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Nuclear factor κ B: a pivotal role in the systemic inflammatory response syndrome and new target for therapy

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Introduction

Pathogenic stimuli that result in the systemic inflammatory response syndrome (SIRS) cause a broad range of host responses that include the production of protein and lipid mediators, expression of cell surface receptors and adhesion molecules, induction of enzymes and production of acute phase proteins, as well as activation of inflammatory cells. SIRS, which is referred to as sepsis when the inflammatory response is induced by infection, can lead to dire consequences, including multiple organ dysfunction syndrome (MODS), the acute respiratory distress syndrome (ARDS) and death. ARDS is a prototypic acute inflammatory lung disease and MODS associated with SIRS is characterized by diffuse organ damage with prominent neutrophilic inflammation. The paradox of SIRS is that host immune responses are critical for defense against infection, but excessive or dysregulated inflammation seems to result in neutrophil-mediated tissue injury and organ dysfunc-

tion. Understanding the molecular events that regulate neutrophilic inflammation could facilitate specific intervention to prevent host tissue injury in SIRS without substantially compromising the host defense.

In SIRS, neutrophilic inflammation appears to be the result of the local production of cytokines, chemokines, endothelial-leukocyte adhesion molecules and enzymes, such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), whose production is regulated by the ubiquitous transcription factor complex NF- κ B (Table 1). NF- κ B is a DNA binding protein necessary for directing high level transcription of many pro-inflammatory genes in tissue culture; however, the extent to which NF- κ B controls specific biological processes in vivo is still being investigated. In this review, we discuss briefly the relevant molecular biology of NF- κ B, including the process of activation of NF- κ B and the role of NF- κ B in the production of cytokines and other pro-inflammatory molecules. Within this context, we summarize information about NF- κ B in human disease and focus on clinically relevant treatment approaches that could attenuate activation of NF- κ B and

Table 1 Human pro-inflammatory molecules that are regulated by NF- κ B

<i>Tumor necrosis factor</i>	<i>Chemokines</i>
TNF α	IL-8
TNF β	Gro α , β , γ
<i>Colony stimulating factors</i>	RANTES
G-CSF	MCP-1/JE
GM-CSF	<i>Adhesion molecules</i>
<i>Interleukins</i>	ICAM-1
IL-1 β	E-selectin
IL-2	V-CAM
IL-6	<i>Enzymes</i>
IL-12	COX-2
<i>Interferons</i>	iNOS
IFN- β	

Table 2 Agents which activate NF- κ B that are involved in the pathogenesis of SIRS, MODS, and ARDS

- Endotoxemia
- IL-1 β
- TNF α
- Ischemia/reperfusion
- Hyperoxia, reactive oxygen species
- Volutrauma, barotrauma

diminish the expression of pro-inflammatory genes, potentially limiting tissue injury and inflammation and improving the outcome of SIRS.

Molecular Biology of Rel/NF- κ B

The Rel/NF- κ B family of transcription factors represents a distinct paradigm of nuclear transactivating factors whose activity is inducible via control of its nuclear localization. These enhancer binding proteins are sequestered in the cytoplasm by inhibitory molecules, termed I κ Bs. I κ B is destroyed upon cell stimulation, and the κ B factors enter the nucleus and associate with their cognate DNA binding sites, initiating gene transcription.

The Rel/NF- κ B family of transcriptional transactivators is a ubiquitous multiprotein complex specialized for rapid response of the cell to a wide variety of both normal and pathogenic agents [1] including those stimuli that are involved in the pathogenesis of SIRS, MODS, and ARDS (Table 2). NF- κ B acts as a transcription factor by binding to a decameric DNA sequence motif found in the promoters and introns of several genes, including those of the immunoglobulin κ light chain, the human immunodeficiency virus long terminal repeats, numerous cytokines, chemokines, adhesion molecules, enzymes and growth factor receptors.

The molecular and biochemical nature of the Rel/NF- κ B complex has been well reviewed [1–4] and will only be discussed in this review in a condensed form. Authentic NF- κ B is a heterodimer consisting of a 50 kD polypeptide (n κ B1 = p50) [5] and a 65 kD polypeptide (RelA = p65) [6]. Other Rel family proteins include c-Rel and p52 (n κ B2) which, along with p50 and RelA, can form various combinations of homodimers and heterodimers that regulate specific target gene transcription. The carboxy terminal domains of RelA(p65) and c-Rel contain strong transcriptional transactivation regions [7]. By contrast, p50 homodimers bind to DNA but are thought to block transcription since it lacks a transactivation domain [8].

I κ B was first described as a cytoplasmic protein which inhibits the DNA binding activity of the heterodimeric NF- κ B complex [9,10]. In the cytosol, I κ B proteins form complexes with heterodimeric NF- κ B and can be inactivated following stimulation of cells with a

wide variety of distinct agents including lipopolysaccharide (LPS), TNF α , IL-1 β , phorbol esters, growth factors, viral proteins and ultraviolet light. Some of these stimuli, endotoxemia, cytokinemia, ischemia/reperfusion, hyperoxia and mechanical stress, are probably relevant to the pathophysiology of SIRS, MODS and ARDS. Like their binding partners (Rel/NF- κ B proteins), the I κ Bs are encoded by a small multigene family [1,4], which includes I κ B- α (MAD3, pp40, RL-IF1, ECI), I κ B- β , I κ B- γ and BCL-3.

Receptor-mediated activation of NF- κ B by TNF- α and IL-1 β

Recently, the signalling pathways from specific receptors to activation of NF- κ B have been worked out for both TNF- α and IL-1 β [11–14]. TNF- α activates NF- κ B primarily through binding to the type 1 TNF receptor (TNFR1), which then induces a signal transduction cascade through several intermediate signalling proteins, resulting in activation of NF- κ B-inducing kinase (NIK), a member of the mitogen-activating protein (MAP) kinase kinase (MAP3 K) family [11]. When TNF- α binds to the TNFR1, there is an association between the cytoplasmic domain of TNFR1 and the TNFR1-associated death domain protein (TRADD), the receptor-interacting protein (RIP), and the TNF receptor-associated factor-2 (TRAF-2) that forms an active signalling complex that interacts with NIK. Activation of NIK results in phosphorylation of I κ B kinases (IKK), which binds to and phosphorylates I κ B- α at serines 32 and 36 [11,12]. Phosphorylated I κ B- α is targeted for destruction by the ubiquitination/proteasome (26 S) degradation pathway, allowing the translocation of NF- κ B to the nucleus [1,15,16]. IL-1 β binding to its receptor also results in NIK and IKK activation, followed by I κ B- α degradation [17–20]. IL-1 β binds to the type 1 IL-1 receptor (IL-1R1) and the IL-1 receptor accessory protein (IL-1RAcP) facilitates an interaction between IL1 receptor-associated kinase (IRAK) and TNF receptor-associated factor-6 (TRAF-6) that results in activation of NIK, IKK and NF- κ B. In combination, these data seems to indicate that NIK is a common mediator in the NF- κ B signalling cascade that results from receptor mediated TNF and IL-1 stimulation. Although it is likely that activation by endotoxin (LPS) follows a similar scheme, the specific link to NIK has not yet been established. A simplified version of the TNF α and IL-1 β pathways is illustrated in Fig. 1.

The role of NF- κ B in human disease

Substantial *in vitro* data suggest that activation of NF- κ B is a critical proximal step in the inflammatory response.

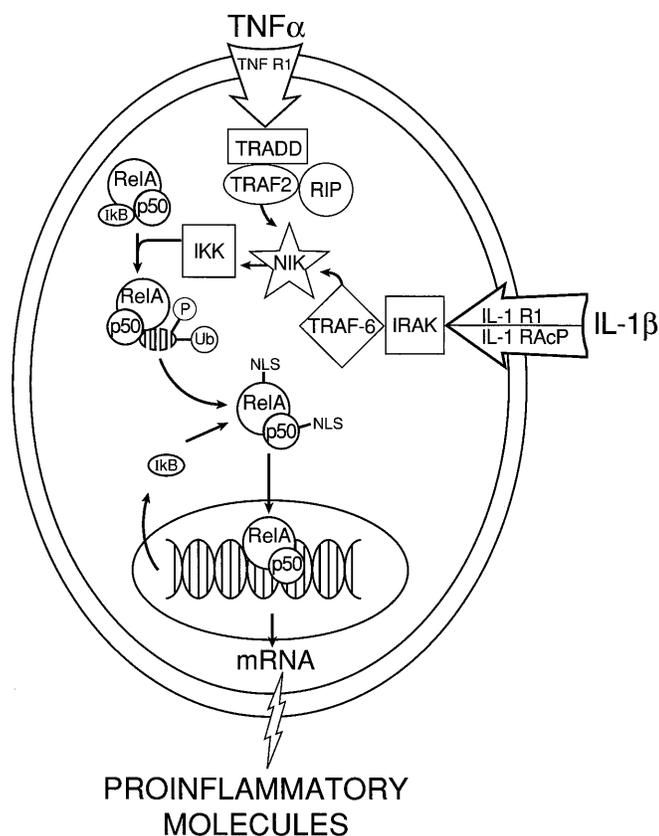


Fig. 1 TNF- α binds to the type 1 TNF receptor (TNFR1), which results in an association with TNFR1-associated death domain protein (TRADD), the receptor-interacting protein (RIP), and the TNF receptor-associated factor-2 (TRAF-2). These cytoplasmic proteins form an active signalling complex that interacts with NF- κ B-inducing kinase (NIK). Activation of NIK results in phosphorylation of I κ B kinases (IKK), which cause phosphorylation I κ B. Phosphorylated I κ B is targeted for destruction by the ubiquitination/proteasome degradation pathway, allowing the translocation of NF- κ B to the nucleus. IL-1 β binds to the type 1 IL-1 receptor (IL-1R1) and the IL-1 receptor accessory protein (IL-1RAcP) which facilitates an interaction between IL1 receptor-associated kinase (IRAK) and TNF receptor-associated factor-6 (TRAF-6). These proteins form an active signalling complex that also results in activation of NIK and IKK leading to the sequence of events that results in activation of NF- κ B. Activation of NF- κ B results in expression of mRNA of a variety of pro-inflammatory mediators (see Table 1) which are involved in the pathogenesis of SIRS, MODS and ARDS. I κ B is also induced by NF- κ B activation and contributes to the down-regulation of this intracellular signalling cascade

In addition, several studies have demonstrated a link between *in vivo* NF- κ B activation with cytokine production and the generation of inflammation in animal models of inflammatory diseases [21–27]. Investigations regarding the role of NF- κ B in humans have only recently been undertaken. In the peripheral blood monocytes of patients with sepsis, NF- κ B activation predicts mortality [28]. Specifically, all the patients in this study who died from

sepsis had increased NF- κ B activation (greater than twice baseline) in the first 6 days, whereas all the patients who survived had NF- κ B activation that remained less than twice baseline at each time point during the 14-day study period. Also, NF- κ B is activated in the alveolar macrophages from patients with ARDS to a significantly higher degree than in alveolar macrophages from critically ill patients with other diseases [29]. This finding correlates with previous reports that IL-8 and TNF α are increased in lung lavage in patients with ARDS [30–31].

Patients with other inflammatory diseases, including rheumatoid arthritis and Crohn's disease have been shown to have increased NF- κ B activation. In the synovium of patients with rheumatoid arthritis, NF- κ B activation is increased compared to patients with osteoarthritis [32]. In patients with Crohn's disease, NF- κ B activation in bowel sections is increased compared with controls [33]. In addition, NF- κ B has been implicated in the pathogenesis of several human malignancies [34] and in Alzheimer's disease [35]. Experimental evidence linking NF- κ B activation in specific cells or tissues with other inflammatory diseases in humans is lacking but should be forthcoming.

Interventions to block activation of NF- κ B

If NF- κ B activation proves to be an important determinant of systemic inflammation, interventions designed to limit NF- κ B activation could be beneficial in SIRS and other inflammatory diseases. Currently, no clinical studies have used inhibition of NF- κ B activation as a goal of therapy; however, several currently available therapeutic approaches could be used to target NF- κ B. Although blocking NF- κ B activation is likely to inhibit neutrophilic inflammation and diminish organ injury secondary to exuberant cytokine production, it is uncertain how this treatment approach would affect host defense functions. In future, it may be important to design clinical trials in SIRS and ARDS with the goal of blocking NF- κ B activation, either in tissue samples or in bronchoalveolar lavage or white blood cells. If NF- κ B activation can be effectively inhibited, the results of NF- κ B inhibition on clinical outcome can be assessed.

One strategy for blocking NF- κ B activation in SIRS is to prevent the interaction of TNF α , IL-1 β or endotoxin with responding cells. The proximal cytokines, as well as endotoxin, are fully capable of producing a sepsis-like syndrome when injected into animals or man and are known to stimulate NF- κ B activation in a variety of cell types *in vitro*. Agents that block TNF α , IL-1 and endotoxin, such as anti-TNF antibodies, soluble TNF receptors, IL-1 receptor antagonist, anti-endotoxin antibodies and soluble CD14, could limit NF- κ B activation and prevent inflammation and organ dysfunction in SIRS. Although these agents have been shown to be ef-

Table 3 Agents which block activation of NF- κ B and might be effective treatments for SIRS, MODS, and ARDS

- Antioxidants
- Inhibition of NF- κ B inducing kinase (NIK)
- Inhibition of the I- κ B kinase (IKK) signalsome
- Proteasome inhibitors
- Corticosteroids
- Induction of endotoxin tolerance

fective in limiting mortality and improving the pathophysiology of experimental SIRS, none has been shown to be efficacious in human studies. Blocking a single mediator, especially after the initiating insult, might be insufficient to inhibit NF- κ B significantly because of the redundancy of proximal mediators with the potential to activate NF- κ B. *In vitro* studies have shown that blocking the individual cytoplasmic components of the TNF α and IL-1 β activation pathways, including TRADD, RIP, TRAF-2 and TRAF-6, is an effective way to block activation of NF- κ B. Strategies that manipulate these intracellular signalling molecules have not been tried in animal models and are far from clinical trials in human diseases. Furthermore, none of these molecules appears to be both a critical and essential pathway for activation of NF- κ B. Several recent reviews, including our own, have addressed many of these anti-cytokine strategies and their limitations [36–37]. Here, we discuss several potential treatment strategies that suppress NF- κ B activation by a variety of stimuli and have the potential globally to down-regulate acute inflammation in SIRS (Table 3). While some of these approaches are already feasible (antioxidants, corticosteroids and induction of endotoxin tolerance), more specific treatments (inhibition of NIK and IKK, and the proteasome inhibitors) will be dependent on advances in technology and close cooperation between pharmaceutical companies and clinical scientists.

Antioxidants

In terms of NF- κ B inhibition, antioxidants are the best studied class of agents. Antioxidants have been investigated as inhibitors of NF- κ B activation because the generation of reactive oxygen species (ROS) is postulated to be a vital link in mediating NF- κ B activation by a variety of stimuli. Four lines of evidence support the role of ROS in NF- κ B activation. First, direct treatment with oxidants such as H₂O₂ activates NF- κ B in some cells [38]. Second, agents that activate NF- κ B in cells (including endotoxin, TNF α , IL-1 β and ionizing radiation) produce oxidative stress [38]. Third, antioxidants have been shown to inhibit NF- κ B activation in a variety of settings both *in vitro* [38] and *in vivo* [22]. Fourth, up-regulation of endogenous oxidant defenses has been

demonstrated to suppress NF- κ B activation [39]. In rats, we have shown that systemic endotoxin treatment induces oxidative stress and NF- κ B activation in lung tissue and that the antioxidant N-acetylcysteine (NAC) can inhibit lung tissue NF- κ B activation following systemic endotoxin treatment [22]. Activation of NF- κ B *in vitro* is also inhibited by a variety of other antioxidant agents including vitamin E derivatives [40–41], pyrrolidine dithiocarbamate (PDTC) [42] selenoproteins [43–44], ascorbic acid [45], dimethyl sulfoxide (DMSO) [46] and S-allyl cysteine (SAC) [47].

Since ROS appear to be important intermediates in NF- κ B activation, inhibiting this step might be beneficial in limiting inflammation in certain clinical settings. Several recent early clinical trials suggest that NAC and related compounds may be an effective treatment for ARDS, and it is possible that this benefit has been derived through blocking the activation of NF- κ B [48–50]. Studies in animal models of ARDS have indicated that effects of NAC have a complex dose-response relationship where low doses improve, but high doses worsen, parameters of lung injury [51].

Blocking NIK and IKK

Enormous efforts have been made to characterize the intracellular signalling process that leads to the activation of NF- κ B [52–60]. The critical common pathway that leads to activation of NF- κ B, activation of IKK by NIK, is rapidly being reported in the literature at an unprecedented pace. Two specific I κ B kinases (IKK α and IKK β) have been identified [11–13], which phosphorylate I κ B α on serine 32 and 36 that is required for processing via the ubiquitin-proteasome pathway. Overexpression of either an inactive IKK α or IKK β mutant associates with both I κ B α and NIK and blocks activation of NF- κ B by TNF α and IL-1 β [11, 13]. These data seem to indicate that the I κ B kinase complex, termed the I κ B kinase (IKK) signalsome, involves aggregation of two distinct kinases, IKK α and IKK β , that are activated by an interaction with NIK [61–62]. An inactive NIK mutant has been shown to block activation of NF- κ B TNF α and IL-1 β , which suggests NIK is a critical step that leads to activation of the IKK signalsome [63]. Recently, it has been shown that NIK activates the IKK signalsome by phosphorylating IKK α , but not IKK β , at serine 176 [14]. Currently there is a frenzied search for compounds that specifically block or antagonize NIK and/or the IKK signalsome since these agents could have broad clinical applications for the treatment of a wide variety of inflammatory diseases that include SIRS, MODS and ARDS. It is likely that a highly specific blockade of NIK and the IKK signalsome will be required because the use of less specific kinase blockers has yielded conflicting results [52–60].

Proteasome inhibitors

The 26 S proteasome degrades I κ B- α after it is targeted by phosphorylation and ubiquitination. Several studies have shown that inhibitors of the proteasome complex block NF- κ B activation and nuclear translocation in cell culture [64–68]. Recently, certain proteasome inhibitors have been given to rodents without significant toxicity. Administration of the proteasome inhibitor calpain inhibitor 1 by intraperitoneal injection in rats suppressed expression of two NF- κ B-dependent genes, iNOS and COX-2 [69]. Chemical proteasome inhibition will probably affect other cellular processes in addition to NF- κ B activation, such as antigen processing, cell cycle regulation and the processing of other transcription factors [70]. However, based on available *in vitro* data, proteasome inhibitors appear to be acceptable agents to suppress NF- κ B activation and could be beneficial for short-term treatment to limit inflammation if toxicity could be overcome.

Corticosteroids

Corticosteroids are a group of compounds with a broad range of effects on the immune system, and have long been known to be potent anti-inflammatory agents and potent *in vitro* suppressors of cytokine production [71]. Some of the anti-inflammatory effects of corticosteroids are mediated through inhibition of NF- κ B activation. Corticosteroids have been shown to block NF- κ B activation *in vitro* in two ways. First, glucocorticoid receptors can interact directly with RelA, the transactivating component of NF- κ B, to inhibit DNA binding [72]. More recently, two separate groups of investigators have shown that dexamethasone, a potent corticosteroid, increases the expression of I κ B- α mRNA and synthesis of functional I κ B- α protein [73, 74]. Although further research is necessary, inhibition of DNA binding of RelA and stabilization of cytoplasmic NF- κ B by a relative excess of newly synthesized I κ B may be an effective dual mechanism by which steroids block the synthesis of cytokines. Although corticosteroids have not proven to be beneficial in post-treatment studies of patients with SIRS and ARDS, sufficient inhibition of NF- κ B may not have been achieved, and glucocorticoids may have other effects that are detrimental in this condition.

Induction of Endotoxin Tolerance

Endotoxin tolerance refers to a phenomenon where by an endotoxin-triggered response is at least partially abrogated by prior exposure to endotoxin. Many investigators have reported that tolerance can be induced in both animal models and human volunteers by treatment

with repeated small doses of endotoxin. A consistent observation is that endotoxin-tolerant macrophages have blunted gene expression of cytokines, including TNF α , IL-1 β , IL-6 and IL-8, in response to treatment with endotoxin. Endotoxin-tolerant cells are cross tolerant to both TNF α and IL-1 β , suggesting that endotoxin tolerance is a fundamental process that occurs at the level of the cell.

We have recently investigated the effect of induction of endotoxin tolerance on NF- κ B-dependent responses by utilizing a rat model of neutrophilic lung inflammation. Rats were rendered endotoxin-tolerant by four daily injections of low dose endotoxin. When endotoxin-tolerant rats were treated with high dose intraperitoneal endotoxin injection, they had decreased NF- κ B activation and chemokine gene expression in lung tissue, as well as attenuated neutrophilic lung inflammation compared to endotoxin-sensitive rats [75–76]. Studies in a rat alveolar macrophage cell line indicate that the mechanism of the tolerance effect is related to depletion of RelA [77]. Other investigators have shown a second mechanism of *in vitro* endotoxin tolerance: increased gene expression of p105 that results in excess production and homodimerization of p50 [78]. This has the overall effect of preventing gene activation, since p50 homodimers do not bind to I κ B and can bind to the decameric NF- κ B DNA sequence, but lack a transactivating domain. Exploiting the natural phenomenon of endotoxin tolerance may be an effective way to suppress NF- κ B-dependent inflammation. Several studies in animal models and clinical trials have suggested that the induction of tolerance might be effective in selected patients with SIRS [79–81]. These data lead us to believe that induction of endotoxin could be used to prevent or relieve at least some sequelae of gram-negative sepsis through inhibition of NF- κ B-dependent inflammatory gene expression.

Summary

NF- κ B is an important transcription factor complex that appears to play a fundamental role in regulating acute inflammation through activation of the cytokine cascade and production of other pro-inflammatory mediators. There is increasing evidence that NF- κ B is important in the pathobiology of disease states such as SIRS, MODS and ARDS; therefore, therapeutic interventions aimed at limiting NF- κ B activation and down-regulating production of inflammatory mediators could prove to be beneficial in decreasing host-derived tissue injury and organ dysfunction. Specific interventions that hold promise for suppressing NF- κ B activation include the use of antioxidants, inhibition of NIK and the IKK signaling, treatment with proteasome inhibitors, induction of endotoxin tolerance and, possibly the use of corticosteroids in selected patients.

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