



Review

The NF κ B/I κ B System in Acute Inflammation

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Abstract. The transcription factor NF κ B is a primary regulator of a wide variety of proinflammatory mediators. Under normal conditions, NF κ B is retained in the cytoplasm bound to inhibitory proteins of the I κ B family. Following cell activation, a number of signal transduction pathways lead to degradation of I κ B proteins which results in nuclear translocation of NF κ B and the ensuing transcriptional activation of proinflammatory genes. Several endogenous regulatory mediators, which function to prevent uncontrolled inflammation, exert their effects by blocking the activation of NF κ B. Thus, NF κ B appears to be at the heart of the acute inflammatory response. The present review discusses the role of NF κ B in the induction and propagation of the acute inflammatory response as well as the regulation and resolution of this process.

Key words: acute inflammatory response; transcription factor; inhibitory I κ B proteins.

The acute inflammatory response is characterized by a complex mediator cascade that serves to activate residential macrophages and recruit blood leukocytes to the site of injury. While acute inflammation is essential for host defense and wound healing, dysregulated inflammatory responses, or inflammatory responses that are disproportionate to the immune challenge, are at the core of most inflammatory and autoimmune diseases. Knowledge of the steps leading to activation of the inflammatory response and an understanding of the regulatory mechanisms that serve to control the progression and/or resolution of the inflammatory response are prerequisite for the development of therapeutic strategies. Recent advances in the field of inflammation have provided important information about the cellular and molecular pathways which positively or negatively regulate the inflammatory response in vital organs.

Much of our work has centered around acute in-

flammatory injury of lung and liver. Lung injury induced in rats by distal airway deposition of IgG immune complexes has been used for many years for the study of lung inflammation¹⁷. The mediator pathways in this model are much like the events occurring during lung injury caused by ischemia³ or bacterial infections^{18, 36}. Ischemia/reperfusion injury of liver results in both local and remote organ injury^{5, 15}. Hepatic ischemia/reperfusion injury may occur during liver resectional surgery¹³, liver transplantation¹⁹, and complications of hemorrhagic shock⁴⁰.

In these models of acute inflammatory injury, the initial insult causes complement activation as well as activation of tissue macrophages^{14, 41}. Activated residential macrophages generate the proinflammatory cytokines, tumor necrosis factor α (TNF- α) and interleukin 1 (IL-1)^{6, 30}. A primary function of these cytokines is the induction of vascular endothelial cell expression

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of adhesion molecules which facilitate adhesion of circulating neutrophils to the vascular endothelium³⁷. Another function of TNF- α and IL-1 is the induction of neutrophil-attracting chemokines³³. The combined efforts of adhesion molecules and chemokines mediate the adhesion and transmigration of neutrophils from the lumen of small blood vessels to tissue interstitium³⁸. In lung and liver, tissue accumulation of activated neutrophils contributes to tissue injury through the release of oxidants and proteases^{16, 39}.

Regulation of Inflammatory Mediator Production by NF κ B

NF κ B is rapidly activated transcription factor which is known to be a primary regulator of the gene expression of many proinflammatory mediators. NF κ B is a general term used to describe a number of dimeric combinations of proteins of the Rel family which possess transcriptional activating properties¹¹. The primary form of NF κ B consists of a heterodimer of p50 and p65 proteins. This complex has the ability to bind with promoter sequences in DNA and to initiate transcription for many proinflammatory mediators. Other combinations of Rel family members have been identified, however, and different configurations of Rel proteins (e.g. p65/p50, p65/p52, etc.) may have preferential sensitivities to different target promoter sequences³². In unstimulated cells, NF κ B resides in the cytoplasm bound to inhibitory proteins of the I κ B family. Currently, seven I κ B proteins have been identified in mammalian cells¹¹. All I κ B proteins contain ankyrin repeat domains which function to facilitate protein-protein interactions. The ankyrin repeat domains of I κ B proteins prevent nuclear translocation of NF κ B by masking nuclear localization sequences of the NF κ B (hetero) dimers. In response to inflammatory stimuli, such as oxidant stress or TNF- α , the NF κ B inducing kinase (NIK) is phosphorylated (Fig. 1)²⁷. NIK then phosphorylates the I κ B kinase (IKK) complex¹⁰. The IKK complex phosphorylates I κ B proteins, which targets I κ B for ubiquitination and degradation. Dissociation and degradation of I κ B results in "activation" of NF κ B, which may be defined as translocation of the NF κ B complex from the cytoplasm to the nucleus. After translocation to the nucleus, NF κ B binds specific promoter elements of DNA and induces transcription of relevant genes. The proinflammatory mediators under the control of NF κ B include cytokines (i.e. TNF- α and IL-1)^{4, 12}, chemokines (i.e. IL-8 family)⁴², and endothelial cell adhesion molecules (i.e. E-selectin, ICAM-1)⁹.

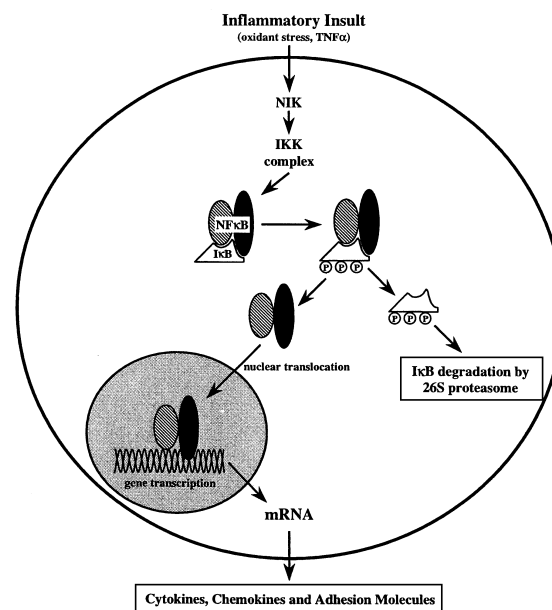


Fig. 1. Intercellular signalling pathways leading to activation of NF κ B. NIK, NF κ B-inducing kinase; IKK, I κ B kinase

The NF κ B/I κ B System during Acute Lung Inflammation

In a rat model of acute lung injury induced by intrapulmonary deposition of IgG immune complexes, the temporal pattern of NF κ B activation has been determined^{21, 22}. In this model, alveolar macrophages are activated by the inflammatory insult and rapidly exhibit (within 30 min) increased nuclear translocation of NF κ B. Activation of NF κ B in alveolar macrophages is associated with enhanced production of the proinflammatory cytokines TNF- α and IL-1³⁰. These proinflammatory cytokines are known to induce the expression of chemokines and vascular adhesion molecules within the lung^{29, 35}. When TNF- α or IL-1 were neutralized with blocking antibodies, lung NF κ B activation was greatly reduced²¹. In addition, when alveolar macrophages were depleted using liposome-encapsulated dichloromethylene diphosphonate, intrapulmonary activation of NF κ B was ameliorated²⁰. In rats depleted of alveolar macrophages, intrapulmonary activation of NF κ B was restored after lung instillation of TNF- α . Thus, it appears that during acute lung inflammation, activation of NF κ B in alveolar macrophages is responsible for proinflammatory cytokine production. Proinflammatory cytokines, such as TNF- α and IL-1, appear to propagate the lung inflammatory response by activating NF κ B in other lung cell types, resulting in the expression of chemokines and vascular adhesion molecules.

The role of NF κ B activation in acute lung inflammation has also been studied with agents that block nuclear translocation of NF κ B. *In vitro*, antioxidants have been shown to prevent the phosphorylation and degradation of I κ B and reduce nuclear translocation of NF κ B¹¹. *In vivo*, administration of the antioxidant, N-acetylcysteine, suppressed lung NF κ B activation induced by intrapulmonary deposition of IgG immune complexes or by intraperitoneal injection of lipopolysaccharide^{1, 21}. However, administration of another antioxidant, catalase, was unable to inhibit lung NF κ B. It was suggested that since N-acetylcysteine is such a small molecule (163 Da) which easily diffuses across cell membranes, and catalase is very large (~240 kDa) and probably does not gain cellular entry, that intracellular oxidants are involved in the activation of NF κ B in lung.

Antioxidants are not specific inhibitors of NF κ B activation. These agents also scavenge oxidants released from activated phagocytes and reduce oxidant-mediated tissue injury. As such, these studies did not provide detailed mechanistic information regarding the role of NF κ B in acute inflammation. Other studies employing anti-inflammatory cytokines known to regulate the production of TNF- α and IL-1 have provided important information both about the inhibitory actions of these cytokines *in vivo* as well as information about the role of NF κ B in the acute inflammatory response. Two of the most potent anti-inflammatory cytokines, IL-10 and IL-13, are known to suppress lung inflammatory injury³¹. The inhibitory effects of IL-10 and IL-13 in lung were found to be associated with almost complete suppression of NF κ B activation²⁴. Both IL-10 and IL-13 prevented NF κ B activation by preserving the cytoplasmic expression of the I κ B protein, I κ B α . Endogenous production of IL-10 and IL-13 in lung serve as a negative feedback loop of the inflammatory response, probably limiting the progression of inflammation by suppressing NF κ B activation^{22, 34}.

Another endogenous anti-inflammatory mediator, a serine protease inhibitor known as secretory leukocyte protease inhibitor (SLPI), has been shown to suppress lung inflammatory injury as well as inhibit the activation of NF κ B^{23, 28}. Similar to IL-10 and IL-13, the inhibitory effects of SLPI were associated with up-regulation of an I κ B protein. In the case of SLPI, however, expression of I κ B α was unaffected, but protein expression of I κ B β was induced. When endogenous SLPI was neutralized with blocking antibody, activation of NF κ B was enhanced and the lung inflammatory response was augmented. It appears that in the lung, NF κ B activation and the ensuing lung injury is regu-

lated by the endogenous production of IL-10, IL-13 and SLPI.

The NF κ B/I κ B System during Acute Liver Inflammation

Acute liver inflammation induced by hepatic ischemia and reperfusion in mice is also associated with increased activation of NF κ B. In this model, activation of NF κ B in the liver was shown to occur shortly after reperfusion^{2, 43, 45}. The time of NF κ B activation is consistent with upregulation of vascular cell adhesion molecules and chemokines within the liver^{6, 7, 25}. Studies of NF κ B activation in specific liver cell types are complicated by the inability to easily isolate different populations of liver cells (i.e. Kupffer cells, hepatocytes, etc.). It is likely that NF κ B activation occurs first in Kupffer cells and then is propagated to hepatocytes and other liver cells, but this hypothesis has yet to be proven. Similar to studies in lung injury models, treatment with antioxidants suppressed NF κ B activation and reduced liver injury⁴⁶. As in lung, administration of IL-10 reduced inflammatory liver injury and inhibited activation of NF κ B⁴³. SLPI also suppresses liver NF κ B activation and attenuates liver injury²⁶. These effects are of interest because, unlike the lung, in which NF κ B activation is associated with degradation of I κ B α , activation of NF κ B during hepatic ischemia/reperfusion occurs without measurable degradation of either I κ B α or I κ B β ⁴⁵. As possible explanation is that exogenously administered IL-10 or SLPI may augment production of I κ B proteins and thus inhibit nuclear translocation of NF κ B. IL-13, however, does not affect NF κ B activation induced by hepatic ischemia and reperfusion. IL-13 effectively suppresses inflammatory liver injury in this model, but these effects were found to be associated with IL-13-induced activation of the transcription factor, STAT6, and not inhibition of NF κ B⁴⁴.

Conclusions

It is now clear that activation of NF κ B is an early, initiating event of the acute inflammatory response in lung and liver. Because NF κ B regulates the gene expression of mediators at every level of the inflammatory response (proinflammatory cytokines, chemokines, adhesion molecules), it represents an attractive target for therapeutic intervention. Endogenous regulation of NF κ B seems to occur through the induction of anti-inflammatory mediators, such as IL-10, IL-13 and SLPI, although the relative contribution of each of these may differ between various organs.

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