0026-895X/05/6704-977–979$20.00
MOLECULAR PHARMACOLOGY
Vol. 67, No. 4
11296/1198132
Mol Pharmacol 67:977–979, 2005
Printed in U.S.A.

PERSPECTIVE

Nuclear Factor-κB Decoys Suppress Endotoxin-Induced Lung Injury

Delphine Baetz, James Shaw, and Lorrie A. Kirshenbaum

The Institute of Cardiovascular Sciences, St. Boniface General Hospital Research Centre, and the Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada

Received January 19, 2005; accepted January 25, 2005

Over millions of years, cells have evolved an elaborate defense systems to ward off and destroy foreign invaders. A key step in the host cells' defense repertoire against bacterial or viral infection includes an inflammatory response mediated by certain cells of the immune system. Once activated, such cells (predominantly T lymphocytes) undergo extensive clonal expansion, resulting in the production and liberation of pro-inflammatory factors such as tumor necrosis factor α, nitric oxide (NO), and others that underlie the sequelae of the inflammatory process. Though crucial for cell survival, excess or uncontrolled activation of inflammatory pathways has been linked to a number of human pathologies including autoimmune diseases, cardiovascular diseases, such as atherosclerosis and myocarditis, and end-organ failure from endotoxic injury. For example, excessive levels of circulating NO from sustained activation of inducible nitric-oxide synthase (iNOS) is believed to account for the increased vascular permeability and vasodilatory responses associated with sepsis-induced lung injury commonly seen in patients with adult respiratory distress syndrome. Interventions designed to modulate immune cell activation and the inflammatory process more generally may have therapeutic advantage in treating patients with systemic inflammatory diseases.

This work was supported by grants from The Canadian Institutes for Health Research (CIHR) (to L.A.K.), and an Interdisciplinary Heart Research program grant from CHIR (CHFNET). D.B. holds a Postdoctoral Research Fellowship from the CIHR-IMPACT program, and L.A.K. is a Canada Research Chair in Molecular Cardiology

Article, publication date, and citation information can be found at http://molpharm.aspetjournals.org.

doi:10.1124/mol.105.011296.

Please see the related article on page 1018.

The NF-κB Signaling Pathway and Inflammation

Because a diverse number of biological signals including reactive oxygen species, adhesion molecule ligation, bacterial endotoxins, viral infection, cytokine and chemokine receptor activation can trigger the inflammatory process, key elements among these diverse stimuli may converge upon one or more common factor(s) to mount an inflammatory response. In this regard, nuclear factor-κB (NF-κB) is a prominent nuclear transcription factor suggested to be a central regulator of genes and end- effectors of the host's inflammatory response. NF-κB was first identified as a key regulatory molecule required for B-lymphocyte proliferation and maturation (Miyamoto and Verma, 1995). Since these original observations, it has become appreciated that NF-κB is a ubiquitously expressed dimeric transcription factor involved in wide range of biological processes that include inflammation, cell adhesion and cell survival (for review, see Karin, 1999).

NF-κB belongs to a family of highly conserved transcription factors that include Rel-A, c-Rel, v-Rel, and *Drosophila melanogaster* dorsal proteins. The major form of NF-κB in cells is a heterodimeric complex composed of 50- and 65-kDa (p50/p65) protein subunits; minor complexes of p50/p50 homodimers have also been reported (Urban and Baeuerle, 1991; Hansen et al., 1992). The p65 subunit is important for gene transcription (Schmitz and Baeuerle, 1991). In unstimulated cells, NF-κB is retained as an inactive complex bound to the cytoplasmic inhibitor protein of IκBα (Baeuerle and Baltimore, 1988; Urban and Baeuerle, 1990; Nolan and Baltimore, 1992; Zabel et al., 1993). A key step in the activation of NF-κB involves phosphorylation and degradation of IκBα (Fig. 1). Insight into the signaling mechanisms that led to IκBα phosphorylation has revealed a kinase complex

ABBREVIATIONS: iNOS, nitric-oxide synthase; NF-κB, nuclear factor κB; IKK, IκB kinases; ODN, oligonucleotides; HVJ, Hemagglutinating virus of Japan.

composed of IκB kinases (IKK) (Scherer et al., 1995; Verma et al., 1995; McKinsey et al., 1996; Van Antwerp and Verma, 1996), IKKα, IKKβ, and IKKγ (Karin, 1999; O'Mahony et al., 2000; Ghosh and Karin, 2002). IKKα and IKKβ are catalytic subunits, whereas IKKγ is a regulatory subunit (for review, see Karin, 1999; Ghosh and Karin, 2002). It is noteworthy that IKKβ seems to be crucial for activating NF-κB (Li et al., 1999) because it phosphorylates critical serine residues 32 and 36 of IκBα (Maniatis, 1999), resulting in the ubiquitination and subsequent degradation of IκBα by the 26S proteasome (for review, see Nabel and Verma, 1993; Karin, 1999). The degradation of IκBα permits NF-κB to translocate to the nucleus and affect gene transcription. Several genes associated with the inflammatory process, including iNOS, cyclooxygenase-2, tumor necrosis factor α and others, contain putative NF-κB binding sites within their promoters, thus highlighting the importance of NF-κB as a key regulator of inflammatory gene activation and identifying it as prime candidate for targeted inactivation. Indeed, several strategies to ameliorate or suppress the inflammatory process associated with different pathologies have been directed toward blocking the biological actions of NF-κB (Scheinman et al., 1995; Brand et al., 1996; Baeuerle and Baichwal, 1997; Desmet et al., 2004; Tanaka et al., 2005).

Role NF-κB in Acute Endotoxic Lung Injury

To test whether functional inactivation of NF-κB could suppress endotoxin-induced lung injury, in this issue of *Molecular Pharmacology,* Matsuda et al. (2005) evaluate the effects of "decoy" 'cis'-acting oligonucleotides (ODN) directed against NF-κB on inflammatory gene expression and pulmonary function in a cecal-ligation puncture model of sepsis. In this report, the authors delivered ODN in vivo using the hemagglutinating virus of Japan-envelope (HVJ), which had been previously reported as an effective vehicle for delivering small ODN into cells in vivo (Morishita and Kaneda, 2002). The premise behind this technique lies in the ability of the NF-κB ODN decoy to suppress simultaneously the expression of several inflammatory genes that contain NF-κB binding elements (Fig. 1).

The authors found that intravenous injection of ODN significantly reduced the increase of NF-κB activity during sepsis, as indicated by electromobility shift analysis. Moreover, NF-κB decoy markedly reduced the expression levels of iNOS, COX-2, histamine H1-receptor, platelet-activating factor receptor, and bradykinin B1 and B2 receptors in the septic lung tissue. It is noteworthy that animals treated with NF-κB ODN displayed an improved outcome with a significant reduction in sepsis-induced lung injury compared with control animals or animals treated with scrambled ODN.

Decoys as Therapeutic Tools For Disease

The strength of the ODN decoy technique lies in its ability to compete for the cis-NF-κB binding elements, thereby blocking the actions of NF-κB. As shown in Fig. 1, the NF-κB decoy inactivates NF-κB activity by competing with the endogenous NF-κB cis elements at the level of the DNA, thereby interfering with NF-κB gene transcription and gene activation. Therefore, such a decoy approach has the potential to block the inflammatory response mediated by one or more genetic pathways. Several reports document the utility of a "decoy" strategy to treat disease manifestation. For example, NF-κB decoys have been shown to reduce the incidence of myocardial cell and neuronal cell damage after ischemic injury (Morishita et al., 1997; Ueno et al., 2001) and to decrease hypoxia-induced apoptosis of human aortic

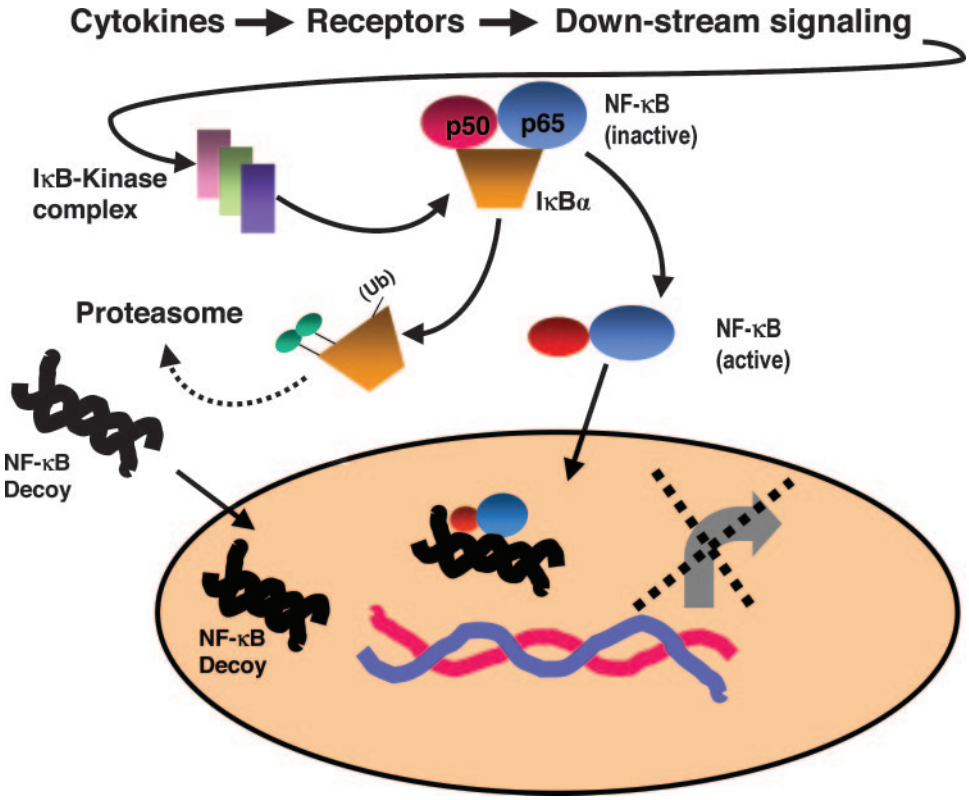

Fig. 1. Inactivation of NF-κB by decoy oligonucleotides. Activation of NF-κB (p50/p65 kDa subunits, red and blue, respectively) by external factors involves the signal induced phosphorylation and degradation of IκBα. This is mediated by the IκB kinase complex, which phosphorylates IκBα at specific serine residues. Phosphorylation of IκBα promotes ubiquitination and its subsequent degradation by the proteasome, which permits NF-κB to translocate to the nucleus and affect gene transcription. Decoy oligonucleotides (ODN) directed against NF-κB (black) inhibit NF-κB-dependent gene transcription by competing with cis' acting elements of putative inflammatory genes that contain NF-κB binding elements. The decoy ODN are delivered in vivo using HVJ-cationic liposomes, which enhance transfection efficiency (Morishita and Kaneda, 2002).

endothelial cells (Matsushita et al., 2000). Furthermore, in vivo transfection of NF-κB decoy has been shown to attenuate the development of autoimmune myocarditis (Yokoseki et al., 2001). This strategy may also be adapted to treat other disease entities involving de-regulated activation of NF-κB including proliferative disorders such as cancer (Biswas et al., 2003, 2004).

Although many studies support the use of "decoy" technology to inactivate certain genes or genetic pathways to "treat" a given pathology, several considerations must be kept in mind. First, given that many genes undergo alternative splicing, thereby resulting in gene products with different functions within the cell, the complete inactivation of a given genetic element and/or pathway may have detrimental cellular consequences. Therefore, it may be important to design decoy molecules that could be selectively "turned on" or "turned off" to prevent untoward effects. Second, specificity of the decoy ODN in question may be an issue because overlapping or cryptic sites within DNA may result in nonspecific effects from inactivation of members of one or more gene family. Third, safety issues regarding vector type and administration technique must be considered, especially given that the ultimate goal is to modulate gene expression in humans.

Nevertheless, given that the inflammatory process represents a major cause of morbidity in patients with endotoxin-induced sepsis, the elegant work of Matsuda et al. (2005) provides new compelling evidence that oligonucleotide decoys directed against the transcription factor NF-κB may be an effective treatment for averting, or at least attenuating, the inflammatory response at the genetic level.

References

Baeuerle PA and Baichwal VR (1997) NF-kappa B as a frequent target for immunosuppressive and anti-inflammatory molecules. *Adv Immunol* **65:**111–137.

Baeuerle PA and Baltimore D (1988) I kappa B: a specific inhibitor of the NF-kappa B transcription factor. *Science (Wash DC)* **242:**540–546.

Biswas DK, Martin KJ, McAlister C, Cruz AP, Graner E, Dai SC, and Pardee AB (2003) Apoptosis caused by chemotherapeutic inhibition of nuclear factor-kappaB activation. *Cancer Res* **63:**290–295.

Biswas DK, Shi Q, Baily S, Strickland I, Ghosh S, Pardee AB, and Iglehart JD (2004) NF-kappa B activation in human breast cancer specimens and its role in cell proliferation and apoptosis 1. *Proc Natl Acad Sci USA* **101:**10137–10142.

Brand K, Page S, Rogler G, Bartsch A, Brandl R, Knuechel R, Page M, Kaltschmidt C, Baeuerle PA, and Neumeier D (1996) Activated transcription factor nuclear factor-kappa B is present in the atherosclerotic lesion. *J Clin Investig* **97:**1715–1722.

Desmet C, Gosset P, Pajak B, Cataldo D, Bentires-Alj M, Lekeux P, and Bureau F (2004) Selective blockade of NF-kappa B activity in airway immune cells inhibits the effector phase of experimental asthma 60. *J Immunol* **173:**5766–5775.

Ghosh S and Karin M (2002) Missing pieces in the NF-kappaB puzzle. *Cell* **109 (Suppl):**S81–S96.

Hansen SK, Nerlov C, Zabel U, Verde P, Johnsen M, Baeuerle PA, and Blasi F (1992) A novel complex between the p65 subunit of NF-kappa B and c-Rel binds to a DNA element involved in the phorbol ester induction of the human urokinase gene. *EMBO (Eur Mol Biol Organ) J* **11:**205–213.

Karin M (1999) The beginning of the end: IκB kinase (IKK) and NF-κB activation. *J Biol Chem* **274:**27339–27342.

Li ZW, Chu W, Hu Y, Delhase M, Deerinck T, Ellisman M, Johnson R, and Karin M (1999) The IKKbeta subunit of IkappaB kinase (IKK) is essential for nuclear factor kappaB activation and prevention of apoptosis. *J Exp Med* **189:**1839–1845.

Maniatis T (1999) A ubiquitin ligase complex essential for the NF-kappaB, wnt/wingless and hedgehog signaling pathways. *Genes Dev* **13:**505–510.

Matsuda N, Hattori Y, Jesmin S, and Gando S (2005) Nuclear factor-κB decoy oligodeoxynucleotides prevent acute lung injury in mice with cecal ligation and puncture-induced sepsis. *Mol Pharmacol* **67:**1018–1025.

Matsushita H, Morishita R, Nata T, Aoki M, Nakagami H, Taniyama Y, Yamamoto K, Higaki J, Yasufumi K, and Ogihara T (2000) Hypoxia-induced endothelial apoptosis through nuclear factor-kappaB (NF-kappaB)-mediated bcl-2 suppression: in vivo evidence of the importance of NF-kappaB in endothelial cell regulation. *Circ Res* **86:**974–981.

McKinsey TA, Brockman JA, Scherer DC, Al Murrani SW, Green PL, and Ballard DW (1996) Inactivation of IkappaBbeta by the tax protein of human T-cell leukemia virus type 1: a potential mechanism for constitutive induction of NF-kappaB. *Mol Cell Biol* **16:**2083–2090.

Miyamoto S and Verma IM (1995) Rel/NF-Kappa B/I Kappa B story. *Adv Cancer Res* **66:**255–292.

Morishita R and Kaneda Y (2002) HVJ (hemagglutinating virus of Japan; sendai virus)-liposome method 100. *Methods Enzymol* **346:**619–627.

Morishita R, Sugimoto T, Aoki M, Kida I, Tomita N, Moriguchi A, Maeda K, Sawa Y, Kaneda Y, Higaki J, and Ogihara T (1997) In vivo transfection of cis element "decoy" against nuclear factor-kappaB binding site prevents myocardial infarction 9. *Nat Med* **3:**894–899.

Nabel GJ and Verma IM (1993) Proposed NF-kappa B/I kappa B family nomenclature (Letter). *Genes Dev* **7:**206319.

Nolan GP and Baltimore D (1992) The inhibitory ankyrin and activator Rel proteins. *Curr Opin Genet Dev* **2:**211–220.

O'Mahony A, Lin X, Geleziunas R, and Greene WC (2000) Activation of the heterodimeric IkappaB kinase alpha (IKKalpha)-IKKbeta complex is directional: IKKalpha regulates IKKbeta under both basal and stimulated conditions. *Mol Cell Biol* **20:**1170–1178.

Scheinman RI, Cogswell PC, Lofquist AK, and Baldwin AS Jr (1995) Role of Transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. *Science (Wash DC)* **270:**283–286.

Scherer DC, Brockman JA, Chen Z, Maniatis T, and Ballard DW (1995) Signal-induced degradation of I kappa B alpha requires site- specific ubiquitination. *Proc Natl Acad Sci USA* **92:**11259–11263.

Schmitz ML and Baeuerle PA (1991) The P65 subunit is responsible for the strong transcription activating potential of NF-kappa B. *EMBO (Eur Mol Biol Organ) J* **10:**3805–3817.

Tanaka A, Konno M, Muto S, Kambe N, Morii E, Nakahata T, Itai A, and Matsuda H (2005) A novel NF-κB inhibitor, IMD-0354, suppresses neoplastic proliferation of human mast cells with constitutively activated C-kit receptors 18. *Blood*, in press.

Ueno T, Sawa Y, Kitagawa-Sakakida S, Nishimura M, Morishita R, Kaneda Y, Kohmura E, Yoshimine T, and Matsuda H (2001) Nuclear factor-kappa B decoy attenuates neuronal damage after global brain ischemia: a future strategy for brain protection during circulatory arrest 5. *J Thorac Cardiovasc Surg* **122:**720–727.

Urban MB and Baeuerle PA (1990) The 65-kD subunit of NF-kappa B Is a receptor for I kappa B and a modulator of DNA-binding specificity. *Genes Dev* **4:**1975–1984.

Urban MB and Baeuerle PA (1991) The role of the p50 and p65 subunits of NF-kappa B in the recognition of cognate sequences. *New Biol* **3:**279–288.

Van Antwerp DJ and Verma IM (1996) Signal-induced degradation of I(kappa)B(alpha): association with NF-kappaB and the PEST sequence in I(kappa)B(alpha) are not required. *Mol Cell Biol* **16:**6037–6045.

Verma IM, Stevenson JK, Schwarz EM, Van Antwerp D, and Miyamoto S (1995) Rel/NF-kappa B/I kappa B family: intimate tales of association and dissociation. *Genes Dev* **9:**2723–2735.

Yokoseki O, Suzuki J, Kitabayashi H, Watanabe N, Wada Y, Aoki M, Morishita R, Kaneda Y, Ogihara T, Futamatsu H, et al. (2001) cis Element decoy against nuclear factor-kappaB attenuates development of experimental autoimmune myocarditis in rats 7. *Circ Res* **89:**899–906.

Zabel U, Henkel T, Silva MS, and Baeuerle PA (1993) Nuclear uptake control of NF-kappa B by MAD-3, an I kappa B protein present in the nucleus. *EMBO (Eur Mol Biol Organ) J* **12:**201–211.

Address correspondence to: Dr. Lorrie A. Kirshenbaum, Institute of Cardiovascular Sciences, St. Boniface General Hospital Research Centre Rm. 3016, 351 Taché Avenue, Winnipeg, Manitoba, Canada, R2H 2A6. E-mail: lorrie@sbrc.ca