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Nuclear Factor-κB — A Pivotal Transcription Factor in Chronic Inflammatory Diseases

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In chronic inflammatory diseases, such as asthma, rheumatoid arthritis, inflammatory bowel disease, and psoriasis, several cytokines recruit activated immune and inflammatory cells to the site of lesions, thereby amplifying and perpetuating the inflammatory state. These activated cells produce many other mediators of inflammation.

What causes these diseases is still a mystery, but the disease process results from an interplay of genetic and environmental factors. Genes, such as those for atopy in asthma and for HLA antigens in rheumatoid arthritis and inflammatory bowel disease, may determine a patient's susceptibility to the disease and the disease's severity, but environmental factors, often unknown, may determine its course. Once established, a chronic inflammatory process appears to take on a momentum of its own. The vicious circle may be suppressed by glucocorticoid or immunosuppressive therapy, but there is no curative treatment for any chronic inflammatory disease.

Our understanding of the molecular mechanisms through which environmental signals alter gene expression has increased considerably. There are genespecific factors that regulate the transcription of target genes by binding to specific recognition elements, which usually are located in the upstream (5') promoter region of the gene. These factors usually increase the rate of transcription of the gene and therefore increase the formation of messenger RNA and protein. Many of these transcription factors are cell-specific and are crucial in cell differentiation and the regulation of specific cellular processes such as proliferation. Other transcription factors are ubiquitous, and their activity may be modulated by environmental signals. It is this latter transcription factors that may have a key role in immune and inflammatory responses. One ubiquitous transcription factor of particular importance in immune and inflammatory responses is nuclear factor-κB (NF-κB).3

NF-κB

NF-κB was first identified as a regulator of the expression of the kappa light-chain gene in murine B lymphocytes4 but has subsequently been found in many different cells. Several different NF-κB proteins have been characterized.5,6 The activated form of NF-κB is a heterodimer, which usually consists of two proteins, a p65 (also called relA) subunit and a p50 subunit. Other subunits, such as rel, relB, v-rel, and p52, may also be part of activated NF-κB, and it is likely that the different forms of NF-κB may activate different sets of target genes. In unstimulated cells, NF-κB is found in cytoplasm and is bound to IκBa and IκBβ, which prevent it from entering the nucleus.7 When these cells are stimulated, specific kinases phosphorylate IκB, causing its rapid degradation by proteasomes8-9 (Fig. 1). The release of NF-κB from IκB results in the passage of NF-κB into the nucleus, where it binds to specific sequences in the promoter regions of target genes.

Because the IκBα gene (previously called MAD-3) has a κB recognition sequence in its promoter region, NF-κB induces the synthesis of IκBα, which enters the nucleus to bind to activated NF-κB and carry the NF-κB to the cytoplasm, thereby terminating the activation of gene expression.10 The targeted disruption of IκBα in mice results in prolonged activation of NF-κB in response to inflammatory stimuli, and the animals die of widespread inflammation.11 By contrast, the synthesis of IκBβ is not induced by NF-κB, so NF-κB is likely to be activated (bound to DNA) for a more prolonged period in the types of cells in which IκBβ predominates.12

Many stimuli activate NF-κB, including cytokines, activators of protein kinase C, viruses, and oxidants5,6 (Table 1). Several signal-transduction pathways may be involved, but all these stimuli act by means of protein kinases that phosphorylate (and thus degrade) IκB. Specific kinases of IκB have recently been characterized.13 Antioxidants, such as pyrrolidine dithiocarbamate and acetyl cysteine, may block the activation of some of these protein kinases,
which suggests that reactive oxygen species have an intermediary role.\textsuperscript{14}

NF-κB regulates the expression of many genes involved in immune and inflammatory responses.\textsuperscript{3} It is not the only transcription factor involved in regulating these genes, however, and it frequently functions together with other transcription factors, such as activator protein 1 (AP-1) and the nuclear factor of interleukin-6, that are also involved in the regulation of inflammatory and immune genes.\textsuperscript{15,16}

NF-κB acts on genes for proinflammatory cytokines, chemokines (chemotactic cytokines that attract inflammatory cells to sites of inflammation), enzymes that generate mediators of inflammation, immune receptors, and adhesion molecules that play a key part in the initial recruitment of leukocytes to sites of inflammation (Table 2). The activation of NF-κB therefore leads to a coordinated increase in the expression of many genes whose products mediate inflammatory and immune responses. For example, the coordinated stimulation of the expression of the genes for E-selectin, interleukin-8, and tumor necrosis factor α (TNF-α) results in the recruitment and activation of neutrophils.

Products of the genes that are regulated by NF-κB also cause the activation of NF-κB. The proinflammatory cytokines interleukin-1β and TNF-α both activate and are activated by NF-κB. This type of positive regulatory loop may amplify and perpetuate local inflammatory responses (Fig. 2).

\section*{THE ROLE OF NF-κB IN INFLAMMATORY DISEASES}

NF-κB increases the expression of the genes for many cytokines, enzymes, and adhesion molecules in chronic inflammatory diseases. One such gene is that for inducible nitric oxide synthase,\textsuperscript{17,27} the expression of which is increased in airway epithelial cells and macrophages in patients with asthma,\textsuperscript{18} in colonic epithelial cells in patients with ulcerative colitis,\textsuperscript{19} and in synovial cells in inflamed joints.\textsuperscript{20} This increased expression is reflected by an increased amount of nitric oxide in the exhaled breath of patients with asthma\textsuperscript{21} and in the colons of patients with active ulcerative colitis,\textsuperscript{22} as well as by elevated urinary nitrite concentrations in patients with rheumatoid arthritis.\textsuperscript{20} Cyclooxygenase-2, another inducible enzyme regulated by NF-κB,\textsuperscript{23} is responsible for the increased production of prostaglandins and thromboxane in inflammatory diseases.\textsuperscript{24}

In all chronic inflammatory diseases, adhesion molecules recruit inflammatory cells, such as neutrophils, eosinophils, and T lymphocytes, from the circulation to the site of inflammation.\textsuperscript{25} NF-κB regulates the expression of several genes that encode adhesion molecules such as intercellular adhesion molecule 1, vascular-cell adhesion molecule 1, and E-selectin.

The production of interleukin-1β, TNF-α, interleukin-6, granulocyte–macrophage colony-stimulating factor, and many chemokytic cytokines (chemokines) is increased in patients with asthma, rheumatoid arthritis, psoriasis, and inflammatory bowel disease. All these cytokines have important roles in the inflammatory process. Interleukin-1β and TNF-α may influence the severity of disease, possibly by the persistent activation of NF-κB. The treatment of patients with rheumatoid arthritis with antibodies to TNF-α can control refractory disease.\textsuperscript{26}

Infections with viruses, such as rhinovirus and influenza virus, can trigger severe acute exacerbations of asthma by initiating a prolonged inflammatory response. Experimental infection with rhinovirus activates NF-κB and stimulates the secretion of inter-
leukin-6 in nasal epithelial cells. In other inflammatory diseases, viruses may activate NF-κB through mechanisms that involve the generation of reactive oxygen intermediates or the activation of protein kinases that result in phosphorylation of IκB. Oxidative stress may also exacerbate inflammation. For example, in animals the inhalation of ozone induces inflammation in the lower respiratory tract and stimulates inflammatory genes controlled by NF-κB.

There have been relatively few direct measurements of the activation of NF-κB in inflammatory cells or in inflamed tissues. The exposure of human peripheral-blood mononuclear cells, epithelial cells, or lung tissue to proinflammatory cytokines such as interleukin-1β and TNF-α or to oxidants results in the marked activation of NF-κB. Similarly, in animals, the activation of T lymphocytes by anti-CD3 antibodies results in marked activation of NF-κB. NF-κB may also be activated in macrophages in the sputum and in epithelial cells and macrophages in bronchial-biopsy specimens from patients with asthma (unpublished data), as well as in synoviocytes and endothelial cells in the joints of patients with rheumatoid arthritis (particularly those with active disease).

Although there are many similarities among the inflammatory responses in patients with arthritis, asthma, inflammatory bowel disease, and other inflammatory diseases, there are also important differences in the type of inflammatory cells involved and in the mediators of inflammation. These differences may relate to the secretion of specific cytokines, such as interleukin-1β and TNF-α or to oxidants results in the marked activation of NF-κB. Similarly, in animals, the activation of T lymphocytes by anti-CD3 antibodies results in marked activation of NF-κB. NF-κB may also be activated in macrophages in the sputum and in epithelial cells and macrophages in bronchial-biopsy specimens from patients with asthma (unpublished data), as well as in synoviocytes and endothelial cells in the joints of patients with rheumatoid arthritis (particularly those with active disease).

**EFFECTS OF GLUCOCORTICOIDs ON NF-κB**

The molecular mechanism of the effects of glucocorticoids on chronic inflammation is not well understood, but there is increasing evidence that they inhibit the action of transcription factors such as AP-1 and NF-κB. In the cytoplasm, glucocorticoids activate glucocorticoid receptors, which then move to the nucleus, where they bind as homodimers to glucocorticoid-response elements in steroid-responsive target genes, resulting in increased transcription. However, glucocorticoids decrease the transcription of the genes involved in inflammation,
and these genes have no identifiable glucocorticoid-response elements in their promoter regions, suggesting that some other mechanism must mediate the inhibitory effect of the hormones. There may be a direct protein–protein interaction between the glucocorticoid receptor and AP-1 and between the receptor and NF-kB. Thus, glucocorticoids activate glucocorticoid receptors that may then bind to activated NF-kB and prevent it from binding to kB sites on genes that have a role in inflammatory processes (Fig. 3). This interaction may occur in the cytoplasm or the nucleus.

Glucocorticoids also increase transcription of the gene for IκBα, thereby increasing formation of this protein, which binds to activated NF-κB in the nucleus. The IκBα protein probably induces the dissociation of NF-κB from κB sites on target genes and causes NF-κB to move to the cytoplasm (Fig. 3). This mechanism has been observed in T cells and monocytes, but it may not occur in all types of cells. Glucocorticoids are potent inhibitors of the activation of NF-κB, which may account for most of their antiinflammatory actions.

In patients with asthma that is resistant to the antiinflammatory effects of glucocorticoids, there appears to be an exaggerated activation of AP-1 that binds to and therefore sequesters activated glucocorticoid receptors inside the nucleus. This would reduce the availability of glucocorticoid receptors to inhibit NF-κB, which is normally active in such patients.

**THERAPEUTIC IMPLICATIONS**

NF-κB is activated by many of the factors (viral infection, oxidants, and antigens) that increase the inflammatory response. This activation in turn leads to the coordinated expression of many genes that encode proteins (such as cytokines, chemokines, adhesion molecules, and enzymes) involved in mediator synthesis and the further amplification and perpetuation of the inflammatory response. NF-κB is therefore an obvious target for new types of antiinflammatory treatment. Glucocorticoids are effective inhibitors of NF-κB, but they have endocrine and metabolic side effects when given systemically. These side effects might not occur with a more spe-
specific NF-κB inhibitor. Antioxidants that inhibit the activation of NF-κB\textsuperscript{14} represent a class of compounds that has not yet been extensively investigated. Because currently available antioxidants, such as vitamins C and E and acetylcysteine, are relatively weak, more potent and long-lasting antioxidants are needed. Aspirin and sodium salicylate also inhibit the activation of NF-κB, albeit only in relatively high concentrations,\textsuperscript{46} and gold salts inhibit the binding of NF-κB to DNA,\textsuperscript{47} which suggests that the antiinflammatory effects of these drugs may be at least in part attributable to the inhibition of NF-κB.

Some naturally occurring inhibitors of NF-κB have been identified; gliotoxin, derived from aspergillus, is a potent and relatively specific inhibitor.\textsuperscript{48} The antiinflammatory cytokine interleukin-10 also inhibits the action of NF-κB, through an effect on IκBα.\textsuperscript{49} Preliminary findings suggest that interleukin-10 has an antiinflammatory effect in patients with inflammatory bowel disease.\textsuperscript{50} The adenovirus-mediated gene transfer of IκBα has been reported to inhibit the activation of endothelial cells.\textsuperscript{51} Now that specific IκB kinases have been characterized,\textsuperscript{13} it is likely that selective inhibitors may be identified by screening libraries of chemical compounds.

It may be unwise to block the activation of NF-κB for prolonged periods, because the factor plays such a critical part in the immune response and other defensive responses. The targeted disruption (or knockout) of the p65 component of NF-κB is lethal because of the associated developmental abnormalities,\textsuperscript{52} whereas the lack of the p50 component results in immune deficiencies and increased susceptibility to infection.\textsuperscript{53} Since NF-κB often works in concert with other transcription factors, it may be possible to achieve a more selective blockade in particular types of cell or a blockade of a restricted set of genes by developing compounds that inhibit the synergistic interactions of several transcription factors.

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**Figure 3. Schematic Diagram of the Effects of Glucocorticoids on NF-κB Activation.**

Activation of NF-κB, for example by cytokines, is blocked by glucocorticoids. Glucocorticoid-receptor complexes bind to the p65 subunit of NF-κB, and this prevents NF-κB activation of inflammatory genes. Synthesis of IκBα is stimulated by the binding of glucocorticoid–glucocorticoid-receptor complexes to a glucocorticoid response element in the promoter region of the IκBα gene. A red X denotes a blocked process, and mRNA denotes messenger RNA.
REFERENCES


