NF-κB activation as a pathological mechanism of septic shock and inflammation

Shu Fang Liu and Asrar B. Malik

Division of Pulmonary and Critical Care Medicine, Long Island Jewish Medical Center, The Long Island Campus for the Albert Einstein College of Medicine, New Hyde Park, New York; and Department of Pharmacology, University of Illinois at Chicago, Chicago, Illinois

Liu, Shu Fang, and Asrar B. Malik. NF-κB activation as a pathological mechanism of septic shock and inflammation. Am J Physiol Lung Cell Mol Physiol 290: L622–L645, 2006; doi:10.1152/ajplung.00477.2005.—The pathophysiology of sepsis and septic shock involves complex cytokine and inflammatory mediator networks. NF-κB activation is a central event leading to the activation of these networks. The role of NF-κB in septic pathophysiology and the signal transduction pathways leading to NF-κB activation during sepsis have been an area of intensive investigation. NF-κB is activated by a variety of pathogens known to cause septic shock syndrome. NF-κB activity is markedly increased in every organ studied, both in animal models of septic shock and in human subjects with sepsis. Greater levels of NF-κB activity are associated with a higher rate of mortality and worse clinical outcome. NF-κB mediates the transcription of exceptional large number of genes, the products of which are known to play important roles in septic pathophysiology. Mice deficient in those NF-κB-dependent genes are resistant to the development of septic shock and to septic lethality. More importantly, blockade of NF-κB pathway corrects septic abnormalities. Inhibition of NF-κB activation restores systemic hypotension, ameliorates septic myocardial dysfunction and vascular derangement, inhibits multiple proinflammatory gene expression, diminishes intravascular coagulation, reduces tissue neutrophil influx, and prevents microvascular endothelial leakage. Inhibition of NF-κB activation prevents multiple organ injury and improves survival in rodent models of septic shock. Thus NF-κB activation plays a central role in the pathophysiology of septic shock.

nuclear factor-κB; septic pathophysiology; cytokines; signal transduction
pertinence to inflammation and septic shock. We will review the current knowledge about the role of NF-κB and NF-κB pathways in septic pathophysiology. To better understand the role of NF-κB activation in septic MODI, we will also discuss the biological functions of NF-κB in a wide spectrum of physiological and pathophysiological processes.

DESCRIPTION

NF-κB family of proteins. NF-κB is a group of structurally related transcriptional proteins that form dimers composed of various combinations of members of the NF-κB/Rel family proteins. NF-κB proteins in mammalian cells include NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), RelB, and C-Rel (83, 119, 226). Except for a truncated form of RelA, RelA (p37), seen in cells overexpressing proto-oncogene c-Myc (37), no new NF-κB protein has been reported since the first description by Sen and Baltimore (213) of NF-κB as a B cell nuclear factor binding to a site in the immunoglobulin κ enhancer. The NF-κB family of proteins is characterized by the presence of a highly conserved 300-amino acid Rel homology domain (RHD) composed of two immunoglobin-like structures. RHD is responsible for dimerization, DNA binding, and association with their inhibitory proteins, IκBα (119, 225). One structural difference between RelB and RelA (or C-Rel) is that RelB protein contains an NH2-terminus-activating domain (225), which may explain the difference in its regulation. NF-κB1 (p50) and NF-κB2 (p52) are synthesized as their precursors, p105 and p100, which contain multiple copies of the ankyrin repeat at their COOH termini, a structural characteristic of all IκB proteins (1, 119, 225). p105 and p100 can serve as p50 and p52 precursors as well as regulatory proteins. Limited proteolysis of p105 or p100 protein at its COOH terminus yields p50 or p52 protein. This proteolytic degradation of p105 and p100 is accelerated under inflammatory conditions, which is one mechanism regulating the inducible NF-κB activation. Interaction between members of NF-κB family of proteins forms an NF-κB dimer of distinct composition, which reflects variation in stimuli, cell types, or signal transduction pathways (12, 119, 226). Those NF-κB dimers can be homodimers or heterodimers, although the most predominant form of NF-κB is the p50/p65 heterodimer. Different forms of the NF-κB dimer exhibit distinct properties in terms of DNA binding preference, selectivity of interaction with IκB isoforms, and transcriptional capability. The RelA/C-Rel dimer binds to the sequence of 5′-HGGARNYYCC-3′, whereas the p50/p65 dimer preferentially binds to the sequence of 5′-GGGRNNYYCC-3′ (12). The RelB/p52 dimer preferentially recognizes a novel NF-κB-binding sequence of 5′-GGAGATTTCG-3′, which is not recognized by the RelA/p50 dimer (24). RelA-containing NF-κB dimers preferentially interact with IκBα and IκBβ (12, 226), whereas p50-containing dimers have a preference for IκBγ and IκBζ (169, 259). NF-κB proteins are constitutively expressed in all cell types with the exception of RelB, the expression of which is restricted to lymphoid tissues (34). Although most NF-κB dimers are activators of transcription, p52/p52 (83), p50/p50, and p65/p65 homodimers are transcriptional repressors. The homodimer of p50 or p65 forms a complex with histone deacetylase (HDAC)-1, binds to DNA, and suppresses NF-κB-dependent gene expression (267). The RelB/p50 or RelB/p52 dimer acts as a transcriptional activator, whereas the RelA/RelB heterodimer represses the transcription of those genes (155). Distinctive properties of different NF-κB dimers increase the ability of NF-κB dimer to differentially regulate gene expression.

IkB family of proteins. NF-κB activities are regulated by the IkB family of proteins, which include IκBα, IκBβ, IκBγ, IκBε, IκBζ, Bcl-3, p105 (NF-κB1), p100 (NF-κB2), and MAIL (molecule possessing ankyrin-repeats induced by lipopoly saccharide). p105 and p100 have similar structural organization, containing p50 or p52 structure at their NH2 termini and IκB structure at their COOH termini (119, 169, 225). The central portion of both proteins contains a glycine-rich region that plays a critical role in processing of the precursors (169). In resting cells, p105 and p100 are partially processed, generating p50 and p52, although the exact mechanisms of this limited processing event is unknown. It is also unclear how and why the proteasome-mediated proteolysis selectively degrades the COOH-terminal portion of p105 and p100 but leaves an intact NH2 terminus (p50 and p52). IκBγ is structurally the COOH-terminal half of p105 protein but is translated from a separately initiated mRNA (226). The two newly discovered IκBs, IκBγ and MAIL, functionally differ from other IκB proteins (see later discussion), although the COOH-terminal portion of these two proteins share high sequence homology with other members of IκB proteins (124, 259). A common structure for all IκBs is the six to eight copies of ankyrin repeats, called ankyrin repeat domain (ARD), which mediate IκB binding to the NF-κB dimers, masking the nuclear localization sequence on NF-κB proteins.

IκB proteins are different in their structures, preference for binding of NF-κB dimers, biological functions, and modes of activation. IκBα, IκBβ, and IκBε, but not other IκBs, have an NH2-terminal regulatory domain, which is required for stimulation-induced IκB degradation (226). Whereas IκBα and IκBβ preferentially interact with dimeric complexes containing the transactivating subunits (RelA, RelB, and C-Rel), particularly those containing RelA (12, 226), IκBγ and IκBζ have a preference for p50-containing dimers (169, 259). IκBα effectively dissociates any prebound NF-κB complex containing RelA, RelB, or C-Rel from their cognate DNA sites, but it is ineffective in promoting the dissociation of DNA-bound p50/p52 homodimer (226). IκBγ interacts stably with p50/p65 dimer but not p65/p65 or C-Rel/C-Rel dimer (169). IκBζ preferentially associates with p50 rather than p65 and inhibits the DNA binding of the p50/p65 heterodimer as well as the p50/p50 homodimer (259). IκBε is exclusively found to be associated with RelA and C-Rel (11). Whereas p105 is preferentially associated with p50-containing dimers (169), p100 is associated with RelB-containing dimers (25). The cytoplasmic retention of RelB-containing NF-κB dimers is mediated exclusively by p100. There are also differences in the mechanism of regulating IκB gene expression. IκBα, IκBγ, and IκBζ are constitutively expressed (119), but IκBζ and MAIL are induced by lipopoly saccharide (LPS) and proinflammatory cytokines (67, 259). IκBα, IκBβ, and IκBε are ubiquitously expressed, whereas IκBγ is expressed only in certain cell types, such as pre-B cells (108). IκBα, IκBζ, and MAIL are NF-κB-regulated genes (67, 110, 119), but the gene expression of IκBβ is not regulated by NF-κB (119). IκB proteins respond differently to NF-κB activators. IκBζ only responds to a
involved in the degradation of NF-κB p105 (94). Upon phosphorylation by IKKs, IkB proteins are recognized by the SCF E3B ubiquitin ligase complex, which ultimately results in the degradation of IkB proteins by the 26S proteasome. IkB degradation causes the release of NF-κB dimers from the nucleus, which then translocate to the cytoplasm where they bind to DNA and activate gene expression.

Mechanisms regulating NF-κB activity. NF-κB is known to be activated by a variety of stimuli, including physical stress, chemical stress, oxidant stress, environmental stress, physiological stress, mitogens, modified proteins, receptor ligands, physiological and pathological mediators, apoptotic mediators, bacteria and their products, fungi and their products, viruses and their products, parasites and their products, proinflammatory cytokines, and a variety of pathological conditions. The signal transduction pathways leading to NF-κB activation are multiple and complex. Some signaling pathways appear to link to a particular stimulus, whereas other pathways are shared by multiple stimuli. One complexity in understanding the NF-κB signaling pathways is that a single stimulus can activate NF-κB through multiple signaling pathways, and multiple signaling cascades that lead to NF-κB activation can utilize a given signaling component. One example for the former is LPS, which causes NF-κB activation by activating multiple signaling pathways. For example, the latter are mitogen-activated protein (MAP) kinase and protein kinase C (PKC) pathways, both of which are involved in multiple signaling processes that lead to NF-κB activation. The signaling pathways that lead to NF-κB activation involve an extremely large number of signaling molecules, particularly kinases, and a detailed description of those molecules is beyond the scope of this review.

Although the upstream signaling pathways that lead to NF-κB activation are multiple and complex, those signaling pathways converge at certain nodal points. There are both canonical and noncanonical pathways of NF-κB activation. Some NF-κB activators act principally through the canonical pathways, whereas others act mainly through noncanonical pathways. Some NF-κB activators activate various signal transduction pathways that ultimately result in the activation of IKKs, which in turn cause the rapid phosphorylation of IkB proteins, resulting in the activation of NF-κB dimers. This process is mediated by the SCF ubiquitin ligase complex, which subsequently results in polyubiquitination of IkB proteins and subsequent proteasomal degradation. IkB degradation causes the release of NF-κB dimers from the nucleus, which then translocate to the cytoplasm where they bind to DNA and activate gene expression.

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Mechanisms regulating NF-κB activity. NF-κB is known to be activated by a variety of activators (with more becoming known every week), including physical stress, chemical stress, oxidant stress, environmental stress, physiological stress, mitogens, modified proteins, receptor ligands, physiological and pathological mediators, apoptotic mediators, bacteria and their products, fungi and their products, viruses and their products, parasites and their products, proinflammatory cytokines, and a variety of pathological conditions. The signal transduction pathways leading to NF-κB activation are multiple and complex. Some signaling pathways appear to link to a particular stimulus, whereas other pathways are shared by multiple stimuli. One complexity in understanding the NF-κB signaling pathways is that a single stimulus can activate NF-κB through multiple signaling pathways, and multiple signaling cascades that lead to NF-κB activation can utilize a given signaling component. One example for the former is LPS, which causes NF-κB activation by activating multiple signaling pathways (Fig. 2). Examples for the latter are mitogen-activated protein (MAP) kinase and protein kinase C (PKC) pathways, both of which are involved in multiple signaling processes that lead to NF-κB activation. The signaling pathways that lead to NF-κB activation involve an extremely large number of signaling molecules, specifically kinases, and a detailed description of those molecules is beyond the scope of this review.

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NF-κB IN SEPTIC PATHOPHYSIOLOGY

Invited Review

NF-κB in Septic Pathophysiology

Fig. 1. NF-κB activation cascade in the canonical pathways. Various NF-κB activators, including inflammatory and stress stimuli, activate IκB kinase (IKK), which phosphorylates IκBα, leading to its recognition and ubiquitination by the SCF family of ubiquitin ligase. This then targets IκBα protein for rapid degradation by 26S proteasome. IκBα degradation exposes the nuclear localization sequence on NF-κB proteins, resulting in its translocation to nucleus where it binds to its consensus sequence in the promoter or enhancer regions of NF-κB target genes. Upon stimulation, p105 and p100 are subjected to similar phosphorylation, ubiquitination, and proteasomal degradation processes, resulting in the release and nuclear translocation of p50- or p52-containing dimers and transcription of NF-κB target genes. The inducible p100 processing is preferentially induced by lymphokine-β (LT-β) and other B cell activation signals, and requires NF-κB-inducing kinase (NIK) activity. The IKK that phosphorylates p100 appears to be IKKζ homodimer. NF-κB activity is regulated by additional regulatory mechanisms including phosphorylation, acetylation, S-nitrosylation and S-glutathionylation of NF-κB proteins. p65 phosphorylation or acetylation increases, and p65 dephosphorylation or deacetylation decreases NF-κB activity. S-nitrosylation of p65 reduces NF-κB activity. NF-κB activity is also regulated by synergistic or antagonistic interactions with other transcription proteins and by positive or negative feedback mechanisms (see text for detail).

NF-κB-regulated Genes

To its consensus sequence on the promoter or enhancer regions of NF-κB-regulated genes, resulting in gene transcription (Fig. 1).

NF-κB activity is regulated by p100 and p105. Upon stimulation with cytokines such as TNF-α, p105 is rapidly phosphorylated by IKKs on Ser927 and 932 in its proline, glutamic acid, serine, and threonine rich region (94). Both IKKα and IKKβ play critical roles in p105 phosphorylation (94). The death domain of p105 protein acts as a docking site for IKK and facilitates an efficient Ser927 phosphorylation (15). p105 phosphorylation generates a binding site for β-TrCP, the receptor subunit of the SCF ubiquitin ligase complex, leading to ubiquitination and subsequent degradation of p105 protein by a proteasome system (12, 119). This releases the associated NF-κB dimer, which translocates into the nucleus and regulates its target gene transcription (Fig. 1). The p105 phosphorylation and degradation processes are facilitated by molecular cross talk with glycogen synthase kinase-3 (GSK-3) (57) and are inhibited by docking of p50 subunit to the ARD (49).

Upon stimulation, p100 is also subjected to the processes of phosphorylation, ubiquitination, and proteasomal degradation, resulting in the release and nuclear translocation of p52-containing NF-κB dimer (25). This p100 processing is tightly...
regulated and involves multiple functional regions of p100 protein. With the help of the COOH-terminal death domain, the COOH-terminal ARD interacts with its NH2-terminal dimerization domain and NLS, thereby bringing the COOH- and NH2-terminal sequences together to form a three-dimensional domain, which is required for the inducible processing of p100 protein (198). The inducible p100 processing requires NF-κB-inducing kinase (NIK) and its downstream kinase IKKα activity but does not require IKKβ and IKKγ, two key components of the classic IKK complex (25). This suggests that the IKKmediating the activation of the p100 NF-κB pathway is an IKKα/IKKβ homodimer complexed directly with NIK. Consistently, the p100-processing based NF-κB activation pathway is not activated by typical NF-κB inducers such as LPS, TNF-α, IL-1β, and dsRNA, but it is rather activated by signals involved in B cell maturation and lymphoid organogenesis, including lymphotoxin-β-receptor activation, engagement of BAFF-R (B cell-activating factor belonging to the TNF family receptor) or CD40 ligand (25). Activation of the IKKα/p100/NF-κB pathway causes the expression of a subset of NF-κB-dependent genes such as organogenic chemokine genes that regulate B cell maturation, B cell function, and lymphoid organogenesis and maintenance of secondary lymphoid organs (25). This p100 processing-based NF-κB pathway has been referred to as noncanonical or alternative NF-κB pathway in the literature to distinguish it from the classic IKK/IκB/NF-κB pathway (Fig. 1). However, the cascade of events leading to the activation of the p100/NF-κB pathway are identical to that responsible for the activation of the IKK/IκB/NF-κB pathway. Activation of both pathways is subjected to the process of phosphorylation, ubiquitination, and proteasomal degradation. It is argued that the p100 NF-κB pathway should be classified as part of the canonical pathway to distinguish it from true noncanonical pathways that will be discussed below.

Noncanonical pathways are signaling pathways leading to NF-κB activation without involving molecular events such as IKK activation, IκBα serine phosphorylation, or IκBα degradation by the ubiquitin proteasome system (193). Noncanonical pathways are divergent signaling pathways without a clear converging point. Hypoxia induces NF-κB activation without causing IκBα serine phosphorylation and IκBα degradation, but rather it causes IκBα phosphorylation at Tyr42 (119). The subsequent release of NF-κB dimer from the tyrosine-phosphorylated IκBα is suggested to be mediated by interaction with phosphoinositide-3 kinase (119). Hypoxia-reoxygenation or pervanadate treatment-induced NF-κB activation is mediated by c-Src-dependent tyrosine phosphorylation of IκBα, but it is independent of IKK activation (70). Hypoxia-reoxygenation also activates p56Lck tyrosine kinase, which causes NF-κB activation by tyrosine phosphorylation of IκBα (152). Hydrogen peroxide (H2O2) activates NF-κB through a combination of Syk tyrosine kinase-mediated tyrosine phosphorylation of IκBα and serine phosphorylation of p65 (235). UV radiation-induced NF-κB activation requires 26S proteasome-mediated IκBα degradation (119) but is independent of IKK activity and IκBα Ser32, Ser36, or Tyr42 phosphorylation (94, 119). The UV radiation-induced NF-κB activation depends on IκBα phosphorylation at a cluster of COOH-terminal sites by casein kinase II (CKII) (94). H2O2 induces NF-κB activation without causing IκBα degradation (30). Studies on mouse embryo fibroblasts deficient in IKKα and IKKβ genes demonstrated that the chemotherapeutic agent doxorubicin induces proteasome-dependent IκBα degradation and subsequent NF-κB activation in the absence of IKKα and IKKβ activities and IκBα Ser32 and Ser36 phosphorylation (94). Hepatitis C virus protein 5A induces NF-κB activation through tyrosine phosphorylation of IκBα at Tyr42 and Tyr305 and IκBα degradation. However, IκBα degradation is not mediated by a proteasome, but rather by the protease calpain (248). Mitochondrial stress-induced NF-κB activation involves the inactivation of IκBβ through calcineurin-mediated dephosphorylation, which is independent of IKKα and IKKβ (21). NF-κB activity is regulated by posttranslational modification of IκBα through mechanisms other than IκBα phosphorylation. Taurine chloramine inhibits NF-κB activation by oxidizing IκBα protein at Met45 (116). Transglutaminase 2 induces NF-κB activation without stimulating IκBα phosphorylation and degradation, but by inducing IκBα polymerization. The polymerization results in NF-κB dissociation and translocation into nucleus where it regulates gene expression (136).

In addition to nuclear translocation as discussed above, NF-κB activity is controlled by additional regulatory mechanisms. These include regulation of nuclear import and export of NF-κB dimers, regulation of the recruitment of NF-κB dimer to the promoter or enhancer sites of NF-κB target genes, regulation of NF-κB transcriptional activity after recruitment, and positive or negative feedback mechanisms. It is reported that low intracellular zinc reduces nuclear import of activated NF-κB dimers and inhibits the transcription of NF-κB-driven genes in human neuroblastoma cells (151). Increase in intracellular [Ca2+] accelerates NF-κB dimer nuclear translocation and promotes NF-κB-mediated transcription (125). Commensal anaerobic gut bacteria, Bacteroides thetaiotaomicron, selectively antagonize virulent salmonella-induced NF-κB activity by enhancing nuclear export of NF-κB dimers (121). This nuclear export does not utilize the traditional chromosomal region maintenance-1 (CRM-1)-dependent nuclear export mechanism but relies on a novel mechanism involving a Bacteroides-induced association between p65 and the nuclear hormone receptor peroxisome proliferator-activated receptor (PPAR)-γ. Subsequently, the p65/PPARγ complex is exported from nucleus, resulting in the attenuation of NF-κB activation (121). This mechanism provides a valid explanation how gut commensal bacteria regulate inflammation.

Regulation of the recruitment of NF-κB dimer to its target genes and regulation of NF-κB-mediated transcriptional activity are primarily mediated by two mechanisms, posttranslational modification of NF-κB proteins and synergistic (or antagonistic) interactions between NF-κB and other transcription proteins, as well as transcriptional coactivators or corepressors. NF-κB and chromatin interaction is also a critical determinant of NF-κB-mediated transcription (171). NF-κB proteins, particularly p65, are subjected to a variety of posttranslational modifications, including phosphorylation (83), acetylation (41), S-nitrosylation (201), and S-glutathionylation (190). NF-κB protein phosphorylation has emerged as an important mechanism regulating NF-κB activity and NF-κB-mediated gene transcription. Both p50 and p65 are phosphorylated by protein kinase A (PKA), resulting in increased DNA binding activity (83, 101). However, p65 phosphorylation is a better-characterized event. A variety of stimuli cause p65 phosphorylation.
phosphorylation (83). Phosphorylation of p65/p50 heterodimer by PKA enhances its DNA binding activity (83). Cells from mice deficient in serine/threonine protein kinase, GSK-3β, and NF-κB-activating kinase (NAK, also known as TBK or T2K) showed normal NF-κB activation in response to a variety of NF-κB inducers when measured by IκB degradation, NF-κB nuclear translocation, and binding to DNA. However, in both cells, NF-κB was unable to drive gene transcription, suggesting that NF-κB protein phosphorylation by these two kinases plays a critical role in regulating NF-κB transactivating activity (83). The phosphatidylinositol 3-kinase (PI3K)/Akt-mediated RelA phosphorylation plays an important role in gram-negative enteric bacteria-induced NF-κB activation (91). Numerous kinases cause p65 protein phosphorylation and enhance NF-κB transactivation activity. Some of them such as PKA (83), PKCζ (94), CKII (36, 83), GSK-3β (27), and TNF receptor-associated factor (TRAF) family member-associated NF-κB activator-binding kinase 1 (TBK1) (28) act directly on p65 protein, whereas others including NIK (83) and PI3K/Akt (83, 232) act indirectly by activating IKKα, which in turn phosphorylates p65. All three IKKs, IKKα, IKKβ, and IKKε, phosphorylate p65 at Ser536 (28). Phosphatase 2A (260) and phosphatase 4 (263) dephosphorylate p65 protein. Regardless of the phosphoacceptor sites, p65 serine phosphorylation, in majority of the cases, enhances NF-κB transcriptional activity. GSK-3β phosphorylates p65 at Ser468, resulting in reduced basal p65 activity (27). However, GSK-3β-mediated p65 serine phosphorylation appears to affect inducible NF-κB activity differently. Cells from mice deficient in the GSK-3β gene showed impaired NF-κB-mediated transcription in response to a variety of NF-κB inducers (83). p65 threonine dephosphorylation increases NF-κB activity (263), implying that p65 threonine phosphorylation decreases NF-κB activity. The mechanisms underlying the enhancement of NF-κB transcriptional activity by p65 serine phosphorylation have also been investigated. It is believed that the COOH-terminal region of nonphosphorylated p65 interacts with RHD, thereby interfering with both DNA and cAMP response element binding protein (CREB) binding protein (CBP)/p300 binding. Phosphorylation of p65 at Ser276 by PKA prevents this intramolecular association, thereby facilitating its DNA binding and interaction with the transcriptional coactivator CBP/p300, enhancing NF-κB transcriptional activity (83). However, it remains unknown whether the same mechanism is applicable to other kinases-induced p65 phosphorylation. Kinases causing p65 protein phosphorylation are multiple and diverse. These kinases do not act on the same phosphoacceptor sites. PKA and CKII phosphorylate p65 at Ser276 and Ser529, respectively (36, 83), whereas PKCζ phosphorylates p65 at Ser311 (94). IKKα, which mediates the effects of NIK and PI3K/Akt, phosphorylates p65 at Ser536 (83, 113), whereas IKKβ, which does not mediate the effects of NIK and PI3K, also phosphorylates p65 at Ser536 (94). It has been reported that differential p65 phosphorylation modulates NF-κB transcriptional activity in a cis-acting element and promoter-specific context, thus leading to a phosphorylation state-dependent gene expression profile (7).

Reversible acetylation of NF-κB protein serves as another mechanism regulating NF-κB activity. Both p50 and p65 subunits can be acetylated at multiple lysine residues, and this acetylation plays an important role in the regulation of NF-κB activity in vivo (41, 80). The transcriptional coactivator/acetyltransferase, CBP/p300, is the major acetyltransferase, and HDAC3 is the major deacetylase, mediating NF-κB acetylation/deacetylation (41). Acetylation of p50 enhances its activity (80). The effect of p65 acetylation on its activity depends on the site of acetylation. Acetylation at Lys221 enhances p65 DNA binding, impairs its assembly with IκBα, and reduces NF-κB dimer nuclear exportation, resulting increased NF-κB activity (42). Deacetylation of p65 protein by HDAC3 promotes its binding to IκB, leading to rapid nuclear exportation of the deacetylated NF-κB complex through a CRM-1-dependent mechanism (41). This p65 deacetylation is believed to be an important mechanism in terminating NF-κB response. Consequently, the cytoplasmic pool of latent IκB/NF-κB complexes is replenished. This readies the cells for the next NF-κB-mediated response. This reversible p65 acetylation also acts as a molecular switch that controls the duration of NF-κB transcriptional activity. Acetylation and deacetylation are key mechanisms regulating chromatin remodeling, which can alter NF-κB transcription activity by affecting their recruitment to target promoters.

NF-κB activity is also regulated by S-nitrosylation (56, 201) and glutathionylation (174, 190). S-nitrosylation inhibits NF-κB activation at several steps in the NF-κB activation cascade. S-nitrosylation inhibits IKKβ activation (201), stabilizes IκB protein, protecting it from degradation, and inhibits NF-κB binding and transcriptional activities (56). S-glutathionylation of p50 protein is responsible for redox-mediated regulation of NF-κB activity (190). The Cys62 of p50 is highly oxidized in the cytoplasm and strongly reduced in the nucleus. The reduced Cys62 is essential for the DNA binding of p50-containing NF-κB dimer (174).

NF-κB proteins interact with large number of transcriptional proteins either through a direct physical interaction or through interaction with a third protein, resulting in an altered NF-κB activity and NF-κB-mediated transcription. This interaction can be either synergistic or antagonistic and can be either reciprocal or nonreciprocal. Interaction between NF-κB and JunD (90), CREB (223), CCAAT/enhancer-binding protein-β (C/EBPβ), also called NF-IL6 (147, 186), interferon (IFN) regulatory factor-1 (IRF-1) (251), Kruppel-like factor 5 (4), POU-domain transcription factor-2 (Oct-2) (214), and nuclear factor of activated T cell (147) is synergistic and reciprocal, leading to augmented transcription activities mediated by both NF-κB and other transcription factors. Interaction between NF-κB and activating transcription factor-2 (20), breast cancer gene 1 (17), high mobility group box 1 (3), Notch (66), really interesting new gene (RING) finger protein, A07 (10), promoter selective transcription factor 1 (142), signal transducer and activator of transcription 6 (STAT6) (222), and transcription factor IIB (254) is synergistic but nonreciprocal, leading to the augmentation or facilitation of NF-κB-mediated transcription without affecting the transcriptional activity mediated by the partner proteins. NF-κB binds to positive transcription elongation factor-b to stimulate transcriptional elongation by RNA polymerase II (14). Interactions between NF-κB and STAT1 (81), E2F transcription factor 1 (43), mammalian transcriptional repressor RBP-J (CBFI) (178), IFN-inducible p202a protein (150), forhead box P3 (FoxP3) (19), and zinc-finger protein ZAS3 (99) are antagonistic and nonreciprocal, inhibiting NF-κB-mediated transcription without affecting the transcriptional activity of the partner proteins. On other hand,
interaction between NF-κB and PTEN (phosphatase and tensin homolog deleted on chromosome ten) or estrogen receptor is antagonistic and reciprocal, resulting in a mutual inhibition of the transcriptional activity mediated by both proteins (68, 88, 242). Reciprocally negative cross talk between NF-κB and AP-1 has also been reported (123), although positive cross talk between those two proteins is more likely (90). NF-κB activity and NF-κB-mediated transcription are inhibited by interactions with its negative regulators, protein inhibitor of activated STAT1 (143) and zinc finger protein A20 (94). NF-κB interacts with various transcriptional coactivators and co-repressors, and a dynamic balance between these coactivators and co-repressors regulates NF-κB-mediated transcription (82).

NF-κB activity is influenced by intranuclear p65 protein abundance and stability, which are also actively regulated. The p65 protein is subjected to a Pin1-dependent prolyl isomerization and ubiquitination-mediated proteasomal degradation. Prolyl isomerization enhances NF-κB activity by inhibiting p65 binding to IkBα, resulting in an increased nuclear accumulation and stability of p65 protein (205). Proteasome-mediated proteolysis of p65 protein reduces its nuclear concentration and, thus, NF-κB activity (205). Intranuclear proteasome can degrade DNA-bound p65 protein, which not only promotes the termination of NF-κB-mediated transcription and response but also reduces intranuclear p65 abundance (206). This accelerated p65 degradation is believed to be a mechanism of inflammation termination and resolution (133).

NF-κB activity is regulated by positive and negative feedback mechanisms. It is reported that RelA increases IkBα phosphorylation and degradation, which serves as a positive feedback loop for high-affinity NF-κB complexes (261). NF-κB activation stimulates its upstream kinases (59), which also form positive feedback. NF-κB also activates several negative feedback mechanisms. NF-κB activation increases IkBa, IκBζ, or MAIL (119, 67, 110), which in turn inhibits NF-κB activation (172). NF-κB activation upregulates the expression of NF-κB-negative regulators, twist-1, twist-2 (231), and p65-interacting inhibitor of NF-κB, SINK (253), which interact directly with NF-κB protein and inhibits its transcription activity. NF-κB also activates negative regulators of upstream signal molecules, resulting in an inhibition of NF-κB signaling (185).

**IKK.** The key step leading to NF-κB activation in the canonical pathways is the activation of IKK. IKK is a large protein complex with a kinase core composed of three subunits: IκKα (IKK1), IκKβ (IKK2), and IκKγ [also called NF-κB essential modulator, NEMO or IKK associated protein, IKKAP] (119). IκKα and IκKβ are the catalytic subunits, and IκKγ is the regulatory subunit. IκKγ associates with IκKα/IκKβ dimer formed between the two catalytic subunits via their leucine zipper motifs to assemble a large IKK holocomplex. This complex assembly is essential for stimulus-dependent IKK activation. IκKγ lacks catalytic domain, but it is essential for IKK activation. It serves an important regulatory function by connecting IκKα/IκKβ dimer to upstream signaling molecules. IκKα and IκKβ share 52% overall sequence identity and 65% identity in their catalytic domains, and IκKγ is not structurally related to the catalytic subunits (119). Depending on the isolation procedure, IKK complex has been reported to be 700–900 kDa as revealed by gel filtration analysis. Because the molecular masses of IκKα, IκKβ, and IκKγ are 85, 87, and 48 kDa, respectively, the large size of the IKK complex indicates the presence of additional components, including IkB and NF-κB proteins as well as upstream kinases. Because IKK is the converging point for multiple and divergent signal pathways, it is likely that the upstream kinase in the IKK complex varies with signaling pathway involved. Although IκKα and IκKβ display similar activity in vitro (119), studies using IκKα or IκKβ knockout mice or transgenic mice overexpressing the inactivatable variant IκKα have revealed distinct functions for the two catalytic subunits (83, 119). IκKβ mediates IkB phosphorylation and degradation, NF-κB nuclear translocation, and NF-κB-dependent gene transcription in response to inflammatory mediators and cytokines, whereas IκKα is largely dispensable for this response (83, 119). In response to cytokines and inflammatory mediators, IκKα contributes to NF-κB-mediated gene transcription by its nucleosomal function. IκKα phosphorylates p65 protein (94) and causes gene-specific phosphorylation (6) and subsequent acetylation of histone H3 (258), promoting the recruitment of NF-κB dimer and enhancing the transcription of NF-κB-regulated genes. IκKα mediates p100 protein processing and the activation of p100-dependent pathway that has been discussed in detail above (25, 94). The IκKβ-dependent pathway mediates the activation of innate immunity and inflammatory responses (25), whereas the IκKα-dependent pathway is involved in the termination and resolution of inflammatory responses (133). The IκKα-dependent pathway suppresses inflammatory response by accelerating RelA and c-Rel protein turnover as well as their removal from promoter of proinflammatory genes (133).

Phosphorylation is an essential step toward IKK activation. Phosphorylation at Ser177 and 181 of the IκKβ activation loop or Ser176 of the IκKα activation loop is required for IKK activation (119). This phosphorylation can be achieved either by the action of an upstream kinase or by an autophosphorylation caused by IKK itself. It is proposed that upstream signaling events induce a proximity mechanism in which the activator contacts each IKK/IKK dimer, increasing their proximity within the higher-order IKK complex and thereby facilitating mutual transautophosphorylation of IKK (83). IKK phosphorylation increases (78, 83, 192, 227, 233), and IKK dephosphorylation by protein phosphatase-2Cβ decreases its activity (194). Numerous kinases are known to phosphorylate and activate IKK, but none has proven to be specific IKK kinase. These kinases include NIK (94), NAK (83), NAK-associated protein 1 (78), MAP/ERK kinase kinase-1 (MEKK1) (119), MEKK3 (94), TGF-β-activating kinase-1 (TAK1) (227), TBK1 (192), and PKCβ (233). Activation of these kinases by various signaling pathways results in IKK phosphorylation and activation and the subsequent NF-κB activation. It is reported that TNF-α- and IL-1α-induced MEKK3 activation results in the formation of an IKK/IκBα/ NF-κB complex, which regulates rapid NF-κB activation, whereas activation of MEKK2 results in the assembly of an IKK/IκBβ/NF-κB complex, which controls the delayed NF-κB activation (210).

IKK has functions other than phosphorylating IkBs. Both IκKα and IκBβ phosphorylate β-catenin. IκKα increases, whereas IκKβ decreases, β-catenin-dependent gene transcription (129). IκKγ has nuclear function and shuttles into the
nucleus where it competes with p65 for binding to CBP, leading to a repression of NF-κB-mediated transcription (243).

IκB kinase-ε (IKKe or IKKi) is a structural homolog of IKKα and IKKβ. LPS, TNF-α, IL-1, and IL-6 induce IKKe activity. Overexpression of IKKe/IKKi causes IκBα phosphorylation at Ser32 and Ser36 and stimulates NF-κB activity (189). However, dominant negative mutant of IKKe/IKKi has no effect on TNF-α or IL-1-induced NF-κB activation, although it blocks NF-κB activation induced by PMA and T-cell receptor activation (189). Cells lacking the IKKe/IKKi gene show normal activation of the canonical NF-κB pathway, suggesting that it is not essential for this pathway (94). IKKe/IKKi plays a pivotal role in integrating inflammatory signals into a coordinated activation of IRF-3 and NF-κB (94) as well as coordinated activation of NF-κB and C/EBPβ or C/EBPδ (73), augmenting the inflammatory response.

**BIOLGICAL FUNCTIONS**

**Regulation of cell proliferation and apoptosis.** NF-κB participates in a variety of cellular activities and plays important roles in diverse biological functions. The two most prominent and well-defined functions for NF-κB are regulation of immunological and inflammatory responses and regulation of cell proliferation and apoptosis. In normal cells, NF-κB proteins are generally antiapoptotic and are regulators of cellular survival pathways. Activation of NF-κB mediates the expression of multiple antiapoptotic or cell survival genes, including bfl-1 (Bcl-2-related genes from a human fetal liver) (61), receptor for activated C kinase-1 (46), inhibitors of apoptosis-1 and -2 (IAP-1 and IAP-2) (224), X chromosome-linked inhibitor of apoptosis protein (141), cellular Fas-associated death domain-like IL-1β-converting enzyme (FLICE) inhibitory protein (162), inhibitor of caspase 8 (224), and survivin (224). NF-κB promotes survival during mitotic cell cycle arrest (165). Inhibition of NF-κB pathway by various means promotes apoptosis (249). Activation of the NF-κB pathway suppresses putative proapoptotic and tumor repressor genes such as PTEN and p53 (54, 242). This inhibition is reciprocal, because these two proapoptotic proteins also inhibit NF-κB activity (54, 88).

Tumor suppressors PTEN and p53 and the proapoptotic protein Fas-associated factor-1 exert their antitumor or proapoptotic action by negatively regulating NF-κB activity and NF-κB-mediated gene transcription (54, 88, 187). The importance of NF-κB in cell proliferation and embryonic development is further evidenced by studies on NF-κB or IKK knockout mice. Mice deficient in p65 or IKKβ and mice deficient in both p50 and p65 died at embryonic day 12.5-14.5 due to massive apoptosis (12, 139). IKKε-deficient mice lack limbs, tails, and rears and exhibit severe deformities in craniofacial and several other organs (119). In tumor cells, however, NF-κB appears to have dual functions, acting as both tumor promoter and tumor suppressor. Numerous lines of evidence support the notion that NF-κB promotes tumorigenesis and tumor progression. A constitutive NF-κB activity is detected in most tumor cell lines but is rarely detectable in normal cells (224). Increased NF-κB activity is also detected in various cancer tissues (224). Inhibition of NF-κB activity in those tumor cells suppresses their proliferation, leading to apoptosis (224). NF-κB proteins are oncogenic. Several putative oncogenes induce cellular transformation though activation of the NF-κB pathway (224).

NF-κB mediates the expression of multiple genes that are associated with tumor cell growth and survival and plays an essential role in every step of tumorigenesis and tumor progression (224). NF-κB activation promotes tumor cell proliferation and migration and mediates tumor invasion and metastasis (224). NF-κB activation also plays important role in angiogenesis (224), which is critical for solid tumor growth. NF-κB activity is essential for tumor maintenance and for cancer cell resistance to chemotherapies or TNF-α therapy (224). In recent years, evidence has also emerged showing that NF-κB activation mediates apoptosis (224). NF-κB mediates the expression of several proapoptotic genes including Fas ligand and c-Myc (224). NF-κB has been reported to mediate p53 tumor repressor gene expression (224) and to stabilize p53 protein (77), although another report has demonstrated that NF-κB inhibits p53 transcription (54). NF-κB activity is required for the induction of apoptosis in several cell lines in response to various apoptosis inducers (224). Human melanoma cells are protected against UV-induced apoptosis by downregulation of NF-κB activity and Fas expression (224). Activators of NF-κB induce apoptosis, and inhibitors of NF-κB inhibit apoptosis (224). Blockade of NF-κB pathway predisposes and triggers tumor formation in the skin (224). These two opposite sets of data are not necessarily contradictory. They may represent a timely switch from one response to another in adaptation to changes in cellular environment. It has been reported that NF-κB can be either proapoptotic or antiapoptotic, depending on the timing of NF-κB activity being modulated relative to the death insult (224). Elucidation of the molecular mechanisms underlying this timely switch may help to better understand the biological basis of tumorigenesis and to identify better targets for tumor prevention.

**Regulation of immunological and inflammatory responses.** NF-κB pathway plays a central role in the immune and inflammatory responses. The important roles of NF-κB in adaptive immunity are evidenced by demonstrations that mice deficient in NF-κB proteins develop a variety of immunological deficiencies. Mice lacking NF-κB1 (p50/p105) exhibit an impaired B cell proliferation, antibody secretion, and defects in B cell-mediated immune response (12, 139). Mice deficient in NF-κB2 (p52/p100) show defects in B cell maturation and T cell activation (12, 139). The RelA (p65) knockout mice display embryonic lethality due to massive apoptosis in the liver (12, 139). RelB knockout mice show defects in hematopoiesis, reduced antigen-presenting dendritic cells in the thymus, impaired cellular immunity, as well as multifocal and mixed infiltration of inflammatory cells in several tissues (12, 139). C-Rel null mice exhibit defective B cell and T cell proliferation, defective immunoglobulin production, and T cell-dependent immune response (12, 139). NF-κB1 and NF-κB2 double knockout mice lack mature B cells and osteoblasts (12, 139). NF-κB1 and RelB double knockout mice died postnatally due to immune deficiencies (12, 139). Mice deficient in IκB proteins also develop various forms of immune deficiencies (12, 139).

Regulation of adaptive immunity includes regulation of immune cell generation and activities. NF-κB proteins are actively involved in those regulations. NF-κB activity is critically required for hematopoiesis and for the differentiation and maturation of both myeloid and lymphoid immune cells, including T lymphocytes, B lymphocytes, natural killing cells,
and dendritic cells (58, 225). NF-kB also plays an essential role in the production of a specialized subpopulation of immune cells (65, 225). NF-kB proteins are key mediators of immune cell antiapoptotic signals and cell survival signals (225). NF-kB activity is critically required for the survival of T lymphocytes, B lymphocytes, and dendritic cells (224, 225). RelA, RelB, and NF-kB2 are required for the development of lymphoid organs (25). IKK activity is also essential for lymphocyte development, maturation, survival, and function (211, 225). Both IKKα and IKKβ are required for mature T cell generation and survival, and IKKβ has an additional role in regulatory and memory T cell development (211, 225).

NF-kB is also a major regulator of immune cell functions. In the Th1 response, induction of intrinsic NF-kB activity is required for postdifferentiation IFN-γ production and clonal expansion (53, 139). IL-12 production by human dendritic cells also requires intrinsic NF-kB activity (128). The switch of immunoglobulin genes to secondary immunoglobulins is an event critical for the generation of functional diversity of a humoral immunity. NF-kB family of proteins have important functions in the regulation of isotype switching. Absence of NF-kB activity reduces the expression of certain germ-line CH genes and impairs class switching and antibody secretion (58).

NF-kB activity is also crucial to innate immunity. NF-kB activity is required for the development and maturation of all hematopoietic cells including macrophages and neutrophils (58, 224), which are at the front line of innate immunity. NF-kB activity is essential for these cells to exert their host defense function against invaded bacterial pathogens. Inhibition of NF-kB pathway or deletion of NF-kB genes results in defective host-defense function. Mice deficient in p50 display a defective bacterial clearance capacity and increased susceptibility to Streptococcus pneumoniae and Listeria monocytogenes infections (216). Overexpression of IκBα selectively in the liver causes a defective bacterial clearance in liver and increased susceptibility to L. monocytogenes infection, despite having intact immunocytes and inflammatory cells (132). Inhibition of NF-kB activation using p65 decoy oligodeoxynucleotides (ODN) also impairs bacterial clearance capacity of Staphylococcus aureus (85). Knockout of NF-kB-dependent genes also impairs bacterial clearance capacity. TNF receptor (TNFR) knockout mice show an impaired bacterial clearance of Escherichia coli (166). ICAM-1 knockout mice display a decreased clearance of Pseudomonas aeruginosa and increased susceptibility to S. aureus infection (207). Interestingly, bacterial pathogen subverts macrophage’s host-defense capability by disrupting the NF-kB pathway (203). During endotoxemia, LPS causes delayed neutrophil apoptosis, which is abrogated by inhibiting NF-kB activation, and NF-kB inhibition accelerates neutrophil apoptosis (47).

Evidence supporting a pivotal role of NF-kB activation in inflammatory response is overwhelming. NF-kB mediates the expression of extremely large number of proinflammatory genes, including cytokines, chemokines, immune receptors, enzymes, and other proinflammatory molecules (11 183). An increased NF-kB activity is observed in virtually every form of inflammation, and inhibiting NF-kB activation prevents the development of those pathological conditions. NF-kB may also play a role in inflammation resolution. However, it appears that different NF-kB subunits are involved in the onset and resolution phase of inflammation (134). The IKKα pathway contributes to the resolution of inflammation (133).

Roles in other physiological and pathological processes. Inappropriate NF-kB activation is one of the pathogenic mechanisms of a list of diseases, particularly those with inflammation or apoptosis as a component. In the nervous system, NF-kB activity increases by many folds in acute neurodegenerative disorders such as stroke, severe epileptic seizures, and traumatic brain injury, and in chronic neurodegenerative conditions, including Alzheimer disease, Parkinson disease, Huntington disease, and amyotrophic lateral sclerosis (157). Depending on where it is activated, NF-kB activation can be deteriorative or protective. In general, activation of NF-kB in microglia promotes the development of neurodegenerative disorders, whereas NF-kB activation in neurons protects them against neuronal degeneration (157). Neuronal NF-kB activation also provides neuroprotection against glutamate-induced excitotoxicity (154). NF-kB mediates glial cell activation, which contributes to neuronal injury (157).

NF-kB activity is developmentally regulated and is required for normal neuronal development and functions (157). NF-kB participates in a differentiation program that triggers the progression of axon-associated Schwann cells into a myelinating phenotype (160). Proper growth and elaboration of neural processes are essential for the establishment of a functional nervous system during development and are integral features of neural plasticity throughout life. Inhibition of NF-kB activity with NF-kB decoy ODN substantially reduces the size and complexity of the neurite arbors of sensory neurons cultured with brain-derived neurotrophic factor (89). NF-kB is activated in association with long-term potentiation (LTP) of synaptic transmission, a process that is believed to be a cellular mechanism of learning and memory (157). Blockade of NF-kB activation reduces LTP and abolishes long-term depression of synaptic transmission (157). Mice lacking p65 protein show a selective learning deficit in the spatial version of the radial arm maze (160).

NF-kB serves as an integrator of diverse signaling pathways that lead to myocardial disorders (114) and has been implicated in the pathogenesis of multiple cardiovascular diseases. NF-kB is proatherogenic, and NF-kB activation contributes significantly to the development of atherosclerosis (51, 114). NF-kB activity and the expression of NF-kB target genes increase in atherosclerotic lesions (51). Proatherogenic factors activate NF-kB, and suppression of NF-kB activity prevents the development of atherosclerosis (51). NF-kB plays an important role in angiogenesis, vascular restenosis, and postinjury vascular fibrosis (114). NF-kB activation is involved in the pathogenesis of several other cardiovascular diseases or pathological conditions, including transplant rejection (114), angina pectoris (114), I/R injury (114), myocardial infarction (114), autoimmune myocarditis (264), congestive heart failure (114), dilated cardiomyopathy (114), and cardiomyocyte hypertrophy (197). Ischemic preconditioning causes NF-kB activation, which provides protection against ischemia myocardial injury induced by subsequent ischemia (114).

In the respiratory system, an increased NF-kB activity contributes to the pathogenesis of multiple airway and lung diseases. NF-kB activation is an integral part of the pathological mechanisms of asthma and other chronic obstructive pulmonary diseases as well as cystic fibrosis (252). NF-kB activation
mediates the development of various forms of acute lung injury and acute respiratory distress syndrome induced by endotoxemia, hemorrhage, mechanical ventilation, allograft rejection, bleomycin, and IgG immune complex deposition (72, 181, 204, 252, 266). NF-κB activation is involved in the pathogenesis of lung interstitial diseases such as sarcoidosis and asbestosis (252). NF-κB is activated by a variety of air pollutants and contributes pulmonary inflammation induced by air pollution (252).

In the gastrointestinal system, NF-κB activation is both injurious and protective. On one hand, NF-κB activation plays a role in gastric ulcer formation and contributes significantly to the pathogenesis of celiac disease (153), collagenous and ulcerative colitis, and Crohn disease (127). On the other hand, NF-κB promotes restitution of wounded intestinal epithelial monolayers (63) and protects against radiation-induced intestinal epithelial cell death and intestinal injury (64). NF-κB is involved in multiple liver diseases in which deregulation of cell apoptosis is the major mechanism (95). Inhibition of NF-κB activation protects mice from T cell-mediated liver injury (107) and prolongs liver allograft survival (256).

In the kidney, NF-κB activation plays a role in the pathogenesis of immune glomerulonephritis (148) and chronic FK506 nephropathy (238). Inhibition of NF-κB activation with NF-κB decoy ODN prevents postreperfusion inflammation in a renal allograft model (245).

Activation of NF-κB pathway mediates I/R injury in multiple organs, including brain (176), heart (114), lungs (204), kidney (31), liver (13), and intestine (269). In general, NF-κB activation mediates I/R organ injury but protects cells from I/R-induced apoptosis (164).

In the joint and skeletal muscle system, activation of the NF-κB pathway is involved in several physiological and pathological processes. Treadmill exercise results in a significant increase in IKK and NF-κB activities in skeletal muscle of rats, suggesting that the NF-κB pathway may play a role in exercise adaptation (96). Mice subjected to 10 days of hindlimb unloading showed an increased NF-κB activity, increased expression of NF-κB-regulated genes, and decreased soleus fiber cross-sectional area (an indicator of muscle atrophy), all of which are abrogated in p105/p50 knockout mice (105), suggesting that NF-κB activation is one of the mechanisms underlying skeletal muscle disuse atrophy. Activation of NF-κB by muscle-specific expression of activated IKKβ transgene causes severe muscle wasting (29) that resembles clinical cachexia, suggesting an important role of NF-κB activation in muscle wasting seen in several pathological conditions, including cancer, sepsis, denervation, and immobilization. NF-κB-mediated muscle wasting requires inducible nitric oxide synthase (iNOS) activity and nitric oxide (NO) production, indicating that the NF-κB effect is mediated by an iNOS/NO pathway (60). Activation of the NF-κB pathway plays an important role in the pathogenesis of rheumatoid arthritis and other forms of arthritis (234).

A dysregulated NF-κB contributes to the pathogenesis of multiple inflammatory and proliferative skin disorders or diseases, including psoriasis,continentia pigmenti, sunburn, Lyme disease, allergic contact dermatitis, and autoimmune diseases, as well as skin cancer (16).

NF-κB activation is involved in or contributes to the development of metabolic disorders such as obesity (8), insulin resistance (8), and diabetes (130). It is reported that glucose-stimulated insulin secretion in pancreatic beta cells requires NF-κB activity (175). NF-κB is involved in the signaling process that initiates parturition. Increased production of surfactant protein A near-term fetal lung causes the migration of fetal amniotic fluid macrophages to the maternal uterus, where they increase the production of IL-1β that activates NF-κB, leading to labor (52).

**ROLE OF NF-κB IN SEPTIC PATHOPHYSIOLOGY**

Signaling pathways that lead to NF-κB activation during sepsis. Consistent with the concept that septic shock is a clinical syndrome with diverse etiologies, NF-κB is activated by a variety of bacteria (Table 1), bacterial products (Table 2), and proinflammatory cytokines released during sepsis (Table 3). NF-κB is the final target of those septic shock inducers. Bacteria and bacterial components trigger inflammatory response by binding to and activating their receptors, Toll-like receptors (TLRs). TLRs are the mammalian homologs of the Drosophila protein, Toll, which belongs to the Toll/IL-1 receptor (TIR) domain-containing superfamily of proteins. At least 13 members of TLRs exist, and 10 of them (TLR1–TLR10) have been identified in humans (241). All TLR subtypes contain an extracellular leucine-rich domain, which mediates the formation of TLR homodimers or heterodimers, and a cytoplasmic TIR domain, which is essential for the assembly

<table>
<thead>
<tr>
<th>Table 1. Bacteria that activate NF-κB</th>
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<tr>
<td>Anaplasma phagocytophilum</td>
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<td>Bacteroides forsythus</td>
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<td>Bartonella henselae</td>
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<td>Bordetella pertussis</td>
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<td>Chlamydia pneumoniae</td>
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<td>Chlamydia psittaci</td>
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<td>Chlamydia trachomatis</td>
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<td>Enteropathogenic Escherichia coli</td>
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<td>Ehrlichia chaffensis</td>
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<td>Enteroinvasive Escherichia coli, Fusobacterium nucleatum</td>
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<td>Gardnerella vaginalis</td>
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<td>Helicobacter pylori</td>
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<td>Hemophilus influenzae</td>
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<td>Haemophilus influenzae</td>
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<td>Lactobacilli Lactobacillus</td>
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<td>Listeria monocytogenes</td>
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<td>Mycobacteria Mycobacterium</td>
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<td>Mycobacterium tuberculosis</td>
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<td>Mycoplasma fermentans</td>
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<td>Neisseria gonorrhoeae</td>
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<td>Neisseria meningitides</td>
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<td>Prevotella intermedia</td>
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<td>Pseudomonas aeruginosa</td>
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<td>Rhodococcus equi</td>
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<td>Rickettsia rickettsii</td>
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<td>Salmonella Dublin</td>
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<td>Salmonella typhimurium</td>
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<td>Shigella flexneri</td>
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<td>Staphylococcus aureus</td>
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<td>Streptococci group A</td>
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<td>Streptococci group B</td>
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<td>Streptomyces californicus</td>
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<td>Ureaplasma urealyticum</td>
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<td>Yersinia enterocolitica</td>
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NF-κB IN SEPTIC PATHOPHYSIOLOGY

L632

Invited Review

AJP-Lung Cell Mol Physiol • VOL 290 • APRIL 2006 • www.ajplung.org

Table 2. Bacterial components and products that activate NF-κB

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<thead>
<tr>
<th>Bacterial components and products</th>
<th>Activation of NF-κB</th>
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<tr>
<td>Cholera toxin from Vibrio cholerae</td>
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<td>Cytolysin from Vibrio vulnificus</td>
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<td>Diphosphoryl lipid A from Rhodobacter sphaeroides</td>
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<td>Enterotoxin A from Bacteroides fragilis</td>
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<td>Enterotoxin A from Staphylococcus</td>
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<td>Enterotoxin B from Staphylococcus</td>
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<td>Flagellin from Pseudomonas aeruginosa</td>
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<td>Flagellin from Salmonella muenchen</td>
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<td>FliC (flagella filament protein) from Salmonella enteritidis</td>
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<td>Flagella filament structural protein from Salmonella enteritidis</td>
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<td>Fumonisin B1 from Fusarium verticillioides</td>
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<td>Gi(Anh) M Tetra from Escherichia coli</td>
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<td>Internalin B from Listeria monocytogenes</td>
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<td>Leukotoxin from Pasteurella haemolytica</td>
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<td>Lipoteichoic acid from gram-positive bacterial wall</td>
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<td>Phospholipases from Listeria monocytogenes</td>
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<td>Porins from Neisseria</td>
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<td>Porins from gram-negative bacteria</td>
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<td>Protein A from gram-positive bacteria</td>
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<td>Toxic shock syndrome toxin-1 from Staphylococcus</td>
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Table 3. Cytokines that activate NF-κB

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<tr>
<td>Tumor necrosis factor-α</td>
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<td>Granulocyte/Macrophage colony-stimulating factor</td>
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leading to NF-κB activation (192), whereas IKKε/IKKι phosphorolytes IkBα (189), which leads to IkBα degradation and NF-κB activation. IKKε/IKKι can also activate IKKs via interaction with an interacting protein, TANK (38).

RIPs (RIP1, RIP2, RIP3, and RIP4) are a group of death domain-containing serine/threonine kinases that mediate signal transduction processes leading to NF-κB activation. RIPs are key signaling molecules for TNF-α receptor activation and play a role in TLR signaling. In response to LPS, RIP1 is associated to TLR4 via interaction with TRAF-2 or TRAF-6, resulting in the activation of PI3K/Akt pathway (244). However, this pathway does not appear to play a major role in LPS-induced NF-κB activation. RIP1 is recruited to TLR3/TRIF complex and mediates TRIF-induced NF-κB activation (94). Because TRIF is an essential signaling molecule for TLR4, it is possible RIP1 interacts directly with TRIF to mediate TLR4 signal. RIP2-deficient macrophages display an impaired NF-κB activation in response to LPS, and RIP2-deficient mice are resistant to the lethal effect of LPS, indicating an important role of RIP2 in LPS/TLR4 signaling (45). RIP4 mediates NF-κB activation induced by multiple stimuli, and dominant negative versions of TRAF-1, TRAF-3, and TRAF-6 block this activation, indicating that RIP4 mediates LPS-induced NF-κB activation via TNF signaling pathways (161).
Activation of TLR4 by LPS leads to the activation of all three classic MAP kinase (MAPK) pathways, the Ras-Raf-MEK1/2-ERK1/2 pathway, the protein kinase R (PKR)-MKK3/6-p38 pathway, and the MEKK1/4-MKK4/7-JNK pathway (26). ERK activation has been reported to act as an important temporal regulator of NF-κB activation and NF-κB-dependent gene expression in response to IL-1 (112). Specific inhibitors of ERK-1/ERK-2, JNK, or p38 have been shown to inhibit NF-κB activation or to suppress the expressions of NF-κB-dependent genes (26, 76, 112). However, a direct molecular link between these three MAPK pathways and NF-κB has not been fully established. The upstream signaling molecules linking PKR [the MAP kinase kinase kinase (MAP3K) for p38 pathway] to TLRs are the TRAF family of adaptor proteins, TRAF-2 and TRAF-5 (84), but the downstream molecules linking p38 to NF-κB remain to be elucidated. Kinases downstream to MAPKs are MAPK-activated protein kinases (MKs). Activation of MAPK pathways can cause NF-κB activation by activating their downstream MKs. The ribosomal S6 kinase-1 is a substrate of ERK1/ERK2 and can physically interact with the IKK complex (184). The mitogen- and stress-activated protein kinase-1, another downstream MKs of ERK1/ERK2, can associate with and phosphorylate p65 at Ser276, enhancing NF-κB-mediated gene transcription (94).

Several MAP3Ks upstream of p38 and JNK, including NIK, NAK, MEKK1, MEKK2, MEKK3, and Tpl2 have been demonstrated to directly phosphorylate and activate IKK (83, 94, 173), suggesting that LPS can induce NF-κB activation by activating those upstream kinases without involving the entire signaling cascades of these three MAPK pathways. Activation of TLR4 by LPS leads to MEKK1 activation, which in turn causes IKK phosphorylation and NF-κB activation (173). MEKK1 is linked to TLR4 via its upstream signaling molecule, TRAF-6, and via interaction with an adapter protein, ECSIT (evolutionarily conserved signaling intermediate in Toll pathways) (94). MEKK3 mediates LPS- and TNF-induced NF-κB activation (94), although different upstream signaling molecules are involved. For LPS, the upstream signaling is transduced by TLR4/MyD88/IRAK/TRAF-6 cascade (103), whereas for TNF, the upstream signaling molecule is RIP, which links to TNFR via TRAFs, leading to MEKK3 activation and subsequent IKK phosphorylation and activation (94). Different MEKK isoforms play different biological roles. It is reported that in response to TNF-α and IL-1α, MEKK3 activation results in the formation of an IκBα/NF-κB/IKK complex, which regulates rapid and transient NF-κB activation, whereas MEKK2 activation leads to the assembly of an IκBβ/NF-κB/IKK complex, which mediates delayed and persistent NF-κB activation (210). Tpl2 appears to play important role in LPS signaling, since Tpl2-deficient mice are resistant to endotoxin lethality. However, those mice display relatively normal NF-κB activation as measured by nuclear translocation of NF-κB dimer in response to LPS (55). The role of Tpl2 in LPS signaling is likely mediated through TNF-α signaling pathways. TNF-α activates the TNFRI/TRAFL-2/RIP1 cascade, leading to the activation of Tpl2, which phosphorylates p65 at Ser276, resulting in NF-κB-mediated gene transcription (55).

LPS activates multiple PKC isozymes, including PKCα, PKCβ, PKCδ (40), PKCζ (94), and PKCe (35). Blocking activation of those PKC isoforms using either isoform-specific antisense oligonucleotides or cells from PKC-deficient mice has demonstrated critical roles of these PKC isoforms in mediating LPS-induced NF-κB activation and NF-κB-dependent gene transcription (35, 40, 94). However, it remains unclear how specific PKC isoforms activate NF-κB. PKCζ phosphorylates p65 directly at Ser511, resulting in enhanced NF-κB activity (94). Upon activation with inflammatory stimuli, PKCζ is associated with the IKK complex and causes IκBα phosphorylation (208), suggesting that PKCζ can also activate IKK. Other PKC isoforms, such as PKCb (233), have also been demonstrated to activate IKK directly upon B cell receptor activation. However, it is unknown whether it plays a role in LPS signaling.

LPS activates PKA, PKCII, and PI3K/Akt, which cause p65 phosphorylation either by direct interaction or by indirect action through the activation of IKKα, leading to increased NF-κB activity and NF-κB-mediated gene transcription (36, 94, 232). PI3K/Akt is linked to upstream signaling molecules IRAK1 by interacting with TRAF-6 and RIP (244). LPS and other bacterial components trigger the release of numerous inflammatory cytokines (Table 3), which contribute significantly to septic pathophysiology. Those cytokines cause NF-κB activation through their respective signaling pathways. The TNF signaling pathways are most well characterized and described here. Upon stimulation, TNFRI recruits TNFR-associated death domain protein (TRADD) (94). TRADD is an adaptor protein that binds to and recruits downstream adapters, TRAF-2 or TRAF-5, to the receptor complex. TRAF-2 or TRAF-5 subsequently recruits RIPs to the receptor complex by direct TRAF/RIP interaction (94). Different RIP isoform interacts with different bridging molecules. RIP1 interacts with TRAF-2 (94), whereas RIP2 interacts specifically with TRAF-1, TRAF-5, and TRAF-6, but not with TRAF-2, TRAF-3, or TRAF-4 (158). Interaction between RIPs and IKKγ leads to the oligomerization of the IKK complex (94), resulting in IKK activation, IκB phosphorylation and degradation, and subsequent NF-κB activation. Recent studies have indicated that TNF signaling cascades are more complicated than this simple module. First, the formation of a TNFR1/ TRAF-2/RIP complex and subsequent recruitment of IKK require the participation of other interacting proteins or kinases. It has been reported that the focal adhesion kinase (FAK) acts as bridge linking TRAF-2 to RIP. TNF-induced TRAF-2/RIP interaction and the subsequent recruitment of IKK complex are not observed in FAK−/− cells (79). RIP is not the only molecule linking TRAF-2 to IKK; NIK is also reported to interact with TRAF-2 and activates IKK complex (230). MEKK3 interacts with RIP and directly phosphorylates IKK and is required for IKK activation (94). MEKK3 functions downstream of RIP and TRAF-2 (94). In response to TNF-α, the MAP3K TAK1 binds to the TRAF-2 and IKK complex, leading to the activation of IKK and NF-κB pathway (94). TAB2 and TAB3 are the adaptor proteins bridging TRAF-2 and TAK1 (109). In addition to the classic TNF signaling cascades, TNF-α activates IKK/NF-κB via PKC-dependent c-Src kinase activation. Activation of PKCα by TNF-α leads to the activation of tyrosine kinase, c-Src, which causes the phosphorylation of IKKβ at Tyr188 and Tyr199 near its activation loop, resulting in NF-κB activation and the expression of NF-κB-dependent genes (104).
Increased NF-κB activity in sepsis and septic shock. Several lines of evidence support the important role of NF-κB activation in septic pathophysiology. As discussed above, NF-κB is activated by numerous bacteria, bacterial toxins, and proinflammatory mediators known to cause septic shock (Tables 1–3). NF-κB activity induced by different bacterial pathogens display different kinetics. NF-κB activity as induced by β-glucans from pathogenic fungus, Pneumocystis carinii, is significantly slower in the induction of NF-κB activity, and the effect persists for a longer period compared with LPS (135). LPS-induced NF-κB activity is biphasic: an early phase occurred at 0.5–2 h, and a late phase occurred at 8–12 h poststimulation. LPS and other early-released inflammatory mediators cause the early phase NF-κB activation, and TNF and IL-1β mediate the late-phase activation (92). The effect of bacterial toxins on NF-κB activity is dramatic and widespread, leading to massive elevation in NF-κB activity in all organs studied (120, 145, 191, 195). This is consistent with the involvement of multiple organs in septic shock.

NF-κB activity is markedly increased in peripheral mononuclear cells from septic patients, and the level of NF-κB activity correlates with the severity of the disease as quantified by Acute Physiology and Chronic Health Evaluation score (9). NF-κB activity is significantly higher in nonsurviving than that in surviving patients (9, 22). A significant increased NF-κB activity is also observed in alveolar macrophages of patients with septic lung injury (212).

Studies on animal models of septic shock induced either by LPS (endotoxin model) or by cecal ligation and puncture (CLP, polymicrobial model) have demonstrated that NF-κB inhibitors with divergent chemical properties and mechanisms of action protect animals from septic lethality (5, 106, 221). Molecules proven to protect mice from lethal endotoxia (102) or to improve survival in severe sepsis patients (18) exert their protective effect by inhibiting NF-κB activation.

One such example is the anti-inflammatory cytokine IL-10. For many years, IL-10 has been known to inhibit proinflammatory cytokine production (87), improve bacteria-induced lung injury (247). COX-2 and iNOS are the two major enzymes mediating septic cardiovascular dysfunction and also contributing to septic MOD/I (see later discussion). Mice knockout of iNOS gene reduces LPS-induced lethality (255). The reduced mortality correlates with decreased neutrophil infiltration in the liver (255). Targeted disruption of ICAM-1 and P-selectin genes improves cardiac function and survival in mice overexpressing TNF-α (86). Polymicrobial sepsis induced by CLP causes a significant increase in intestinal wall permeability in wild-type mice, and this response is dramatically reduced in mice deficient in IL-6 (247). COX-2 and iNOS are the two major enzymes mediating septic cardiovascular dysfunction and also contributing to septic MOD/I (see later discussion). Mice knockout of iNOS gene reverses the depressed vasconstrictor response, ameliorates the impaired vasodilator response, prevents lung injury, and improves survival rate in both LPS and CLP models of septic shock (39, 98, 126). COX-2 knockout mice are also resistant to LPS-induced inflammation and death (26). 5-LO is another NF-κB-regulated gene that contributes to septic MOD/I. Deletion of the 5-LO gene reduces LPS-induced tissue neutrophil influx and injury in multiple organs (50).

Roles of NF-κB in pathophysiology of septic shock. The pathophysiological abnormalities of septic shock include cardiovascular dysfunction, intravascular coagulation, neutrophil activation, and infiltration into multiple organs, increased microvascular endothelial barrier permeability, and MOD/I. Blockade of NF-κB pathway appears to correct all these alterations in experimental animal models (Fig. 3). A major feature of septic pathophysiology is cardiovascular dysfunction manifested as refractory systemic hypotension, depressed cardiac contractility, vascular hyporeactivity to vasoconstrictors, and impaired endothelium-dependent vasodilator response. Compelling evidence indicates that excessive production of NO and vasodilator prostaglandins are mainly responsible for the septic cardiovascular dysfunction (26, 39, 98, 111, 122,
188). NOS inhibitors reverse systemic hypotension in both animal models of septic shock and septic patients (122, 188). These inhibitors also restore vascular responsiveness to vasoconstrictors and ameliorate the depressed myocardial contractility associated with septic shock (122, 188). Compared with wild-type mice, iNOS knockout mice show attenuated systemic hypotension and improved vascular contractility in both LPS and CLP models of septic shock (39, 98). Blocking prostaglandin production with the COX inhibitor ibuprofen protects against hypotension and depression of cardiac function associated with endotoxic shock and reduces endotoxic mortality (26, 111). Thus iNOS-generated NO and COX-2-produced vasodilator prostaglandins are mainly responsible for septic cardiovascular dysfunction.

Fig. 3. NF-κB activation plays a central role in the pathophysiology of septic shock. Bacteria and bacterial components activate NF-κB through multiple signaling pathways, leading to 1) expression of inflammatory cytokines such as TNF-α, IFN-γ, IL-1β, IL-6, IL-12, and IL-18, which cause fever and neurological abnormalities and more importantly activate NF-κB and its upstream signaling molecules, amplifying and perpetuating NF-κB-mediated inflammatory response; 2) expression of tissue factor (TF), factor VIII, and plasminogen-activator inhibitor type-1 (PAI-1), leading to the activation of extrinsic and intrinsic coagulation cascades and an impaired fibrinolytic, resulting in disseminated intravascular coagulation (DIC); 3) expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), and excessive production of nitric oxide (NO) and vasodilator prostaglandins, resulting in systemic hypotension, depressed cardiac contractility, and vascular hyporeactivity; 4) expression of adhesion molecules (ICAM-1, VCAM-1, E-selectin, P-selectin) and chemokines [IL-8, macrophage inflammatory protein (MIP)-1/2, monocyte chemoattractant protein (MCP)-1/2, cytokine-induced neutrophil chemoattractant (CINC)], leading to neutrophil infiltration and activation, and the release of reactive oxygen species (ROS), reactive nitrogen species (RNS), and proteolytic enzymes, which cause microvascular endothelial injury, increased endothelial barrier permeability and resultant multiple organ injury; and 5) expression of COX-2, 5-lipoxygenase (5-LO), 5-LO-activating protein (FLAP), leading to the production of proinjurious prostaglandins (PGs), leukotrienes (LTs), and thromboxane A2 (TBXA2) that cause tissue injury. Those cascades of events are interrelated. Activation of coagulation promotes inflammatory mediator production and amplifies the process of inflammation. Systemic hypotension and coagulation cause tissue hypoperfusion and hypoxia, setting up the precondition for the development of organ injury.
gene and protein expressions in vital organs in vivo (22). NF-κB also mediates TNF-α-induced factor VIII promoter activity and gene expression (183). TF and factor VIII are the two key factors initiating the extrinsic and intrinsic coagulation pathways, leading to the activation of coagulation. Coagulation can cause the release of inflammatory mediators such as thrombin, which activates NF-κB and induces NF-κB-dependent gene expression (200), forming a positive feedback loop to amplify the coagulation. NF-κB mediates cytokine-induced expression of plasminogen activator inhibitor type 1 (PAI-1) (100). Increased PAI-1 level leads to impaired fibrinolysis, further promoting coagulation. NF-κB activation also mediates cytokine-induced downregulation of thrombomodulin (TM) expression (229). TM regulates coagulation by decreasing the procoagulant activities of thrombin and by converting protein C to activated protein C, which inactivates procoagulant factors FVa and FVIIIa. Impairment of TM-mediated anticoagulation mechanism further exacerbates coagulation, leading to the development of DIC. DIC is one of the key factors contributing to microvascular dysfunction and multiple organ injury in sepsis (163). Activation of coagulation also promotes the release of inflammatory mediators, which further amplify the inflammatory response (163). Inhibition of NF-κB activation prevents coagulation, resulting in improved outcome of septic shock (22).

MOD/I is a devastating complication of sepsis and is the major cause of death in these patients. As discussed above, NF-κB activation triggers a cascades of events that lead to systemic hypotension and intravascular coagulation, which cause tissue hypoperfusion and hypoxia, setting up the conditions for the development of MOD/I. NF-κB mediates the expression of multiple proinjurious and proinflammatory cytokines, chemokines, adhesion molecules, and enzymes and enzyme cofactors (11, 183, 215). Proinjurious cytokines cause or promote organ inflammation and injury directly and increase endothelial barrier permeability (218). Proinflammatory enzymes, COX-2, 5-LO, and 5-LO-activating protein (FLAP) synthesize and release proinjurious eicosanoids, and iNOS synthesizes NO, leading to the subsequent formation of RNS, all of which cause endothelial injury and contribute to the pathophysiology of septic MOD/I (26, 74, 140). Adhesion molecules and chemokines mediate neutrophil infiltration, adhesion, and activation at sites of inflammation (1, 32, 196), which lead to the release of ROS, RNS, and proteolytic enzymes, resulting in microvascular endothelial injury, increased endothelial barrier permeability, and ultimately MOD/I. Thus NF-κB activation is involved in multiple steps in the cascades of molecular events that lead to the development of MOD/I. Studies on animal models have demonstrated that inhibition of NF-κB activation diminishes gene and protein expressions of those proinflammatory mediators, reduces tissue neutrophil infiltration, and prevents LPS- or cytokine-induced injury in multiple organs (1, 144, 145). Inhibition of IKK activity has also been shown to reduce cytokine production and to decrease neutrophil infiltration in lungs, colon, and liver and to improve survival rate in a polymicrobial model of sepsis (268). The clinical relevance of these animal studies is supported by studies showing that alveolar macrophages from septic acute respiratory distress syndrome patients (212) and neutrophils from patients with postoperative organ dysfunction (75) have significantly increased NF-κB activity. Moreover, bronchoalveolar lavage fluid from acute lung injury patients is capable of activating NF-κB in cultured epithelial cells (177), and plasma from patients with postoperative organ dysfunction stimulates NF-κB activity in healthy neutrophils, an effect that is suppressed by NF-κB inhibitors (75).

The impact of NF-κB activation on the development of septic MOD/I is both profound and extensive. NF-κB activation is an integral part of the pathological response that leads to the development of organ dysfunction/injury. In the rat brain, LPS and IL-1β cause LTP in dentate gyrus, which is attenuated by the NF-κB inhibitor SN-50 (120). Inhibition of NF-κB activation with NF-κB decoy ODN attenuates neuronal damage (240). In the heart, cardiac-specific overexpression of IκBα protects against cardiac injury during trauma (33), and inhibition of NF-κB activation prevents LPS-induced increase in microvascular permeability (145). In the liver, inhibition of NF-κB activity suppresses LPS-induced inflammatory cytokine and adhesion molecule expression, reduces neutrophil influx, and prevents LPS-induced increase in microvascular endothelial permeability (144, 145). Intracellular delivery of the NF-κB decoy ODN in vivo suppresses endotoxin-induced inflammatory cytokine production and fatal liver failure in mice (180). NF-κB activation also plays important role in septic lung injury (72, 145). NF-κB activity is significantly elevated in patients with septic lung injury (212). Inhibition of NF-κB activation suppresses LPS-induced cytokine and adhesion molecule expressions, reduces lung neutrophil influx, and prevents LPS-induced increase in lung microvascular endothelial barrier permeability in experimental animals (145). In the kidney, NF-κB is activated by LPS (191). Gene transfer of truncated IκBα prevents the tubulointerstitial injury (236).

Increased NF-κB activity is detected in various regions of gastrointestinal tract during endotoxemia (195). NF-κB activation mediates TNF-α-induced increase in intestinal epithelial tight junction permeability (149). Anti-inflammatory cytokine TGF-β inhibits gut inflammation by inhibiting NF-κB activation. Failure of this TGF-β negative regulatory mechanism leads to a sustained NF-κB activation and gut inflammation (1, 168). As discussed above, NF-κB activation contributes to the development of muscle wasting, which is another feature of septic shock (29).

Effects of NF-κB inhibition on the outcome of septic shock. Studies using an LPS model of septic shock have consistently demonstrated that blocking the NF-κB pathway improves the outcome of septic shock. Inhibition of NF-κB activation using inhibitors with diverse chemical properties and mechanisms of action or using antisense ODN to p65 significantly increased the survival rate of septic animals (Table 4). Some of the reported results are impressive, improving survival rate from 0–20% (LPS alone) to 80–100% (LPS plus NF-κB inhibitors). However, studies using bacterial models of septic shock have given conflicting results (Table 4). Studies using a CLP model showed that inhibition of NF-κB significantly improved survival rate in three studies but significantly reduced the survival rate in one study (Table 4). Blockade of NF-κB activation using p65 antisense ODN had no effect on the survival rate of septic mice induced by intravenous injection of S. aureus (85). Mice deficient in the p50 subunit of NF-κB showed a reduced survival time compared with wild-type mice when challenged by intraperitoneal injection of S. pneumoniae (216). There are likely important differences between LPS and bacterial models
in that bacterial challenge involves the effects not only of bacterial toxins but also of bacterial infection, including the activation of a robust host-defense response. In the LPS model, blockade of NF-κB pathway typically inhibited or terminated the inflammatory and injury-promoting responses and, therefore, improved survival. In the bacterial model, however, blocking NF-κB activation inhibited both the inflammatory and injury-promoting responses as well as the salutary host-defense responses. The beneficial effects of inhibiting inflammatory response can be compromised by the impairment of bacterial clearance capacity as a result of NF-κB inhibition. Thus the consequences of blocking NF-κB action in the bacterial model of septic shock may depend on the balance of the two opposite effects of NF-κB inhibition. It has been demonstrated that treatment of mice with morphine markedly inhibited NF-κB activation and proinflammatory gene expression, reduced tissue neutrophil infiltration but increased bacterial burden in lungs, spleen, and blood with a resultant increase in mortality (246), consistent with this concept.

SUMMARY AND CONCLUDING REMARKS

NF-κB is a group of pleiotropic transcription factors activated by numerous stimuli (>460 and still counting). Studies in the past 10 years have uncovered multiple new pathways and new mechanisms regulating NF-κB activity. In addition to the canonical pathways, NF-κB activity is regulated by multiple noncanonical pathways that lead to NF-κB activation without involving IKK activation, IkB serine phosphorylation, and proteasomal degradation. NF-κB transcriptional activity is determined not only by the nuclear translocation of NF-κB dimers but also by other processes, including posttranslational modification of NF-κB protein, regulation of NF-κB dimer recruitment to target promoters, modification of histones surrounding NF-κB binding sites, engagement or disengagement of transcriptional cofactors and modulators, and interaction with positive and negative regulators.

NF-κB controls or contributes to the transcription of >200 genes that are involved in a variety of physiological and pathophysiological processes, particularly in immunity and inflammation. NF-κB is a critical regulator of cell differentiation, proliferation, and apoptosis and plays pivotal roles in normal organ development and tumorigenesis. Aberrant regulation of NF-κB activity has been implicated in the pathogenesis of multiple diseases, including autoimmune diseases, immune deficiencies and disorders, inflammatory diseases, I/R injury, cancers, and neurodegenerative disorders.

NF-κB activation is a central molecular event leading to the development of septic shock (Fig. 3). NF-κB is activated by a variety of bacteria and bacterial components and is the final target of all these septic shock-inducing agents. Studies using animal models of septic shock have shown that NF-κB activity is markedly elevated in multiple organs. NF-κB activity is also markedly increased in patients with septic shock, and the degree of NF-κB activity correlates with the severity of the disease. Greater NF-κB activity is associated with higher rates of mortality and worse clinical outcome. NF-κB activation mediates the transcriptional expression of large number proinflammatory genes that play important roles in pathophysiology of sepsis. Mice deficient in those NF-κB-dependent genes are resistant to the development of septic shock and sepsis-related death in both endotoxin and CLP models. Importantly, blockade of NF-κB pathway corrects the pathological abnormalities seen in septic shock in animal models. Inhibition of NF-κB activation reduces multiple inflammatory gene expression, restores systemic hypotension, prevents myocardial dysfunction, diminishes intravascular coagulation, and decreases tissue neutrophil influx and microvascular endothelial barrier permeability. Inhibition of NF-κB activation is also coupled to the prevention of multiple organ injury and improves the survival in rodent models of septic shock.

The critical role of NF-κB activation in septic pathophysiology and the effectiveness of inhibiting NF-κB activation in correcting septic abnormalities indicate that targeting NF-κB is a desired therapeutic strategy for the treatment of septic shock. This idea is supported fully by the significantly improved survival rate in the LPS model of septic shock. However, in response to bacterial challenge, the bacterial burden remains a major issue, because inhibition of NF-κB activation also im-

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CLP, cecal ligation and puncture; PDTC, pyrrolidone dithiocarbamate; ODN, oligodeoxynucleotide; GaIN + LPS, α-galactosamine (20 mg ip) followed by LPS (5 mg ip); IRFI 042, 5-emissuccinoyl-2-[2-(acetylthio)ethyl]-2,3-dihydro-4,6,7-trimethylbenzofuran; YS 51, 1-(beta-naphthylmethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline; 15d-PGJ2, 15-deoxy-delta(12,14)-prostaglandin J2.
pairs the bacterial clearance capability. Unfortunately, the innate immune response controls both bacterial clearance and the inflammatory response that causes the pathophysiology of septic shock, and in this sense the two are intertwined. Therefore, at this time it would be unrealistic to inhibit one response without affecting the other. One potentially useful strategy would be to inhibit NF-κB activation in a cell-specific manner such as in endothelial cells. Endothelial cells play an important role in pathology of sepsis, and endothelial activation is an initiating step of sepsis-induced MOD/I. If inhibition of NF-κB role in pathology of sepsis, and endothelial activation is such as in endothelial cells. Endothelial cells play an important role in pathology of sepsis, and endothelial activation is an initiating step of sepsis-induced MOD/I. If inhibition of NF-κB activation in endothelial cells does not interfere with the host-defense function, then this might be a strategy for disconnecting the proinflammatory and host-defense functions of NF-κB activation. Another possible approach may be a selective modulation of NF-κB activity. This strategy requires an initial assessment of the immune state and bacterial load; thus, in the absence of clear bacterial burden, inhibition of NF-κB may be beneficial in mitigating the inflammatory response, whereas a significant bacterial burden would lead to a detrimental outcome following inhibition of NF-κB. It is possible that with the development of targeted cell-specific strategies inhibiting NF-κB activation, the consequences of sepsis inducing lung injury and multiorgan failure can be prevented.

REFERENCES
Invited Review


NF-κB in Septic Pathophysiology

Invited Review

L641


NF-kB in SEPTIC PATHOPHYSIOLOGY


NF-κB IN SEPTIC PATHOPHYSIOLOGY

Invited Review

L643


B IN SEPTIC PATHOPHYSIOLOGY


