

Special Topic: NF- κ B, Immunity and Cancer

Nuclear Factor- κ B: Fine-Tuning a Central Integrator of Diverse Biologic Stimuli

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The nuclear factor (NF)- κ B family of proteins is a key regulator of inflammation, innate immunity, and cell survival and differentiation. Components of these pathways are potential targets of intervention for inflammation, infectious diseases, and cancer. However, therapeutic interventions that dampen the host response to infection and injury must also recognize the autoregulatory loops in the “resolution” phase of inflammation and infection. A more precise fine-tuning of these pathways leading to NF- κ B activation will require dissecting temporally the different phases of activation and endogenous autoregulatory deactivation programs in diseases and redefining end-points after drug/inhibitor treatment to correlate changes in these stages.

Keywords Nuclear factor-kappa B, inflammation, therapeutics, signal pathways

NF- κ B is a family of highly conserved transcription factors that can be activated by different Toll-like receptors (TLRs), cytokine receptors including interleukin (IL)-1 receptor (IL-1R1) and tumor necrosis factor receptor (TNFR), as well as cytosolic pattern-recognition receptors such as nucleotide-binding and oligomerization-domain (NOD) proteins, caspase recruitment domain (CARD), and ICE protease-activating factor (IpaF) (Fig. 1) (reviewed in Refs. 1–3). TLRs are sensors of microbes

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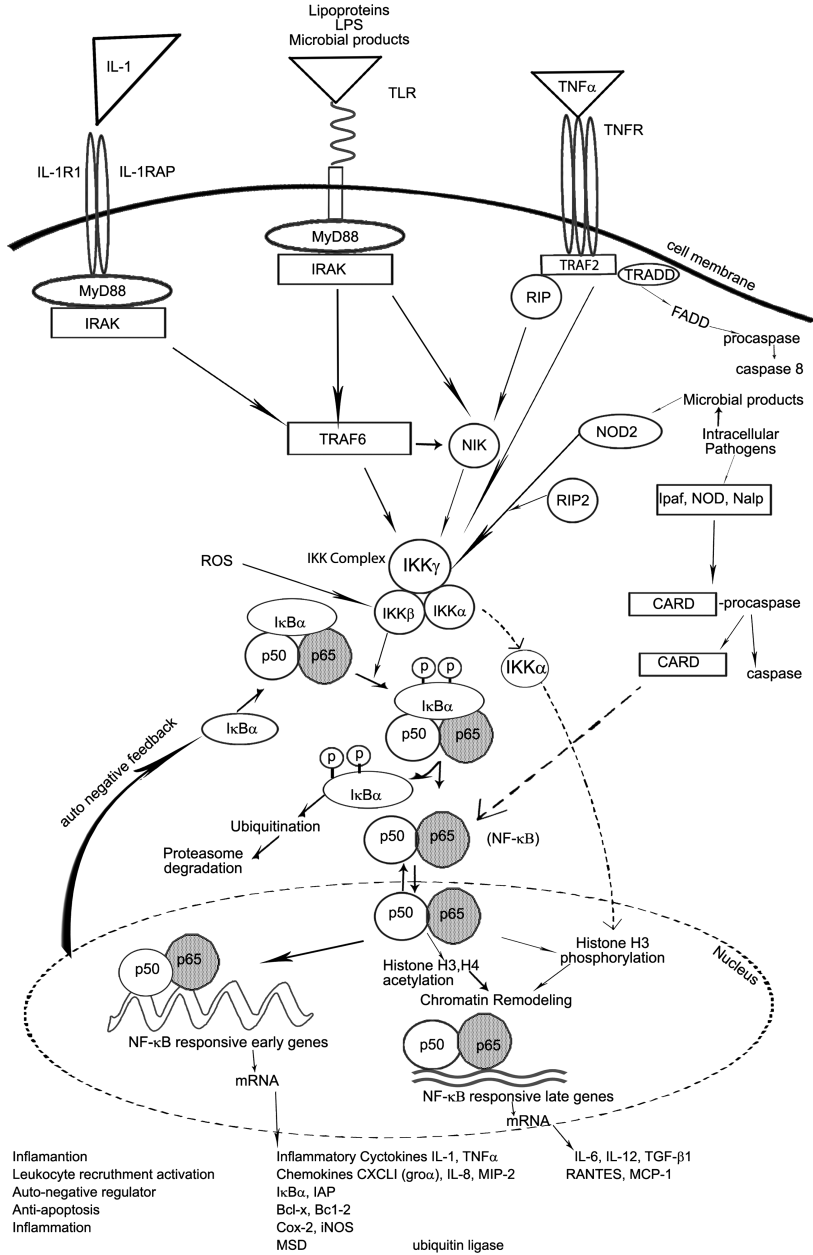


FIGURE 1 Molecular pathways leading to NF- κ B activation. Abbreviations used in this figure: CARD, caspase recruitment domain; COX-2, cyclooxygenase 2; FADD, Fas-associated death domain protein; IAP, inhibitory apoptotic

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as they bind to specific pathogen-associated molecular patterns that are common to bacteria, fungi, and viruses [4, 5]. The downstream recruitment of other proteins/adapters after the binding of microbial products or proinflammatory cytokines to these receptors culminates in the activation of I κ B kinases (IKK).

In resting cells, NF- κ B dimers are bound noncovalently to inhibitory proteins I κ Bs (I κ B α , I κ B β , I κ B ϵ) and remain in an inactive form in the cytoplasm [1, 6]. Upon stimulation, I κ B is phosphorylated by IKK, polyubiquitinated and degraded by the proteasome [7, 8]. The release of I κ B unmasks the nuclear localization site on NF- κ B and allows it to be translocated into the nucleus where it binds to promoter region of target genes and activates transcription.

Transcriptional activity of NF- κ B is dependent on the presence of transcriptional activation Rel domains present in the transcription factors of p65(Rel A), Rel B, and c-Rel but absent in other NF- κ B members, p50 and p52 [3, 9, 10]. Although the most common activated form of NF- κ B is the p50 and p65 heterodimer, homodimers of p50 exist and act as repressors as they lack transactivating domains. The ability of different family members to homodimerize or heterodimerize and their cell- and tissue-specific expression contributes to the specific and diverse cellular responses to different stimuli.

The central kinase, IKK complex, consists of IKK α , IKK β , and IKK γ with IKK β being the main kinase for cytosolic phosphorylation of the inhibitor I κ B α [11]. However, optimal induction of certain NF- κ B-dependent genes such as ICAM-1 and MCP-1 is dependent on IKK α translocation to the nucleus [12]. Furthermore, IKK α regulates chromatin remodeling including phosphorylation of histone H3 and removal of co-repressors from some NF- κ B-dependent genes [13].

protein; I κ B, inhibitor of NF- κ B; IKK, I κ B kinase; IL-1, interleukin 1; IL-1RAP, IL-1 receptor accessory protein; iNOS, inducible nitric oxide synthetase; Ipaf, IL-1 β converting enzyme protease-activating factor; IRAK, IL-1 receptor associated kinase; MCP-1, macrophage chemoattractant protein 1; MIP-2, macrophage inflammatory protein 2; MSD, manganese superoxide dismutase; MyD88, myeloid differentiation factor 88; Nalp, pyrin domain-containing protein; NIK, NF- κ B inducing kinase; NOD, nucleotide oligomerization domain; RIP, receptor-interacting protein; RANTES, regulated upon activation, normal T cell expressed and secreted; TGF- β 1, transforming growth factor β 1; TNF, tumor necrosis factor; TRAF2, TNF receptor associated factor 2; TRADD, TNF receptor associated death domain protein.

NF- κ B regulates the transcription of genes for many cytokines (IL-1, IL-2, IL-6, IL-12, TNF α , TNF β , GM-CSF), chemokines (IL-8, RANTES, MCP-1, MIP-1 α , eotaxin, gro α), cell adhesion molecules (ICAM-1, VCAM-1, E-selectin), acute phase proteins (SAA, CRP), matrix metalloproteinase, manganese superoxide dismutase, antiapoptotic proteins (Bcl-2, Bcl-x), and the inducible enzymes, nitric oxide synthetase (iNOS) and cyclooxygenase 2 (COX-2)[11, 14]. The activation of NF- κ B therefore leads to the inducible expression of many of the mediators involved in inflammation and cell survival and represents an attractive therapeutic target for suppressing inflammation resulting from infection and injury.

Recently, kinetic analysis and chromatin immunoprecipitation assays revealed at least two different classes of promoters with NF- κ B binding sites, some with immediate accessible NF- κ B binding sites, capable of recruiting NF- κ B dimers immediately upon entry in the nucleus [15, 16]. However, promoters of other genes, which are expressed later, require other signals or co-activators to modify chromatin structure for NF- κ B binding. Some co-activators have intrinsic histone acetyltransferases to remodel chromatin structure to allow accessibility to NF- κ B [17, 18]. The regulation of these “late” expressed genes (IL-6, MCP-1, RANTES) is more complex, as cofactors can have synergistic or antagonistic effects [19]. Finally, the quantity and quality of different stimulators and the duration of stimulation can also play a role in gene induction.

The transcriptional activity of NF- κ B can be regulated at multiple steps, including the amount of I κ B present; phosphorylation of I κ B, which is controlled by IKK activity; and subsequent degradation of I κ B and other co-activating transcriptional factors, such as activator protein 1 and cAMP responsive element-binding proteins. In addition, endogenous positive and negative feedback loops exist to regulate NF- κ B activity. Thus, IL-1 and TNF α , cytokines that are stimulated by NF- κ B, are themselves activators of the NF- κ B pathway and serve to further amplify the inflammatory response. NF- κ B autoregulates itself by binding to I κ B α genes and induces their transcription, thereby down-regulating itself [19]. Already a reversible proteasome inhibitor, bortezomib, targeting the ubiquitin-proteasome pathway, has been approved for treating multiple myeloma [20]. Malignant cells constitutively expressed activated NF- κ B and are more sensitive to the cytotoxic effects of proteasome inhibition.

In earlier times, when our understanding of biochemical mechanisms of action was limited, the design of agents to achieve specific results was mostly empirical. Striking successes (e.g., aspirin, corticosteroids)

were achieved by this “cut and try” method. The development of specific anti-inflammatory COX-2 inhibitors illustrates difficulties encountered in the drug-development process. Intense search for COX-2-specific inhibitors started with the discovery of a new inflammation-inducible enzyme, COX-2, and structural design of small-molecule inhibitors to target COX-2 activities. This strategy of selective COX-2 inhibition has been successful in producing a handful of nonsteroidal, anti-inflammatory drugs with low gastrointestinal toxicity. However, it has also simultaneously revealed surprising side effects of COX-2 inhibition: thrombogenic risk, renal adverse events, and interference with mucosal healing.

By its very nature, the inflammatory process is exceedingly complex, being both necessary to the organism’s self-protective response to external chemical and biological challenges and itself damaging to the organism. Inflammation is often followed by the host internal homeostatic regulatory mechanism to downregulate or “resolve” inflammation. Therefore, the therapeutic interventions that address inflammation must recognize that agonistic as well antagonistic manipulations may be necessary. In addition, because the pathways involved in inflammation consist of numerous steps and include both branches and feedback loops that may be positive or negative, pharmacologic intrusion at any one point may have pleiotropic and surprising effects. Finally, because of complex interconnected signal transduction pathways, a given inhibitor may affect more than one pathway, giving rise to still another class of pleiotropic effects. The newer technology of DNA microarray analysis can be used to identify the secondary effects of new drugs as well as the effects of old ones. Thus, the gene expression approach could also provide new insights to pathways or molecules as common drug targets. Identifying the genes expressed or suppressed and the temporal pattern of their expression could pinpoint the timing of delivery of natural inhibitors to the “resolving” phase of diseases.

Complete blockade of NF- κ B would likely produce unwanted side effects as NF- κ B is essential in maintaining normal host defense and generating innate immune responses to pathogens and microbial products. Mice deficient in NF- κ B proteins illustrate the important functions of NF- κ B as IKK-deficient mice show abnormal morphogenesis and die shortly after birth [21]. NF- κ B has constitutive function as it appears to be required to maintain low levels of antiapoptotic proteins to prevent loss of mitochondrial transmembrane potential. An ideal NF- κ B inhibitor would ablate the proinflammatory arm while preserving the autoregulatory, anti-inflammatory loop and other restoration

mechanism to readjust these multiple interrelated processes. It is tempting to speculate that a new drug targeting NF- κ B can also be delivered to a specific microenvironment (cell/tissue/organ) as certain receptors, intracellular adapters, types of NF- κ B heterodimers or homodimers, and inhibitory proteins are cell specific and therefore influence physiologic outcome.

REFERENCES

- [1] A. Hoffmann and D. Baltimore, Circuitry of nuclear factor kappaB signaling. *Immunol Rev* **210**: 171–186, 2006.
- [2] M.S. Hayden and S. Ghosh, Signaling to NF- κ B. *Genes Dev* **18**: 2195–2224, 2004.
- [3] A.S. Baldwin Jr., The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu Rev Immunol* **14**: 649–683, 1996.
- [4] L.A. O'Neill, How Toll-like receptors signal: what we know and what we don't know. *Curr Opin Immunol* **18**: 3–9, 2006.
- [5] B. Beutler, Inferences, questions and possibilities in Toll-like receptor signaling. *Nature* **430**: 257–263, 2004.
- [6] A. Hoffmann, A. Levchenko, M.L. Scott, and D. Baltimore, The I κ B-NF- κ B signaling module: temporal control and selective gene activation. *Science* **298**: 1241–1245, 2002.
- [7] M. Karin and Y. Ben-Neriah, Phosphorylation meets ubiquitination: the control of NF-kappa B activity. *Ann Rev Immunol* **18**: 621–663, 2000.
- [8] J. DiDonato, F. Mercurio, C. Rosette, J. Wu-Li, H. Suyang, S. Ghosh, and M. Karin, Mapping of the inducible IkappaB phosphorylation sites that signal its ubiquitination and degradation. *Mol Cell Biol* **16**: 1295–304, 1996.
- [9] A. Hoffmann, T.H. Leung, and D. Baltimore, Genetic analysis of NF- κ B/Rel transcription factors defines functional specificities. *EMBO J* **22**: 5530–5539, 2003.
- [10] S. Ghosh, M.J. May, and E.B. Kopp, NF- κ B/ and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu Rev Immunol* **16**: 225–260, 1998.
- [11] M. Karin, Y. Yamamoto, and Q.M. Wang, The IKK NF- κ B system: a treasure trove for drug development. *Nat Rev Drug Discov* **3**: 17–26, 2004.
- [12] G. Gloire, E. Dejardin, and J. Piette, NF-kappaB activation by reactive oxygen species: fifteen years later. *Biochem Pharmacol* **72**: 1493–1505, 2006.
- [13] G.Y. Park, X. Wang, N. Hu, T.V. Pedchenko, T.S. Blackwell, and J.W. Christman, NIK is involved in nucleosomal regulation by enhancing histone H3 phosphorylation by IKK α . *J Biol Chem* **281**: 18684–18690, 2006.
- [14] P.P. Tak and G. S. Firestein. NF-kappaB: a key role in inflammatory diseases. *J Clin Invest* **107**: 7–12, 2001.
- [15] S. Sacconi, S. Pantano, and G. Natoli, Two waves of nuclear factor kappaB recruitment to target promoters. *J Exp Med* **193**: 1351–1359, 2001.
- [16] G. Natoli, S. Sacconi, D. Bosissio, and I. Marazzi, Interactions of NF-kappaB with chromatin: the art of being at the right place at the right time. *Nat Immunol* **6**: 439–445, 2005.
- [17] B. Tian, D.E. Nowalk, and A.R. Brasier, A TNF-induced gene expression program under oscillatory NF-kappaB control. *BMC Genomics* **6**:137–155, 2005.

- [18] K.Y. Lee, K. Ito, R. Hayashi, E.P.I. Jazrawi, P.J. Barnes, and I.M. Adcock, NF-kappaB and activator protein 1 response elements and the role of histone modifications in IL-1beta-induced TGF-beta1 gene transcription. *J Immunol* **176**: 603–615, 2006.
- [19] S.C. Sun, P.A. Ganchi, D.W. Ballard, and W.C. Greene, NF-kappa B controls expression of inhibitor I kappa B alpha: evidence for an inducible autoregulatory pathway, *Science* **259**: 1912–1915, 1993.
- [20] D. Chauhan, T. Hideshima, and K.C. Anderson, Targeting proteasomes as therapy in multiple myeloma. *Adv Exp Med Biol* **615**: 251–60, 2008.
- [21] A.A. Beg, W.C. Sha, R.T. Bronson S, Ghosh, and D. Baltimore, Embryonic lethality and liver degeneration in mice lacking the RelA component of NF- κ B. *Nature* **376**: 167–170, 1995.