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Nuclear Factor-κB Activation and Innate Immune Response in Microbial Pathogen Infection

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ABSTRACT. Human pathogenic microorganisms have developed a variety of strategies to infect the host organism successfully, whereas the host has evolved a series of defense mechanisms. In most cases, the epithelial cell layer represents the first barrier for the bacterial pathogen and triggers the innate and inflammatory responses in the host. Epithelial cells release proinflammatory mediators including cytokines and chemokines, leading to the subsequent attraction of monocytes/macrophages. Therefore, epithelial cells represent an immediate-early warning system in the host organism. Subsequent to the colonization of the epithelial layer, invasive microbial pathogens often induce an acute inflammatory response, which functions to activate residential macrophages and recruits blood leukocytes to the site of infection. Distinct receptors of the Toll family on the cell surface of immune cells mediate antibacterial responses in mammals as well as in *Drosophila*. One of the most important cellular factors involved in the regulation of the host innate antimicrobial response is the immediate-early response transcription factor nuclear factor (NF)-κB. Microbial pathogens activate cellular signal transduction pathways that induce NF-κB activation, but pathogens also find ways to overcome the innate immune response through active manipulation of the NF-κB signal transduction pathways. Exploration of the mechanisms that influence NF-κB activity could contribute to a better understanding of the molecular pathogenesis of microbial infections and could be important for potential therapeutic intervention that may be relevant in a wide variety of inflammatory diseases. BIOCHEM PHARMACOL **60**;8:1109–1114, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. NF-κB; signal transduction; innate immune response; inflammation; *Helicobacter pylori*

The induction of proinflammatory cytokine genes and other genes with immunomodulatory function represents an essential part of the host response to infection with microbial pathogens. Cytokines were initially thought to be produced solely by cells of the immune system; however, it is now evident that non-immune cells, including epithelial cells, also produce cytokines and provide an essential first-line defense [1]. Induction of cytokine/chemokine genes is mediated by the immediate-early response transcription factor NF-κB,† which has an important function in the regulation of the host inflammatory response. The ubiquitous dimeric transcription factor NF-κB can be induced in different cell types by certain microbial components and translocates into the nucleus upon cellular stimulation, where it binds target genes and regulates their transcription. Cellular inhibitors within the NF-κB system, the IκB molecules, block the nuclear translocation of NF-κB. In response to a variety of extracellular stimuli, the IKK complex phosphorylates and marks IκB molecules for degradation and subsequent ubiquitination [2]. The ubiquitin ligase complex that recognizes phosphorylated IκB molecules consists of an F-box/WD-domain protein, β-transducin repeat-containing protein (β-TrCP)/Slimb, Skp1, Cdc53/Cul1, and ROC1 [3]. The IKKs (IKKα, IKKβ, and NF-κB essential modulator [NEMO] or IKKγ) themselves form a complex and respond to a number of cellular NF-κB upstream activators [4].

NF-KB ACTIVATION IN INFECTED EPITHELIAL CELLS

Due to their localization between the external environment and the internal tissue, epithelial cells have the first contact with pathogenic microbes in most cases. Thus, epithelial cells and the mucosa form a barrier that impedes the invasion of microorganisms and their products. Epithelial cells thus actively participate in mucosal immunity and inflammation. Inflammatory responses at mucosal sites involve an array of mediators, including prostaglandins, leukotrienes, and cytokines [5]. Epithelial cytokine responses to bacteria were first studied using epithelial cell

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† *Abbreviations:* AP-1, activator protein-1; GM-CSF, granulocyte-macrophage colony-stimulating factor; IκB, inhibitor kappa B; IKK, IκB kinase; IRAK, IL-1 receptor-associated kinase; JNK, c-Jun N-terminal kinase; IL, interleukin; LPS, lipopolysaccharide; MCP-1, monocyte chemotactic protein-1; MKK, mitogen-activated protein kinase kinase; NF-kappaB, nuclear factor-kappaB; NIK, NF-κB-inducing kinase; PAI, pathogenicity island; TLR, Toll-like receptor; TNF-alpha, tumor necrosis factor-alpha; and Yop, Yersinia outer proteins.

lines from the human urinary tract. *In vivo*, the rapid local cytokine response to urinary tract infection (less than 30 min) indicates that bacteria induce mucosal cytokine production directly [6]. Subsequent studies have shown that cytokine production occurs during a variety of infections in human [1]. Thus, the ability to produce cytokines in response to microbial pathogens appears to be a general feature of epithelial cells.

In infection by *Neisseria gonorrhoeae*, the etiologic agent of gonorrhea, the rapid production of proinflammatory cytokines by epithelial cells involves the activation of NF-κB. Nuclear translocation and DNA binding of NF-κB appear rapidly (within 10 min) and transiently (last for 3 hr) and are induced even at a multiplicity of infection of 5 (MOI 5), indicating the high specificity for NF-κB activation. The NF-κB heterodimer activated by *N. gonorrhoeae* is composed of the p50 and p65 subunits. *N. gonorrhoeae* infection induces the inflammatory cytokines/chemokines TNF-α, tumor growth factor (TGF)-β, GM-CSF, IL-1α, IL-1β, IL-6, IL-8, IL-12p40, and MCP-1 in various epithelial cell lines. A serine protease inhibitor, TPCK (*N*-tosyl-L-phenylalanine-chloromethyl ketone), which blocks IκBα degradation, thereby inhibits up-regulation of cytokine mRNAs of TNF-α, GM-CSF, IL-6, IL-8, and MCP-1. Thus, NF-κB plays a major role in the transactivation of these genes in response to *N. gonorrhoeae* infection. The activation of NF-κB and cytokines also occurs in *N. gonorrhoeae*-infected and cytochalasin D-treated cells (this compound interferes with the integrity of actin filaments, which are required for the bacterial entry process), indicating that the cellular signaling is independent of bacterial invasion. LPS from the gram-negative *N. gonorrhoeae* does not induce NF-κB in epithelial cells [7]. LPS effectively induces cytokine production from macrophages, but only poorly induces epithelial cytokine responses. The poor LPS response can be easily explained by the lack of CD14 and TLR4 on epithelial cells (see below). Additionally, *N. gonorrhoeae* induces the transcription factor AP-1, involving activation of the JNK [7, 8]. The distinct signaling leading to NF-κB activation has been not studied so far in *N. gonorrhoeae*-infected epithelial cells. The notion that epithelial cells represent an integral component of the host's non-specific immune response in bacterial infection is consistent with other reports. For example, *Escherichia coli* induces IL-1α, IL-1β, IL-6, and IL-8, but not TNF-α in epithelial cells [6, 9]. Other microorganisms such as the *Salmonella*, *Shigella*, *Listeria*, and *Helicobacter* species induce NF-κB and a wide range of cytokines including MCP-1, GM-CSF, IL-8, and TNF-α [10].

Bacterial attachment enhances the induction of cell cytokine responses by an unknown mechanism. Fimbriae (in *E. coli*)-mediated cell contact may increase the concentration of bacterial products at the cell surface that activate NF-κB and cytokines [6, 11]. Additionally, certain bacterial virulence factors such as the PAI-encoded proteins of *Helicobacter pylori* influence the magnitude of the epithelial cytokine response and the mechanism of cytokine activation. The epithelial cytokine/chemokine response may be particularly important at the early stages of *H. pylori* infection, whereas inflammatory mediators produced by infiltrated polymorphonuclear leukocytes and mononuclear phagocytes could directly damage the surface epithelial layer, leading to loss of microvilli, irregularity of the luminal border, and vacuolization [12]. The events that commonly follow the infection consist of gastritis, peptic ulcer and, more rarely, gastric cancer and low-grade B-cell mucosa-associated lymphoid tissue lymphomas [13]. The major disease-associated, genetic difference in *H. pylori* strains is the presence or absence of a pathogenicity island containing 31 genes that code for proteins involved in a specialized type IV secretion machinery [14]. *H. pylori* infection modulates the host cells by bacterial protein translocation. Several groups have shown for the first time that *H. pylori* translocates the CagA protein by a type IV secretion system. CagA is tyrosine-phosphorylated and induces changes in the tyrosine phosphorylation state in the epithelial cell [15–19]. Knockouts of certain PAI genes suppressed or reduced the activation of NF-κB [20–24] or AP-1 [24], and affected the secretion of cytokines/chemokines such as IL-8, RANTES, growth-related oncogene (GRO)-α, macrophage inflammatory protein (MIP)-1α, epithelial neutrophil-activating peptide (ENA)-78, MCP-1, TNF-α, IL-6, and IL-1, which are induced by *H. pylori* infection [25]. Similar to *N. gonorrhoeae* infection of epithelial cells, the *H. pylori*-directed NF-κB activation is transient, direct, and very specific. In response to *H. pylori* infection, IKKβ activation, as well as IκBα phosphorylation and degradation, was observed within 30 min. Upstream of the IKK complex, NIK is crucial for NF-κB activation in *H. pylori*-infected epithelial cells* (Fig. 1). The role of *H. pylori*-induced p21-activated kinase 1 (PAK-1) in NF-κB activation, which is necessary in *H. pylori*-directed JNK and AP-1 activation [24], is currently under investigation.† Since the integrity of the type IV secretion machinery is an absolute requirement for NF-κB activation in *H. pylori* infection, it is reasonable to speculate that the component(s) which induce this transcription factor must either be injected into the eukaryotic target cell or activate contact-dependent eukaryotic cell-surface receptors. This hypothesis is supported by the notion that only those PAI factors (CagA, CagF, and CagN) that are not necessary for the functional integrity of the type IV secretion apparatus do not affect NF-κB activation. Thus far, the identity of the effector molecule(s) of *H. pylori* and the mechanism of integration of the signals that induce NF-κB are not known. Since gastric inflammation is a hallmark of *H. pylori* infection, the understanding of the host cell response mechanism could contribute to treatment of the disease.

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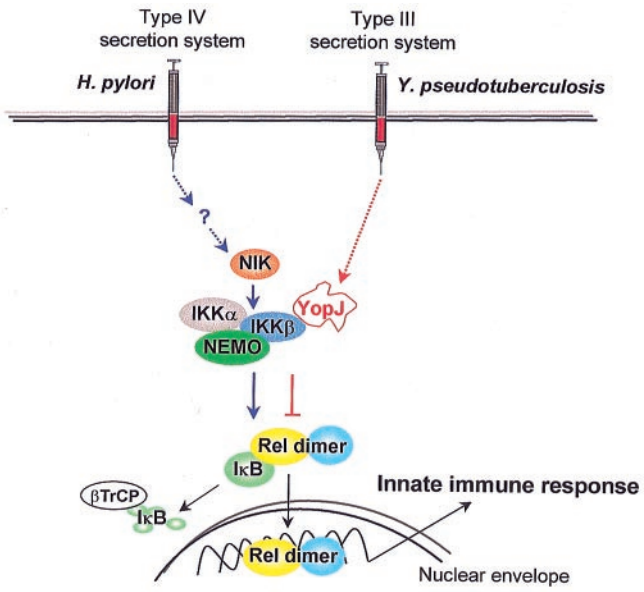

FIG. 1. Activation and inactivation of NF-κB by microbial pathogens in epithelial cells. A specialized type IV secretion machinery in *H. pylori* infection modulates the host cell signaling by bacterial protein injection. Knockouts of certain genes coding for type IV secretion components suppress or reduce the activation of NF-κB or AP-1 in epithelial cells, and affect the secretion of cytokines/chemokines. The *H. pylori*-directed activation and nuclear translocation of NF-κB is transient and involves IKKβ activation as well as IκBα phosphorylation and degradation. Upstream of the IKK complex, the NIK is crucial for NF-κB activation in response to *H. pylori* infection. *Yersinia pseudotuberculosis* inject the virulence factor YopJ into the eukaryotic cell via a specialized type III secretion apparatus. The YopJ protein inhibits activation of NF-κB and the production of certain proinflammatory cytokines by directly binding to IKKβ and inhibiting its kinase activity. βTrCP, β-transducin repeat-containing protein.

NF-KB INACTIVATION AS A MECHANISM TO AVOID THE INNATE IMMUNE RESPONSE

The central role of NF-κB in the innate immune response suggests that many pathogenic microorganisms have evolved mechanisms to interfere with the function of NF-κB or modulate NF-κB signal transduction. Currently, however, few examples of such microorganisms indicating an active process of NF-κB inhibition directed by the pathogen have been determined. The bacterial pathogen genus *Yersinia*, including the species *Y. enterocolitica*, *Y. pseudotubercolosis*, and *Y. pestis*, contains a virulence plasmid encoding a type III secretion system to inject several virulence factors into target cells. Upon infection, this system delivers virulence factors known as Yops into host cells. The Yops disrupt host signaling functions to avoid the immune response [26]. A virulence factor from *Y. pseudotuberculosis*, YopJ, is a 33-kDa protein that inhibits both the activation of NF-κB and that of the signaling pathways for extracellular-regulated kinase (ERK), JNK, and p38 kinase [27–31]. The disruption of these pathways by YopJ blocks the production of proinflammatory cytokines in the infected epithelial cells as well as in macrophages. The YopJ was shown to bind to IKKβ and the superfamily of mitogen-activated protein MKKs and to inhibit phosphorylation and subsequent activation of these kinases [32]. Thus, by blocking IKKβ and MKKs, *Yersinia* inhibits the release of cytokines and other immunomodulatory factors during innate immune response. YopJ-related proteins in other organisms (AvrA of *Salmonella typhimurium*) and in plant pathogens (AvrRxv of *Xanthomonas campestris*) suggest that this family of proteins could play a fundamental role in the modulation of host signaling responses [33, 34].

NF-KB ACTIVATION BY TOLL-LIKE RECEPTORS IN IMMUNE CELLS

Infection by invasive microorganisms often leads to an acute inflammatory response, which functions to activate residential macrophages and recruit blood leukocytes to the site of the inflammatory insult. Gene expression of endothelial adhesion molecules and CXC chemokines, which mediate neutrophil adhesion and transmigration from blood vessels to tissue interstitium, is also controlled by NF-κB [35]. This response is an essential protective mechanism for the host to remove injurious agents and restore normal tissue structure and function.

Recent advances in the molecular characterization of innate immune response mechanisms in multicellular organisms such as mammals and insects have revealed striking similarities, suggesting that they share a common evolutionary ancestry [36]. Within the fruit fly *Drosophila melanogaster*, distinct cell-surface receptors belonging to the Toll family mediate antibacterial responses. They do so by inducing genes that encode antimicrobial peptides [37]. Additionally, six human genes related to Toll have been reported [38–40]. The transmembrane receptors contain leucine-rich repeats in the external domain and the Toll homology domain in the cytoplasmic part, which is also present in members of the interleukin-1 receptor family [39]. Only if invasive pathogens overcome the epithelial barrier can they activate TLRs and thereby amplify the innate immune reaction. In mammals, two distinct TLRs expressed in macrophages discriminate between different microbial pathogens or their products [41], and are central to the intensity and specificity of the innate immune response (Fig. 2). TLR4 represents the receptor recognizing gram-negative bacteria and LPS [42, 43]. It is suggested that TLR4 cooperates with the glycosylphosphatidylinositol-anchored membrane protein CD14, the principal LPS binding protein on the surface of mononuclear cells [44, 45]; CD14, however, does not directly participate in the initiation of the intracellular signal transduction. Instead, TLR4 is probably activated by the complex of CD14 and LPS binding protein (LBP) with LPS, and induces transmembrane signaling [41]. In contrast to TLR4, TLR2 transduces the signal evoked by lipoteichoic acid [46], bacterial lipopeptides [47–49], peptidoglycan [46], and whole gram-positive bacteria [50].

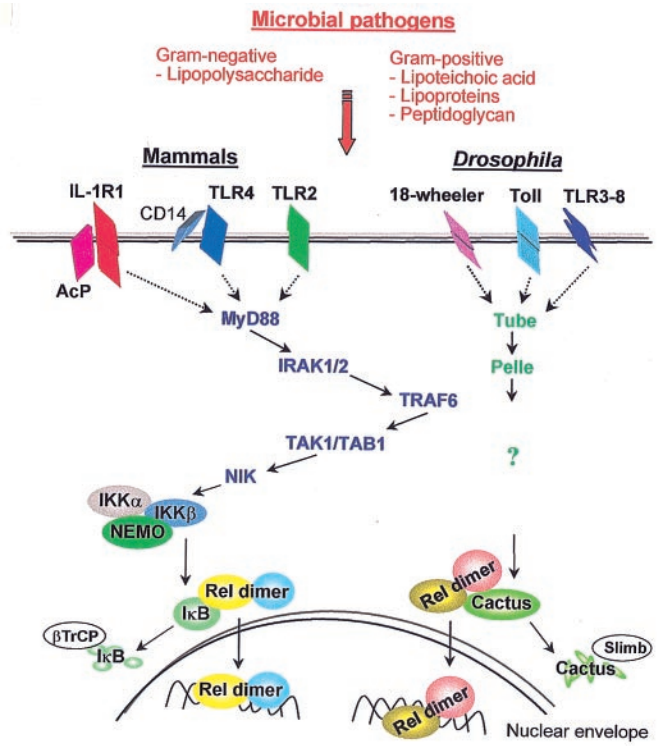

FIG. 2. Schematic representation of the signaling downstream of Toll receptors and IL-1 receptor 1 in immune cells. Ligand binding induces recruitment of adaptor molecules (MyD88 in mammals and Tube in *Drosophila*) and the kinases IRAK (mammals) and Pelle (*Drosophila*). In mammals, the TLR4 responds to LPS of gram-negative bacteria, which binds to the CD14 receptor via the LPS binding protein, LBP. Lipoteichoic acid, bacterial lipoproteins, and peptidoglycan of gram-positive bacteria are recognized by the TLR2. In *Drosophila*, the receptors triggering an antibacterial response and the underlying mechanisms are not completely understood. In mammals, the activated TLRs or the IL-1 receptor activate a cascade involving the activity of TRAF6 and certain kinases which directs the activation of the IKK complex (IKKα, IKKβ, NF-κB essential modulator [NEMO] or IKKγ). Transforming growth factor β-activated kinase 1 (TAK1) and its adaptor TAK1-binding protein 1 (TAB1) bind to TNF receptor-associated factor 6 (TRAF6) after IL-1 stimulation and both interact with NIK. NIK phosphorylates IKKα and activates IKKβ, and activated IKKβ phosphorylates serines within IκB molecules (e.g. IκBα, p105). An IKK molecule in *Drosophila* is not known so far. Once phosphorylated, the IκB–Rel dimer complex or the Cactus–Rel dimer complex associates with ubiquitin ligase (whose recognition subunit is called β-transducin repeat-containing protein (βTrCP) in mammals and Slimb in *Drosophila*). This complex ubiquitinates IκB molecules or Cactus and targets them for degradation by the 26S proteasome. Removal of the inhibitor relieves the nuclear localization signal of the Rel dimer and allows nuclear translocation and activation of target genes.

Biochemical experiments in mammalian cells and genetic analysis in *Drosophila* have defined a signaling cascade after activation of Toll receptors that directs nuclear localization of the NF-κB/Rel proteins (Dorsal, Dif, and Relish in *Drosophila*) (Fig. 2). At the receptor, an adaptor protein (MyD88 in mammals and Tube in *Drosophila*) and a kinase (IRAK in mammals and Pelle in *Drosophila*) assemble. *Pelle* encodes a serine/threonine kinase with an aminoterminal death domain, which mediates interaction with a similar death domain in Tube. In the mammalian system, IL-1R1 and TLR4 associate with MyD88, which has a TIR domain [51]. Receptor stimulation leads to IRAK autophosphorylation and dissociation of MyD88, and subsequently to activation of the downstream targets TNF receptor-associated factor 6 (TRAF6) and evolutionary conserved signaling intermediate in Toll pathways (EC-SIT) [52]. Two TRAF molecules have been identified in *Drosophila* [53], but their role in the innate immune response is unclear. In mammals, the IL-1 signaling downstream of TRAF6 activates the MAPKK kinase transforming growth factor β-activated kinase (TAK1) together with TAK1-binding protein 1 (TAB1), NIK, and the IKK complex [54]. Remarkably, missense mutations in mouse NIK demonstrate a role of this protein in immune system development, but no direct function in the IL-1 signaling pathway [55]. Targeted mutations of the two kinases of the IKK complex reveal different phenotypes and make clear that the IKKα and IKKβ kinases have different functions. Mice that lack IKKα die at the end of embryogenesis with defects in skin and skeleton. Homozygous mutant fibroblasts from these animals standardly activate NF-κB in response to IL-1 [56–58]. IKKβ-deficient mice die at mid-gestation with a phenotype similar to p65 knock-out mice, and IKKβ-deficient fibroblasts fail to activate NF-κB in IL-1-stimulated cells [59, 60]. Downstream of the IKK complex (an IKK homologue has not been found so far in *Drosophila*), the substrates (IκBα, IκBβ, IκBε, p105 in mammals and Cactus in *Drosophila*) are phosphorylated and subsequently degraded by the 26S proteasome. Further components contributing to the Toll family signaling pathways will be identified in future; already, two-hybrid screens have identified novel proteins that interact with TRAF6 and Pelle [52, 61].

CONCLUSIONS

NF-κB signaling and activity induced by microbial pathogens in epithelial cells as well as in immune cells determine the outcome of the cellular innate immune defense. To some extent, bacteria have evolved strategies to overcome this host response through active manipulation of the NF-κB signal transduction pathways. In other cases, the infection leads to a chronic inflammatory state, which in turn leads to the destruction of the epithelial structure and severe diseases. In the case of *H. pylori* infection, the chronic inflammatory situation in the mucosal surface seems to be a prerequisite for the development of gastric adenocarcinoma. Therefore, exploitation of the mechanisms causing NF-κB activation or of the regulatory machinery that controls NF-κB represents an important field of potential therapeutic intervention that may be relevant to a wide variety of inflammatory diseases.

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