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Review

The nuclear IκB family of proteins controls gene regulation and immune homeostasis

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ABSTRACT

The inhibitory IκB family of proteins is subdivided into two groups based on protein localization in the cytoplasm or in the nucleus. These proteins interact with NF-κB, a major transcription factor regulating the expression of many inflammatory cytokines, by modulating its transcriptional activity. However, nuclear IκB family proteins not only interact with NF-κB to change its transcriptional activity, but they also bind to chromatin and control gene expression. This review provides an overview of nuclear IκB family proteins and their role in immune homeostasis.

Contents

1. Introduction

The mammalian NF-kB family of proteins forms two distinct sub-families: NF-κB proteins, including NF-κB1 (p50) and NF-κB (p52) and the Rel proteins, including p65 (RelA), RelB, and c-Rel. The NF-κB and Rel proteins control various biological events, such as immune responses, cell growth, and survival [1,2]. p50 and p52 proteins are encoded within N-terminal regions of p105 and p100, respectively [3]. p105 is cleaved by the proteasome, forming p50 from the N-terminal region, and IκB-γ from the C-terminal region. This cleavage depends on activation of the NF-κB-inducing kinase NIK [4]. IκB-γ was shown to be rapidly degraded after proteolytic processing of p105 [4]. p100 becomes phosphorylated at site-specific serine residues (866 and 870), and then the N-terminal protein, p52, and a C-terminal protein, IκB-δ are generated due to proteolytic cleavage [5,6]. This pathway is referred to as non-canonical NF-κB activation [7]. Although p100 is the precursor of the NF-κB subunit p52, it also inhibits translocation of the NF-κB subunit, into the nucleus [8].

Classical regulation of NF-κB occurs via the canonical NF-κB activation pathway which is dependent on the inhibitory IκB family of proteins [7]. The first member of the inhibitory IκB family of proteins to be identified, IκB-α, associates with NF-κB in the cytoplasm through six ankyrin repeat domains to inhibit the translocation of NF-κB into the nucleus [9,10]. Various stimulatory signaling pathways involving molecules such as Toll-like receptors, cytokines, T cell receptors, and co-stimulatory molecules trigger the activation of the IκB kinase complex, including IKKα and IKKβ, along with the NF-kB essential modulator, NEMO. After activation, phosphorylation and ubiquitination of IκB-α leads to its degradation by proteasomes [11]. When IκB-α is

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degraded, multiple NF-κB dimers are released, and they translocate into the nucleus. These activated NF-κB dimers control the expression of many genes, including inflammatory cytokines [12]. The IκB-α promoter region has NF-κB-binding elements that positively regulate IκB-α expression [13]. Therefore, this feedback loop mechanism is important for both initiating and suppressing inflammatory reactions. Newly synthesized IκB-α was reported to enter the nucleus, bind NF-κB, then be exported [14]. IκB-α-deficient mice were first reported in 1995 [15]. The IκB-α-deficient mice were smaller than their littermates at postnatal day 4, and they showed a poorly defined basal skin layer at postnatal day 6 [15]. The IκB-α deficiency also led to constitutive activation of NF-κB and elevated levels of NF-κB responsive genes, including vascular cell adhesion molecule-1 (VCAM-1) and G-CSF, in splenocytes [15]. A second group generated IκB-α-deficient mice; these mice also developed dry flaky skin and showed elevated TNF-α mRNA in the skin 4–6 days after birth [16].

IκB-β was the second IκB family protein identified [17]. IκB-β also has six ankyrin repeat domains, allowing it to complex with NF-κB dimers, inhibiting NF-κB transcriptional activity [18]. IκB-β is degraded upon stimulation with LPS, thus resulting in the persistent activation of NF-κB [19]. This means that IκB-α and IκB-β have similar NF-κB inhibitory activities, and equal volumes of these complexes exist within cells [20]. However, NF-κB does not have transcriptional activity at the IκB-β locus, indicating that IκB-β does not have a positive feedback loop mechanism like IκB-α [19]. IκB-β-deficient mice were reported to resist LPS-induced endotoxin shock and showed a decrease in TNF-α/IL-6 serum levels [21]. These findings were supported by a different group who also reported that IκB-β-deficient mice were resistant to LPS-induced endotoxin shock and exhibited a reduction in TNF-α production in macrophages in response to LPS stimulation [22].

IκB-ε has six ankyrin repeat domains and can form complexes with NF-κB to inhibit NF-κB transcriptional activity [23,24]. IκB-ε is degraded upon stimulation with various factors, such as LPS or PMA + ionomycin, and it is then resynthesized [25]. Depletion of cytoplasmic IκB proteins (IκB-α, IκB-β, and IκB-ε) in mouse embryonic fibroblasts resulted in constitutive activation of NF-κB, higher expression levels of p100 and p105, and higher expression levels of NF-κB target genes in steady state conditions when compared to wild type, IκB-$\alpha^{-/-}$, IκB-$\alpha^{-/-}$IκB-$\beta^{\text{knock-down}}$ or IκB-$\varepsilon^{-/-}$IκB-$\beta^{\text{knock-down}}$ cells [26]. However, depletion of cytoplasmic IκB proteins resulted in impaired NF-κB binding activity in response to stimulation [26]. IκB-ε-deficient mice showed a normal response to pathogen challenge and normal hematopoietic cells maturation [27]. Interestingly, IL-1α and IL-1β expression levels were constitutively increased in macrophages from IκB-ε-deficient mice [27].

Several well-known nuclear IκB family proteins are IκB-ζ, $I\kappa B_{NS}$, and Bcl-3 [28]. They also have ankyrin domain repeats, which play an important role in complex formation with NF-κB to control its transcriptional activity in the nucleus. In addition, a new member of the nuclear IκB family was identified in 2010, called IκB-η [29]. Nuclear IκB family proteins can be up-regulated upon stimulation with various factors to directly regulate target gene expression in the nucleus (Table 1). This review focuses on the role of IκB-ζ, $I\kappa B_{NS}$, Bcl-3, and IκB-η in immune homeostasis and gene regulation and their relationship with each other.

Table 1
Structure, function, and localization of IκB family proteins.

	Ankyrin Repeat	Size (amino acids)	Localization	Function
IκB-α		317	Cytosol & nuclear	NF-κB Inhibitor
IκB-β		356	Cytosol & nuclear	NF-κB Inhibitor
IκB-ε		500	Cytosol	NF-κB Inhibitor
p105	435 p50 IκB-δ	968	Cytosol	NF-κB Precursor
p100	405 p52 IκB-γ	900	Cytosol	NF-κB Precursor
IκB-ζ		618	Nuclear	Positive/Negative regulate NF-κB signaling
$I\kappa B_{NS}$		313	Nuclear	Positive/Negative regulate NF-κB signaling
Bcl-3		454	Nuclear	Positive/Negative regulate NF-κB signaling
IκB-η		516	Nuclear	Positive/Negative regulate NF-κB signaling

2. IκB-ζ

IκB-ζ was first identified in macrophages in response to LPS stimulation [30]. IL-1β stimulation also induced IκB-ζ expression in macrophages through the MyD88 pathway [31]. However, TNF-α stimulation in macrophages failed to induce IκB-ζ expression [32]. When Takeshige et al. used the protein inhibitor Actinomycin-D, LPS stimulation failed to induce IκB-ζ expression [32]; thus, the target genes of IκB-ζ (so called 'secondary response genes') including IL-6 and Lipocalin-2 were not upregulated in immune cells. In the case of human NK cells, IκB-ζ expression was upregulated by IL-12/IL-18 stimulation. It formed a complex with NF-κB, became enriched on the IFN-γ promoter region (which included a NF-κB binding element), and positively regulated IFN-γ gene expression [33]. Furthermore, IκB-ζ positively regulated the expression of the human β-defensin-2 gene, which included C/EBPs and NF-κB binding elements, but it negatively regulated the expression of the endothelial-leukocyte adhesion molecule 1 (ELAM-1) gene, that included NF-κB binding elements [34]. IκB-ζ complexes with Akirin2, which can be bridged by NF-κB and chromatin remodeler SWI/SNF complexes to control IL-6 gene expression in macrophages [35]. A more recent study has shown that human DCs stimulated by β-glucan can induce IκB-ζ expression through the IL-1β feedback loop mechanism to positively regulate IL-23A gene expression. This mechanism is NF-κB-dependent [36]. Thus, the induction of IκB-ζ expression is stimulus-specific and provides selective control over the expression of NF-κB target genes.

IκB-ζ expression is upregulated in T cells in response to TGF-β1 + IL-6 stimulation, and it positively regulates IL-17 gene expression in cooperation with RORγt [37]. IL-1 stimulation can induce IκB-ζ expression in T cells, which positively regulates the development of Th17 independently of IL-6 signaling [38]. Thus, IκB-ζ expression in T cells by both an IL-6-dependent and -independent pathway plays a pivotal role in the development of Th17 cells. The regulation of IL-17A expression by IκB-ζ is well described. The IL-17A gene has many CEBP/β binding elements, and IκB-ζ can directly bind conserved non-coding sequence2 (CNS2) with RORγt. Thus, CNS2-deficient T cells fail to generate Th17 cells in response to TGF-β + IL-6 stimulation [39]. However, the IκB-ζ-mediated induction of IL-17A gene expression is dispensable for NF-κB transcriptional activity. c-Rel is a subunit of NF-κB. c-Rel-deficient T cells fail to express IL-17A, because RORγ and RORγt (a master regulator of Th17) expression in these cells is reduced [40]. Moreover, IκB-ζ was shown to bind to the promoter or enhancer region of Th17-related genes (IL-17F, IL-21, and IL-23 receptors), positively regulating their expression. Therefore, IκB-ζ-deficient mice are resistant to Th17-dependent experimental autoimmune encephalomyelitis. However, in CNS2-deficient mice, Th17 cells developed in the intestinal lamina propria, and the percentage of Th17 cells was comparable to that in control mice [39]. Thus, IκB-ζ enrichment at the CNS2 locus is dispensable for lamina proprial Th17 cell generation. IκB-ζ-deficient mice develop another type of autoimmune disease with age, similar to Sjögren's syndrome [41]. The lacrimal glands of IκB-ζ-deficient mice showed caspase 3 processing; thus, the caspase inhibitor Z-VAD-FMK ameliorated inflammation. Interestingly, Rag2 and IκB-ζ double KO mice did not develop Sjögren's syndrome-like diseases with age; however, when control- or IκB-ζ-deficient $CD4^+$ T cells were transplanted into double KO mice they developed Sjögren's syndrome-like diseases [41]. These results suggested that T cells have an important role in augmenting the severity of Sjögren's syndrome in IκB-ζ-deficient mice, but IκB-ζ expression in T cells is dispensable for