truncation of the ANK in IκBL resulted in a failure to inhibit NF-κB activity. This evidence suggests that IκBL functions as an IκB. We and others have reported that IκBL is localized in the nucleus in an NLS-dependent manner (9, 43). An NLS-truncated form of IκBL as well as an ANK-truncated mutant had no suppressive effect on NF-κB-driven luciferase activity. These results strongly suggest that IκBL is a novel nuclear IκB (9). Intriguingly, we found that IκBL binds specificity to the RelB NF-κB subunit through its ANK domain, whereas all other nuclear IκB proteins have been shown to bind to the p50 subunit of NF-κB (our unpublished observation). As RelB was originally identified as a Rel-related protein that had an inhibitory effect on NF-κB-dependent gene activation, IκBL might be involved, coordinately with RelB, in the transcriptional repression of inflammatory cytokines (45). As seen in IκBη, IκBL is also expressed at the basal level, but is gradually induced after exposure to TLR ligands, such as LPS and CpG, for up to 24 h. Therefore, it is plausible that IκBL plays a role in the later phase as well as the acute phase of the inflammatory response. In agreement with the results of our studies, it was shown that IκBL inhibits both LPS-induced NF-κB and IRF activation via association with Cactin, which interacts with *Drosophia* cactus and is a mammalian ortholog IκBα (46). Taken together, these results suggest that IκBL plays a unique role in the suppression of NF-κB activity downstream of TLR signaling.

Roles of classical IκBs in the nucleus

Some recent evidence clearly indicates that classical IκBs, such as IκBα and IκBβ, also act in the nucleus as well as

in the cytoplasm. IκBα contains a nuclear export signal (NES) in its N-terminus and continuously shuttles Rel-containing dimers between the cytoplasm and nucleus via its NES (2). Targeted knock-in mice that harbored a mutation in the NES were generated and showed a reduced number of mature B cells and altered formation of secondary lymphoid tissue (47). These might be attributed to the reduction in c-Rel and p100 expression and impairment of both classical and alternative NF-κB activation. However, this does not clearly explain why nuclear export of IκBα is crucial for maintenance of constitutive and/or signal-induced NF-κB activation.

Like IκBα, IκBβ is also degraded and subsequently re-synthesized upon LPS stimulation. Newly synthesized IκBβ is hypophosphorylated and can be detected in the nucleus. Two recent studies have shown that *de novo* synthesized and poorly phosphorylated IκBβ forms a complex with the RelA/c-Rel dimer and facilitates recruitment of the complex to the promoters of genes such as *IL-1β*, *IL-6* and *TNFα*, but not to *Cxcl2* promoters (48, 49). IκBβ-deficient macrophages show rapid loss of IL-1β, IL-6 and TNFα mRNA and decreased production of these cytokines. Therefore, nuclear IκBβ associating with RelA/c-Rel dimers may prolong the expression of certain genes. IκBβ-deficient mice display delayed onset and decreased severity of CIA and a reduced amount of TNFα in serum (48). These data suggest that IκBβ plays dual roles in the inflammatory response, as a cytoplasmic inhibitor of NF-κB and a nuclear activator of some target genes. Finally, characteristics of nuclear IκBs described above are summarized in Table 1.

Table 1 Characteristics of nuclear IκBs.

IκB	Knockout mice	Specificity to NF-κB	Expression		Cytokine expression	Reference
			Basal	Inducible		
Bcl-3	Die earlier by multi-organ inflammation due to mTEC defect Abnormal splenic architecture and germinal center formation Sensitive to LPS-induced endotoxin shock	p50 or p52 homodimer	+	++	Inhibition: TNFα	(14, 15, 18–21, 23)
IκBζ	Atopic dermatitis-like lesion Prevents EAE development	p50 homodimer	-	+++	Activation: IL-6, IL-12p40, IL-17A	(30, 33, 34)
IκBNS	Sensitive to DSS-induced colitis and LPS-induced endotoxin shock Defect of B cell development	p50 homodimer	-	+++	Inhibition: IL-6, IL-12p40 Activation: IL-2	(37–40)
IκBη	Not reported	p50 homodimer (RelA-containing dimer?)	++	+	Activation: TNFα, IL-6, IL-12p40	(8)
IκBL	Earlier onset (KO) and reduced severity (Tg) of CIA	RelB	++	++	Inhibition: IL-6, IL-1β	(9, 12)
IκBβ (hypophosphorylated)	Reduced severity of CIA and LPS-induced endotoxin shock	RelA and c-Rel dimer	+	+++	Activation: TNFα, IL-12p40	(48, 49)

mTEC, medullary thymic epitherial cell; EAE, experimental autoimmune encephalomyelitis; DSS, dextran sodium sulfate; KO, knockout; Tg, transgenic; CIA, collagen-induced arthritis.

Spatiotemporal regulation of NF-κB activity by nuclear IκBs

The transcriptional activity of inflammatory cytokine genes can be divided into three different states: poised, activated and silenced (Figure 2) (50). Nuclear IκBs, as described above, act in distinct states. Under homeostatic conditions, NF-κB binding elements in the promoters of inflammatory cytokine genes, such as TNFα, are occupied by the p50 homodimer. The p50 homodimer lacks transcriptional activity but recruits a corepressor complex, including NCoR and SMRT, to the inflammatory cytokine gene and keeps the loci in the 'poised' state (5, 50). This state is actively maintained by Bcl-3, which blocks poly-ubiquitination and subsequent degradation of p50. Consequently, the gene loci are held poised for transcription (Figure 2A) (19).

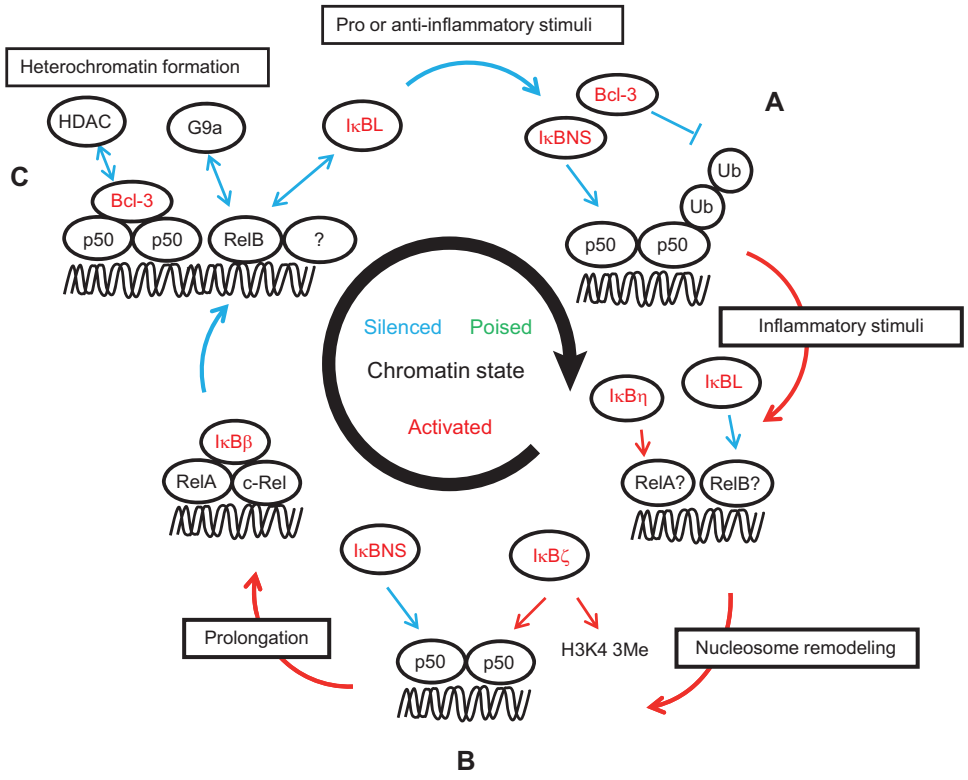

Figure 2 Triphasic nature of transcriptional potency during the inflammatory response.
(A) Under homeostatic conditions, NF-κB binding elements in the promoters are occupied by transcriptional inactive p50 homodimers, which are stabilized by their association with IκBNS and Bcl-3. Bcl-3 prevents p50 polyubiquitination and subsequent degradation. (B) Inflammatory stimuli rapidly induce nuclear entry of the NF-κB dimer and convert the gene loci to the activated state. IκBη and IκBL, which are expressed at basal levels, respectively promote and inhibit NF-κB-mediated inflammatory cytokine gene expression. IκBζ and IκBNS are rapidly induced by TLR agonists and modulate NF-kB activity via association with p50 homodimer. IκBζ facilitates transcription of secondary response genes through its transcriptional activation potency or induction of H3K4 trimethylation, a marker of active transcription, at the promoters. In contrast, IκBNS exerts an inhibitory effect on transcription by stabilization of the p50 homodimer and blockade of RelA-containing dimer binding to the promoters. Newly synthesized and hypophosphorylated-IκBβ is involved in the sustained expression of inflammatory cytokines. (C) DNA-bound RelB can recruit the histone methyltransferase G9a and histone deacetylases (HDACs) to the promoter region resulting in the induction of facultative heterochromatin formation. IκBL, which associates with RelB, might be involved in RelB-mediated transcriptional termination. Bcl-3, which binds to p50 homodimer on the promoter, also recruits HDAC and induces termination of transcription. Bcl-3 and IκBNS, both of which are induced by pro- or anti-inflammatory stimuli, maintain the silenced but poised chromatin structure for transcription. The red and blue arrows indicate stimulatory and inhibitory effects on transcription, respectively. Nuclear IκBs are shown in red text. Ub, ubiquitin.

Once activated via TLR signaling, the NF-κB dimer, which translocates into the nucleus, can initiate a series of transcriptional programs of various inflammatory cytokine genes (Figure 2B). First, primary response genes such as TNFα are rapidly induced without *de novo* protein synthesis. It was reported that RNA polymerase II constitutively binds to the proximal promoter regions of most primary response genes as well as housekeeping genes, even under homeostatic conditions. The RelA-containing dimer, which binds to the κB element of the promoter, can induce histone H4 acetylation, P-TEFb recruitment and subsequent phosphorylation of RNA polII at Ser2 (51). TLR-induced phosphorylation of PolII at Ser2 results in productive elongation and subsequent processing of mRNA. Concurrently, inducible transcriptional modulators, such as IκBζ, C/EBPδ and ATF3, are also induced by the RelA-containing dimer (6, 28, 52).

For the induction of most secondary response genes, *de novo* synthesized IκBζ is required. IκBζ deficiency results in a failure to promote H3K4 trimethylation, which is a histone marker for the transcriptionally active state, and a failure to recruit RNA polII and TBP to the promoters of secondary cytokine genes such as *IL-6* and *IL-12p40*. However, recruitment of Brg1, an ATP-dependent chromatin-remodeling factor, is not influenced by IκBζ deficiency. Thus, pre-initiation complex recruitment, but not

nucleosome remodeling, at secondary response gene loci is controlled by IκBζ (31).

In contrast to IκBζ, IκBNS is an inducible repressor of secondary response genes. IκBNS deficiency leads to prolonged association of RelA on the IL-6 promoter, whereas forced expression of IκBNS completely inhibits RelA recruitment to the promoter. Therefore, IκBNS has the opposite effect of IκBζ on the transcriptional activation of secondary response genes (38, 40). IκBβ acts as an activator and an inhibitor of inflammatory cytokine transcription. Upon LPS stimulation, IκBβ-deficient cells showed a rapid loss of mRNA after its expression reached maximum, resulting in reduced production of IL1β and TNFα. Unlike IκBζ, which associates with the p50 homodimer, the hypophosphorylated form of IκBβ specifically binds to the RelA/c-Rel heterodimer and facilitates prolonged production of TNFα and IL1β. It has been suggested that those cytokines that are largely regulated by c-Rel are the targets of IκBβ-containing complexes (48). Therefore, IκBβ and IκBζ have distinct roles in the temporal regulation of inflammatory cytokine expression, even though they share some target genes. Basal expression of IκBη is considered crucial for the regulation of a wide variety of cytokines and chemokines, including both primary and secondary response genes. Although the precise role of IκBη in the epigenetic control of transcription is currently unknown, IκBη can form a complex with RelA and the p50 heterodimer in the promoter region of the IL-6 gene, raising the possibility that IκBη facilitates inflammatory cytokine expression in cooperation with RelA (8).

The termination of inflammatory cytokine gene transcription and facultative heterochromatin formation at these loci are thought to prevent excessive inflammatory response (Figure 2C). RelB was originally identified as a Rel-related protein that showed an inhibitory effect on NF-κB-dependent gene activation (45). The RelB-mediated inhibitory effect is due to its competitive binding to RelA and/or c-Rel binding sites and induction of facultative heterochromatin formation through recruitment of histone methyltransferase, histone deacetylase and DNA methyltransferase (50, 53–55). We found that IκBL bound to RelB and was required to maintain transcriptional silencing of inflammatory cytokines such as IL-1β. Short hairpin RNA-mediated knockdown of IκBL resulted in the breakdown of LPS-induced endotoxin tolerance (our unpublished observations). LPS-induced delayed expression of Bcl-3 is also required for the repression of TNFα (18). Bcl-3-deficient macrophages showed sustained expression of TNFα in the later phase of inflammation. Bcl-3 is induced by LPS in a p50-dependent manner and associates with p50 itself. HDAC1 is also induced by LPS stimulation with kinetics similar to Bcl-3, and it forms a complex with Bcl-3. HDAC activity is indispensable for Bcl-3-mediated TNFα repression. Taken together, IκBL and Bcl-3, coupled with RelB and p50, respectively, might be involved in active transcriptional silencing and prevention of excess inflammatory cytokine production.

Concluding remarks

Recent progress in the field of nuclear IκBs has provided important insights into the transcriptional regulation of inflammatory cytokines controlled by NF-κB. Nuclear IκBs may contribute to the epigenetic modulation of gene loci for optimal activation and transcriptional silencing. However, how each nuclear IκB coordinately regulates temporal transcription through NF-κB is almost completely unknown. Thus, further studies using mice or cells lacking two or more nuclear IκB proteins are needed to elucidate the transcriptional circuitry of NF-κB target genes controlled by nuclear IκBs. Understanding the epigenetic control of cytokine gene loci by nuclear IκBs in the later phase as well as in the acute phase of inflammation may shed light on the pathogenesis of inflammatory disorders.

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