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RESEARCH HIGHLIGHT

IκBβ is a positive and negative regulator of NF-κB activity during inflammation

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Nuclear factor κB (NF-κB) transcription factors play key roles in many physiological processes including immune responses and inflammation. The NF-κB family consists of p65, cRel, RelB, p50 and p52, which form dimers and bind to κB sites in the promoters of target genes [1]. Among them, p65, cRel and RelB have a transactivating domain (TAD) and induce transcription by recruiting transcriptional co-activators. p50 and p52 lack a TAD and thus suppress or stimulate transcription in a context-specific manner. In unstimulated cells, NF-κB is sequestered in the cytoplasm through its close association with a family of specific inhibitory proteins: inhibitors of NF-κB (IκB).

NF-κB activators, such as tumor necrosis factor α (TNFα) and lipopolysaccharide (LPS), lead to the phosphorylation of IκB, and abolish the inhibitory effects of IκB through its ubiquitination and degradation by proteasomes. The released NF-κB translocates to the nucleus and binds DNA, regulating the expression of a wide range of genes encoding inflammatory cytokines such as TNFα and interleukin-6 (IL-6). The regulation of NF-κB activity is mediated by complicated mechanisms involving many proteins with various functions, and plays a pivotal role in the initiation and resolution of inflammation. A recent study by Rao and colleagues published in *Nature* revealed an unexpected role for IκBβ in the regulation of NF-κB and inflammation [2].

The IκB protein family comprises eight members and is classified into three functional groups: the typical IκB proteins (IκBα, IκBβ and IκBε), the precursor proteins (p100 and p105) and the atypical IκB proteins (IκBζ, BCL-3 and IκBNS). IκBα is the prototypical protein of the IκB family, and is present in the cytoplasm of unstimulated cells. Following cell stimulation, it undergoes rapid phosphorylation by IκB kinase β (IKKβ) and is then subjected to ubiquitin-mediated proteasomal degradation that results in the release of bound, cytoplasmic NF-κB dimers (Figure 1A). Cytoplasmic NF-κB then translocates to the nucleus where it drives gene expression, including that of IκBα, which facilitates the termination of NF-κB activation by binding and retaining NF-κB dimers in the cytoplasm. Thus, the primary function of IκB proteins was thought to be the suppression of NF-κB activity.

However, recent studies have revealed that IκB proteins are not simple inhibitors of NF-κB activity but rather pleiotropic NF-κB cofactors and more complex regulators of gene expression. In particular, the atypical IκB family member proteins have been revealed to act as both positive and negative regulators of gene expression. Bcl-3, which was originally identified as a proto-oncogene, has a typical TAD in its C-terminal region. Bcl-3 facilitates transcription by providing transcriptional activation ability to p50 and p52 homodimers, the NF-κB dimers lacking TAD, when it is subjected to proper post-translational modifications including phosphorylation and ubiquitination [3]. Bcl-3-associated p50 and p52 homodimers promote the expression of cyclin D1 and control proliferation and tumor growth. However, in the absence of a proper modification, Bcl-3 functions as an inhibitor of NF-κB-induced gene expression by stabilizing p50 homodimers in a DNA-bound form and preventing the binding of other transcriptionally active NF-κB dimers. After stimulation with LPS, p50 is ubiquitinated and degraded gradually in Bcl-3-deficient macrophages, then active NF-κB dimers bind to κB sites previously occupied by p50 homodimers [4]. Bcl-3 promotes p50 homodimer occupancy of target gene promoters by inhibiting the ubiquitination and subsequent degradation of p50, and preventing replacement with active NF-κB dimers. Bcl-3-mediated suppression specifically occurs at κB sites with a strong binding preference for p50 homodimers, and reduces expression of TNFα. Furthermore, Bcl-3 restricts inflammation by suppressing the proinflammatory cytokine IL-23 [5] and by inducing the expression of the

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anti-inflammatory cytokine IL-10 [6].

Dual functions of IκB are also reported in other atypical IκB family proteins. IκBζ, a Bcl-3-homologous IκB protein, is an important regulator of NF-κB activity during the inflammatory response. IκBζ is not expressed constitutively, but is rapidly induced by LPS and IL-1 through specific NF-κB signaling pathways mediated by MyD88. Newly synthesized IκBζ associates with p50 homodimers that are bound to specific κB sites on the IL6 promoter, and then mediates its expression [7]. However, like BCL-3, IκBζ functions as either a positive or a negative regulator of NF-κB in a context-specific manner. As IκBζ is capable of transactivation when bound to p50 but not when bound to p65, it enhances expression of specific NF-κB target genes but suppresses other specific genes [8]. Another atypical IκB protein, IκBNS, not only inhibits the induction of NF-κB target genes such as IL-6 and limits inflammation [9], but also increases the expression of a small subset of genes [10].

Unlike atypical IκB proteins, members of the typical IκB family protein were thought to be simple inhibitors. IκBβ, like IκBα, was thought to act as an inhibitor by sequestering NF-κB dimers in the cytoplasm. Indeed, IκBβ prevents nuclear import of NF-κB more potently than IκBα. In addition, although IκBα knockout mice display severe postnatal developmental defects and constitutive NF-κB activation [11], knockin mice created by replacing *IκBα* with *IκBβ* survive and develop without any apparent abnormalities, suggesting a functional redundancy between IκBα and IκBβ [12]. However, Rao and colleagues have changed this concept by showing that IκBβ acts as both a positive and negative NF-κB regulator, inhibiting as well as facilitating inflammatory responses. They generated IκBβ$^{-/-}$ mice and investigated the functions of IκBβ *in vivo*. Surprisingly, IκBβ$^{-/-}$ mice show a dramatic reduction of TNFα production in response to LPS and are also resistant to LPS-induced endotoxin shock. TNFα expression, but not that of IL-6, is drastically reduced in IκBβ$^{-/-}$-deficient macrophages, indicating that IκBβ acts as a positive regulator of NF-κB, which specifically promotes TNFα transcription.

Why does IκBβ specifically enhance the expression of *TNFα*? The answer to this may depend on the distinct properties between IκBβ and IκBα. First, IκBβ and IκBα exhibit different preferences for NF-κB dimers. IκBα associates with p65:p50 and cRel:p50, forming IκBα:p65:p50 and IκBα:cRel:p50 complexes, whereas IκBβ associates with p65:cRel, forming the IκBβ:p65:cRel complex. Second, the association of IκBβ with the NF-κB p65 protein does not prevent the DNA binding activity of p65, and thus the IκBβ:p65:cRel complex is able to bind to the κB site and promote transcription of its target gene [13]. Furthermore, as binding of the IκBβ:p65:cRel complex to DNA is resistant to IκBα, it may enhance and prolong the expression of genes once this complex is formed. Third, IκBβ is subjected to phosphorylation and degradation in a different manner to IκBα. IκBβ exists in a phosphorylated form in the cytosol, and is subjected to further slow phosphorylation and degradation. Newly synthesized IκBβ enters into the nucleus in a hypophosphorylated form and forms the IκBβ: p65: cRel complex. From these observations, Rao and colleagues proposed the following model of IκBα and IκBβ showing their different mode of regulation and function (Figure 1A and 1B).

LPS stimulation induces the rapid phosphorylation and subsequent degradation of IκBα. Released p65:p50 heterodimers then translocate to the nucleus and promote various genes including TNFα, IκBα, IκBβ and c-Rel. Newly synthesized IκBα binds and sequesters NF-κB complexes in the cytoplasm and then terminates the transcription of most NF-κB-dependent genes. IκBβ selectively binds p65 or c-Rel and stabilizes the p65:cRel heterodimer in resting cells. In this state, IκBβ exists in a hyperphosphorylated form and acts as an inhibitor. Following LPS stimulation, further slow phosphorylation and degradation of IκBβ occurs, resulting in the release of the p65:c-Rel heterodimer. p65:c-Rel enters into the nucleus and binds to selective κB sites of the TNFα promoter leading to its expression. Then, newly resynthesized hypophosphorylated IκBβ enters into the nucleus and associates with the p65:cRel complex, forming the IκBβ:p65:cRel complex which is capable of continued interaction with the TNFα promoter κB site and subsequent expression. IκBβ promotes not only TNFα gene expression, but also several cytokines such as IL-12 that depend on p65 and cRel for their expression. IκBβ does not affect IL-1 and IL-6 gene promoters. Thus, only a selective group of NF-κB-regulated genes is a target of IκBβ-mediated gene expression.

Many diseases are linked to inflammation and there is considerable interest in the signaling pathway of NF-κB activity as a potential therapeutic target. However, recent studies have shown that NF-κB has dual roles, acting not only as an initiator of inflammation but also in the complex process of the resolution of inflammation. Thus, it will be necessary to identify therapeutic targets that can provide therapeutic benefits without harmful effects. Does the IκBβ-NFκB-TNFα pathway lead to the development of more selective approaches for NF-κB inhibition? TNFα plays a key role in inflammation, and anti-TNFα antibody therapies are effective treatments for rheumatoid arthritis [14]. Rao and colleagues investigated whether the course of collagen-induced arthritis could be altered in the absence of IκBβ, and found that IκBβ$^{-/-}$ mice displayed a delayed onset and decreased severity of arthritis due to reduced chronic production of TNFα [2]. From these findings, IκBβ has been proposed as a new target of treatment for chronic

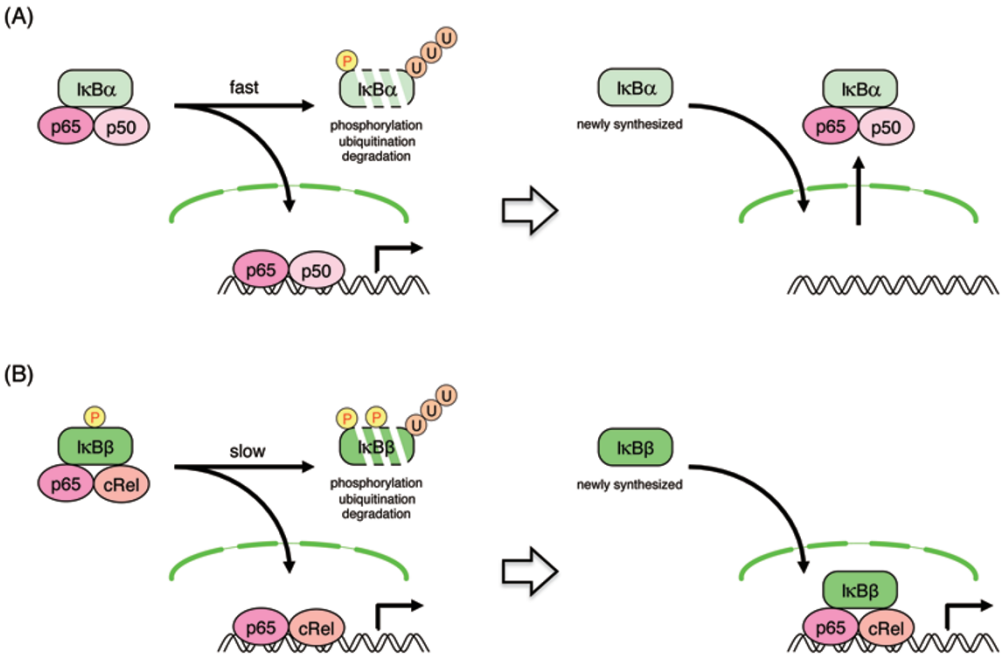

Figure 1 Model of differential regulation of IκBα **(A)** and IκBβ **(B)** after LPS stimulation.

inflammatory diseases.

Interestingly, NF-κB induces many IκB family proteins that not only lead to the termination of NF-κB activity, but also promote specific cytokine gene expression. As described above, newly synthesized IκBζ binds to characteristic κB sites of the IL-6 gene promoter, thus promoting its expression [7], whereas IκBβ binds to the TNFα gene promoter and prolongs its production [2]. These results indicate that individual cytokine expression is regulated by specific IκB: NF-κB complexes. Furthermore, the function of IκB proteins can be altered by post-translational modifications. Each of these IκB specific functions and their modifications may provide plausible therapeutic targets for the treatment of inflammatory diseases. To this end, it may be necessary to clarify the mechanism of IκBβ regulation itself as well as crosstalk between other IκB proteins including IκBα and atypical IκB proteins.

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