The IκB/NF-κB system: a key determinant of mucosal inflammation and protection

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Jobin, Christian, and R. Balfour Sartor. The IκB/NF-κB system: a key determinant of mucosal inflammation and protection. Am. J. Physiol. Cell Physiol. 278: C451–C462, 2000.—The ubiquitous transcription factor NF-κB is a central regulator of the transcriptional activation of a number of genes involved in cell adhesion, immune and proinflammatory responses, apoptosis, differentiation, and growth. Induction of these genes in intestinal epithelial cells (IECs) by activated NF-κB profoundly influences mucosal inflammation and repair. NF-κB activation requires the removal of IκB from NF-κB by inducible proteolysis, which liberates this transcription factor for migration to the nucleus, where it binds to κB-regulatory elements and induces transcription. IκBα degradation is incomplete and delayed in IECs, resulting in buffered responses to luminal stimuli. The stimulatory environment partially determines whether the effect of NF-κB is protective or deleterious for the host. κB-dependent proinflammatory gene expression, particularly chemokines, major histocompatibility complex class II antigens, and adhesion molecules may be extremely important in early protective responses to mucosal pathogens but, when dysregulated, could lead to the development of chronic inflammation, as seen in inflammatory bowel diseases. The key role of NF-κB in regulating expression of a number of proinflammatory genes makes this protein an attractive target for selective therapeutic intervention.

cytokines; signal transduction; bacteria; gene manipulation; intestinal epithelial cells

AN EMERGING AREA OF RESEARCH in intestinal homeostasis and inflammation is focused on the role of transcription factors in regulating intestinal epithelial cell (IEC) gene expression. This review discusses recent findings concerning the IκB/NF-κB signaling pathway and the importance of this transcriptional system in IEC biology, mucosal inflammation, and infection.

IMPORTANCE OF IECs IN THE MUCOSAL IMMUNE SYSTEM

IECs form a single layer of cells that isolate the host from the hostile gut luminal environment. Aside from their classical absorptive and physical barrier roles, an emerging concept views IECs as immunological sentinels of the intestinal mucosa (67). IECs are capable of responding to a wide array of biologically active agents commonly found in the lumen of the distal intestine, including bacterial products, adherent and invasive bacteria, cytokines, and short-chain fatty acids. IECs exert their immunological functions by processing and presenting antigens to T cells (45, 79), expressing cell adhesion molecules (30, 56, 68, 72), secreting various cytokines, particularly chemokines (32, 63, 66), releasing eicosanoids (28, 33, 60), producing nitric oxide (69, 70), and expressing costimulatory molecules (24, 79).

The strategic location of IECs allows them to interact both with luminal antigens and with resident intraepithelial and lamina propria mononuclear cells to form a complex network of interrelated immunologically active cells that recognize and respond to mucosal infection, injury, and inflammation. Communication between IECs and mucosal myeloid, lymphoid, and mesenchymal cells might be critical for maintaining gut immune homeostasis (67). Because of the repertoire of molecules they produce when activated by microbial agents or proinflammatory cytokines, IECs can function as sensors of mucosal injury and actively participate in the mucosal response to intestinal inflamma-
tion and infection (67). In response to bacterial invasion (66), bacterial products (57, 66), and proinflammatory cytokines (55), IECs produce a wide variety of chemokines, adhesion molecules, major histocompatibility complex (MHC) class II molecules, and inflammatory mediators that influence the adjacent immune and mesenchymal mucosal cells and recruit circulating inflammatory cells to the mucosa (15, 67, 71, 84). In turn, proinflammatory molecules such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ), produced by recruited inflammatory and immune cells, reciprocally stimulate adjacent IECs. Whereas lamina propria mononuclear cells may be predominantly responsible for chronic, immunemediated inflammation, IECs likely are quite important in maintaining mucosal homeostasis and responding to environmental challenges that injure the intestine. Therefore, IECs not only represent the gut’s first line of defense but are also part of a complex and well-orchestrated mucosal immune system.

Although these in vitro studies strongly suggest a role for IECs in intestinal inflammation, it is still unclear whether they participate in the initiating phase of inflammation. For example, transgenic mice overexpressing IL-8 in epithelial cells in the small intestine do not show signs of neutrophilic extravasation or tissue damage (110). However, genetic alteration of the keratin 8 or N-cadherin gene results in intestinal inflammation (9, 44). In addition, a human IEC xenograft model of Escherichia histolytica infection indicates that IECs participate in the initiating phase of inflammation (106). More recently, stimulation of the peroxisome proliferator-activated receptor or expression of the galanin-1 receptor of IECs has been shown to inhibit or potentiate intestinal inflammation, respectively (43, 112).

Most of the molecules synthesized during the course of IEC stimulation are the result of a highly integrated complex cascade that includes transmission of a membrane signal to the nucleus via activation of a series of protein kinases and phosphatases. This sequence of events ultimately leads to the upregulation of a characteristic set of genes (66, 130). Most of the immune genes that are upregulated in stimulated IECs are transcriptionally controlled, belonging to a class known as immediate-early (IE) genes, for which induction occurs without new protein synthesis. One of the transcription factors that binds to the promoter/enhancer region of many IE genes is the nuclear factor κB (NF-κB).

**IkB/NF-κB System and Gene Regulation**

NF-κB is an inducible transcription factor comprised of subunits that can include cRel, RelA, RelB, p50 and p52 (the latter two synthesized as p105 and p100 precursors, respectively) (7, 10). In most cells the NF-κB prototype is a heterodimer composed of the RelA (p65) and NF-κB1 (p50) subunits. This variant is the most potent gene transactivator among the NF-κB family (8, 97) and is the major NF-κB protein found in the nucleus of cytokine-stimulated IECs (55, 59).

<table>
<thead>
<tr>
<th>Table 1. Inducers of NF-κB</th>
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<tbody>
<tr>
<td>Cytokines and Growth Factors</td>
</tr>
<tr>
<td>Interleukin-1β*</td>
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<td>Interleukin-2*</td>
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<td>Interleukin-17*</td>
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<tr>
<td>Interleukin-18*</td>
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<tr>
<td>Lymphotixin</td>
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<tr>
<td>Leukotriene B4*</td>
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<tr>
<td>Tumor necrosis factor-α*</td>
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<tr>
<td>Macrophage colony-stimulating factor</td>
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<td>Platelet-derived growth factor*</td>
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<tr>
<th>T Cell Mitogens</th>
<th>Viruses and Viral Products</th>
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<tbody>
<tr>
<td>Antigen</td>
<td>Adenovirus*</td>
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<tr>
<td>Anti-CD2</td>
<td>Epstein-Barr virus</td>
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<tr>
<td>Anti-CD3</td>
<td>Human immunodeficiency virus type 1</td>
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<tr>
<td>Anti-CD28</td>
<td>Human T cell leukemia virus type 1</td>
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<tr>
<td>Calcium ionophores</td>
<td>Hepatitis B virus B</td>
</tr>
<tr>
<td>Lectins (PHA, ConA)</td>
<td>Herpes simplex virus type 1</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>Double-stranded RNA</td>
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<tr>
<td>Oxidative Stress</td>
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<tr>
<td>Oxygen</td>
<td>Membrane protein</td>
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<tr>
<td>Reactive oxygen intermediates*</td>
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</tbody>
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* Documented to stimulate intestinal epithelial cells. LPS, lipopolysaccharide; PG-PS, peptidoglycan-polysaccharide; ConA, concanavalin A; PHA, phytohemagglutinin.

NF-κB is activated by a wide variety of agents, including phorbol esters, IL-1, TNF-α, lipopolysaccharide (LPS), double-stranded RNA, cAMP, bacteria, and viral transactivators (7, 10) (Table 1). Once activated, NF-κB transcriptionally regulates many cellular genes implicated in early immune, acute phase, and inflammatory responses, including IL-1β, TNF-α, IL-2, IL-6, IL-8, IL-12, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), intercellular adhesion molecule-1 (ICAM-1), vascular cellular adhesion molecule-1 (VCAM-1), T cell receptor-α (TCR-α), and MHC class II molecules (7, 10) (Table 2). Thus the inducers

<table>
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<th>Table 2. Molecules regulated by NF-κB in IEC</th>
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<tr>
<td>Product</td>
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<tr>
<td>Cytokines and chemokines</td>
</tr>
<tr>
<td>Interleukin-1β</td>
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<tr>
<td>Interleukin-6</td>
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<tr>
<td>Interleukin-8</td>
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<tr>
<td>GRO-α, GRO-β</td>
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<tr>
<td>RANTES</td>
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<tr>
<td>Macrophage inflammatory protein-2</td>
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<tr>
<td>Cell surface receptor</td>
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<tr>
<td>Interleukin-2R</td>
</tr>
<tr>
<td>CD95/ APO-1 (Fas)</td>
</tr>
<tr>
<td>Adhesion molecule</td>
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<tr>
<td>ICAM-1</td>
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<tr>
<td>Inflammatory enzyme</td>
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<tr>
<td>Inducible nitric oxide synthase</td>
</tr>
<tr>
<td>Cyclooxygenase-2</td>
</tr>
<tr>
<td>Stress proteins</td>
</tr>
<tr>
<td>Complement factors B, C3, C4</td>
</tr>
<tr>
<td>Immunoregulatory molecule</td>
</tr>
<tr>
<td>Major histocompatibility complex I and II</td>
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IEC, intestinal epithelial cell; GRO, growth-related oncogene; RANTES, regulated on activation, normal T cell expressed and secreted; ICAM-1, intercellular adhesion molecule-1.
and products of NF-κB activation are highly relevant to intestinal inflammation (37, 100).

Endogenous cytoplasmic inhibitors, known as IκBs, tightly regulate NF-κB activation by complexing with the transcription factor and trapping it in the cytoplasm. IκB molecules form a distinct family of proteins that include IκBα, IκBβ, IκBε, IκBγ, Bcl3, p105, and p100 (41, 116). Proteins in this family are characterized by an ankyrin repeat domain involved in protein/protein interaction (11, 41).

The most characterized and studied NF-κB inhibitor is IκBα. This protein binds avidly to the p65 (RelA) subunit of NF-κB through the association of ankyrin repeat domains of IκBα with the nuclear localization signal and the Ig-like domain of p65 (52, 53). During activation of NF-κB, numerous stimuli, including IL-1 and TNF-α, activate a complex of IκB kinases (IKK) that phosphorylate IκBα on the amino terminus at serine residues 32 and 36 (Fig. 1) (21). Phosphorylation of these serine residues is a necessary step for inducible IκB degradation; replacement of these two amino acids by site-directed mutagenesis prevents IκB degradation and NF-κB activation (16, 17, 21, 62, 118). Phosphorylated IκBα is then selectively ubiquinated and rapidly degraded via a nonlysosomal, ATP-dependent 26S proteolytic complex composed of a 700-kDa proteasome (73, 89, 103). Evidence for the role of ubiquination comes from recent work showing that target inactivation of a specific IκB-ubiquitin ligase inhibits NF-κB activation (131, 132). In the final steps of the activation cascade, phosphorylation and proteolytic degradation of IκB allows the release and nuclear transmigration of NF-κB (36, 116). Degradation of IκB exposes the NF-κB nuclear localization signal, resulting in transportation of NF-κB into the nucleus (6).

Together with IκBα (37 kDa), the proteins IκBβ and IκBγ (each 45 kDa) are the most abundant and potent mammalian NF-κB inhibitors. Although each of these three proteins acts to inhibit NF-κB, there are differences among them with regard to their affinity for NF-κB and their mechanism of action (6, 42, 108, 109, 117, 119, 126). Regulation and activation of IκBα differs considerably from that of IκBβ and IκBε. In most mammalian cells IκBα is rapidly and completely degraded (<10 min) following inducible phosphorylation but is quickly resynthesized (60 min) in an NF-κB-dependent manner (Fig. 1) (113). Newly synthesized IκBα complexes with nuclear NF-κB to terminate gene transcription (4, 6, 93, 120). Thus stimulation of NF-κB induces its own inhibitor to regulate cellular activation. In contrast, IκBβ transcription is not regulated by NF-κB; therefore, the protein is slowly degraded (2 h) on IL-1 and LPS stimulation and leads to persistent activation of NF-κB (>20 h) (65, 117, 124). The mechanism of persistent activation on IκBβ degradation may involve a chaperone-like role of unphosphorylated, unubiquitinated IκBβ.

**Fig. 1.** NF-κB is kept latent in the cytoplasm by the inhibitor protein IκB. Appropriate stimuli induce selective IκB phosphorylation, which is then ubiquitinated and targeted for degradation by the proteasome pathway. Free NF-κB migrates to the nucleus by virtue of its nuclear localization signal and induces transcription of multiple NF-κB-dependent genes. NF-κB is then inactivated by newly synthesized IκB both in the cytoplasm and the nucleus. IL, interleukin; TNF, tumor necrosis factor; LPS, lipopolysaccharide.
newly synthesized IκBβ that allows transport of NF-κB to the nucleus without being trapped by IκBα (114). Preliminary reports show that degradation and resynthesis are slower for IκBε than for IκBα and that the inhibitory effect of IκBε is exerted in the cytoplasm (108, 109, 126). This pool of variably responding IκB molecules, with different kinetics of degradation, synthesis, and affinity, may allow cells to activate NF-κB differentially and, consequently, to regulate downstream genes differentially in response to the wide array of stimulating agents.

Another level of control for NF-κB activation involves cytokine-induced phosphorylation of RelA/p65, which modulates its transactivation capacity (29, 123, 136). The kinase(s) responsible for p65 phosphorylation remains to be formally identified, but potential candidates include IKK and casein kinase II (12, 80). Therefore, similar to c-jun regulation, NF-κB transactivational activity could be induced by phosphorylation, independently of its DNA-binding activity.

**Cytokine and Bacterial Products Signaling Through the IκB/NF-κB Pathway**

Recent findings in cytokine signaling have provided a better understanding of proximal events involved in NF-κB activation, with elucidation of the mechanisms by which cytokines transduce their signals through the IκB/NF-κB system (Fig. 2). Most of the cytokine and microbial product receptors have no intrinsic kinase activity.
activity and, therefore, rely on scaffolding and adaptor proteins to transmit their extracellular signal inside the cells. On ligand binding, these receptors aggregate and form a multimer complex followed by the recruitment of downstream scaffolding and adaptor proteins to the cytoplasmic tail portion of the receptor. For example, following TNF-α stimulation, the TNF receptor-1 (TNFR1) trimerizes and recruits the TNF receptor-associated factor (TRAF)-2 and the receptor interacting protein (RIP) to the cytoplasmic portion of the TNFR1 via the intermediate action of TNF receptor 1-associated death domain (TRADD) (49, 50). In another example, stimulation by the cytokine IL-1β initiates a signaling cascade that requires the participation of the IL-1 receptor accessory protein (IL-1RACP), MyD88, and the IL-1 receptor-associated kinase (IRAK). These proteins act together to associate with and activate TRAF-6 (18–20, 125). In each case, the signal coming from the respective TRAF/RIP protein is transmitted to the NF-κB-inducing kinase (NIK) (77), which in turn associates/activates the IKK complex (Fig. 2) (75, 111, 121).

The IKK complex is composed of at least IKK-α, IKK-β, and IKK-γ/Nemo subunits and a scaffolding protein named IKK complex-associated protein (IKAP) (102). Differences are seen in the roles of the IKK complex proteins from in vitro studies compared with in vivo studies. In vitro studies suggest that all of the IKK complex proteins are critical in mediating/controlling cytokine-induced IκB phosphorylation (23, 81, 96, 128, 134). However, recent findings using gene deletion technology provide a different picture regarding the contribution of each kinase in NF-κB activation in vivo (78). From these studies, it appears that IKK-β, but not IKK-α, mediates cytokine-induced NF-κB activation (74), whereas the role of IKK-α in vivo seems to be restricted to transmitting signals involved in skeletal development (51, 115). These results suggest that strategies aimed at manipulating the cytokine inflammatory cascade during inflammation should target IKK-β rather than IKK-α.

Another question concerns which upstream kinase mediates cytokine-inducible NF-κB activation in vivo. A puzzling observation is that IKK-β kinase activity is preferentially activated by the mitogen-activated protein kinase kinase kinase-1 (MEKK-1), whereas the NF-κB-inducing kinase NIK has a higher affinity for IKK-α (83, 85). Gene deletion experiments with MEKK-1 and NIK would help clarify the physiological contribution of each kinase in cytokine-induced NF-κB activation. Interestingly, adenoviral-mediated delivery of a constitutively active NIK (AdSNIK) strongly induces IκB phosphorylation/degradation, NF-κB activation, and IL-8 secretion in Caco-2 cells (64). This result suggests that NIK activates the critical IKKβ subunit responsible for NF-κB induction.

There are additional levels of regulation for NF-κB induction by cytokine-induced phosphorylation. Regulation of IKKβ activity appears to be mediated by a cluster of serine residues located next to the helix-loop-helix (HLH) domain (27). The progressive phosphorylation of these serine residues seems to weaken the interaction between the HLH domain and the kinase domain, resulting in a decrease in kinase activity and a consequent decrease in IKKβ phosphorylation activity.

Bacterial products such as LPS and peptidoglycan-polysaccharide (PG-PS) are found in high concentrations in the colon and are able to stimulate IECs (67). Therefore, the elucidation of bacterial products signal transduction through the IκB/NF-κB system is highly relevant for mucosal homeostasis. Recently, it was demonstrated that LPS signaling involved the Toll-like receptors (TLR) (48, 129). Moreover, LPS was shown to signal through the NF-κB system by utilizing components of the IL-1 pathway such as MyD88, IRAK, and TRAF-6 in endothelial and monocytic cell lines (135). Therefore, it would be important to determine whether LPS and PG-PS signal through the NF-κB system in a similar manner in IECs.

Because the signaling cascade leading to NF-κB activation involves the participation of multiple proteins, this complex network provides many potential targets for therapeutic intervention. Recent studies have focused on the role of NF-κB in the clinically important field of intestinal inflammation.

**UNIQUE CYTOKINE SIGNAL TRANSDUCTION AND IκBα/NF-κB ACTIVATION IN IECs**

In the past few years, there has been increased interest in how cytokines, bacteria, and bacterial polymers induce IEC gene expression. IEC gene expression must be tightly regulated to avoid overreaction to normal microbial flora while at the same time remaining adequately responsive to environmental pathogens. The IECs from the distal ileum and colon are in constant contact with a rich microbial flora, with luminal contents having as much as 10^9 aerobic and 10^{11–12} anaerobic enteric bacteria/g wet wt and 80 µg LPS/g feces, yet, under normal conditions, no pathological inflammation is present. This observation, in conjunction with our observation that mature IECs are relatively refractory to IL-1β, LPS, and PG-PS activation, suggests that IECs have developed unique protective responses that allow them to remain quiescent in a hostile environment (Table 3).

The unique signaling pathways associated with cytokine-induced NF-κB activation in IECs has been investigated using various approaches. Adenoviral gene delivery of a truncated TRAF-2 protein revealed that TNF-α partially mediates NF-κB activation and IL-8 expression through TRAF-2 in HT-29 and IEC-6 cells (59). In addition, in HT-29 cells, although not in several

Table 3. Unique cytokine-induced signal transduction pathways that buffer NF-κB responses in IECs

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
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<tbody>
<tr>
<td>(14)</td>
<td>Diminished responses to IL-1β in differentiated cells</td>
</tr>
<tr>
<td>(55)</td>
<td>Lack of IRAK degradation following IL-1β stimulation</td>
</tr>
<tr>
<td>(64)</td>
<td>Decreased IKK activity and IκBα serine-32 phosphorylation</td>
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<tr>
<td>(59)</td>
<td>Unique cytokine-induced NF-κB activation in IECs</td>
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Numbers in parentheses are Reference numbers. IL-1β, interleukin-1β; IRAK, IL-1 receptor-associated kinase; IKK, IκB kinase.
other cell types (26), TRAF-2 is required for IL-1β-induced NF-κB and IL-8 gene expression (59). These data suggest that interactions occur between the TRAF-2 protein and several different cytokine receptors in IECs in contrast to signal transduction in many bone marrow-derived cells, where TRAF-2 interacts solely with the TNF receptor family. Native colonic epithelial cells and most IEC lines have delayed and incomplete IκBα degradation following cytokine activation (55), in addition to a strong decrease in IKK activity (64). Although viral delivery of NIK induces a strong IκBα phosphorylation and NF-κB-activated gene expression in HT-29 cells, only a marginal degradation of IκBα steady-state level is observed in these cells (64). By contrast, ectopic expression of NIK and IKK induces almost complete IκB degradation in Caco-2 cells (54, 64). In addition, the IL-1β signaling kinase IRAK is rapidly and completely degraded in IL-1β-stimulated Caco-2 cells but not in HT-29 cells (14, 64). Because the proteasome pathway mediates degradation of both IκBα and IRAK, it is tempting to speculate that HT-29 cells have a defect in their proteasome pathway, although there is currently insufficient evidence to support this hypothesis. The physiological mechanisms of IκB resistance to degradation and its relevance to intestinal homeostasis remain to be determined.

The intestinal mucosa is composed of a dynamic cell population in perpetual change from a proliferative and undifferentiated stage (crypt-base) to mature surface villus epithelial cells (1, 92, 127). Migration of cells from the crypt to the surface of the colon is accompanied by cellular differentiation that leads to important morphological and functional changes. Although several studies have shown that this process involves substantial changes of cellular morphology, growth, proliferation, and expression of biochemical markers (76, 137), little is known about the alteration of immunological functions as IECs mature. Interestingly, the IL-1β signaling pathway leading to IKK and NF-κB activation is downregulated in differentiated HT-29 cells (surface-like cells) compared with undifferentiated cells (crypt-like cells) (14). In addition, bacterial invasion is reduced in differentiated IECs (22). These findings suggest that a gradient of NF-κB activation may exist along the crypt-surface axis in response to stimulation by proinflammatory cytokines and bacteria.

IEC apoptosis is an important phenomenon in mucosal homeostasis, assuring a constant balance between cell production and cell loss. Recently, NF-κB was shown to play a protective role against apoptosis mediated by some death signals such as TNF-α and radiation in many cell types (5). Therefore, NF-κB activation status may have an impact on intestinal hyperplasia through cell removal by apoptosis in a manner similar to experimental rheumatoid arthritis (82). Of interest, cellular differentiation dramatically sensitized HT-29 cells to Fas-mediated apoptosis (M. P. Russo, R. B. Sartor, and C. Jobin, unpublished observations). The combined effect of IEC differentiation on downregulation of IL-1β signaling and increased susceptibility to Fas-mediated apoptosis may represent a mechanism to maintain mucosal homeostasis. However, the role of NF-κB in the IEC apoptotic process remains to be established, since this transcription factor also seems to play a proapoptotic role in HT-29 cells (40).

Although these data shed some light on the complex cytokine-induced NF-κB signal cascade in IECs, many questions remain unanswered. For example, the relative contribution and role of various adaptor proteins and kinases in cytokine-induced NF-κB activation remain unclear. The responsiveness of native IECs at various stages of differentiation to physiological stimuli needs to be determined to address the hypothesis that IECs have dampened NF-κB-regulated responses that preserve homeostasis in an aggressive luminal environment.

**THE IκB/NF-κB SYSTEM IN INTESTINAL INFLAMMATION AND INFECTION**

NF-κB regulates the transcription of a number of proinflammatory molecules involved in acute responses to injury and in chronic intestinal inflammation, including IL-1β, TNF-α, IL-6, IL-8, IL-12, iNOS, ICAM-1, VCAM-1, TCR-α, and MHC class II molecules (Table 2) (7, 10, 87). In addition, activation of NF-κB in IECs has been demonstrated in vivo (95). NF-κB activation as indicated by increased DNA binding activity and p65 nuclear translocation has been documented in the intestine of patients with Crohn’s disease, ulcerative colitis, and self-limited colitis (86, 95, 105), as well as in rodents with experimental colitis (86). The amount of activated NF-κB correlates with the degree of mucosal inflammation. Immunohistochemistry performed on tissue sections isolated from patients with inflammatory bowel disease (IBD) demonstrates the presence of activated NF-κB in IECs located at the crypts but not at the surface region (95).

Because IECs are surrounded by commensal bacteria and are the first cells affected by pathogens, the effect of bacteria on NF-κB activation in IECs has been examined. Savkovic et al. (101) reported that T84 cells infected with enteropathogenic Escherichia coli secrete IL-8 through a NF-κB-dependent mechanism. However, IL-8 gene expression was not induced in IECs infected with nonpathogenic E. coli, suggesting that IECs discriminate between pathogenic and nonpathogenic bacteria with respect to NF-κB activation (101). This discriminatory mechanism may ensure proper IEC regulation in response to the natural enteric flora and its constituents. These data further suggest that specific bacterial genes activate the IEC signaling cascade leading to NF-κB activation and gene expression. Hobbie and colleagues (47) have shown that Salmonella infection of IECs triggers IκBα degradation and IL-8 release. A number of invasive bacteria as well as high doses of bacterial cell wall polymers (LPS, endotoxins) and PG-PS induce NF-κB-dependent chemokines in a wide variety of IEC lines (57, 67). Additional studies have shown that bacterial invasion activates the IKK complex in IECs, thereby triggering NF-κB activity (35). These findings provide a link between bacterial invasion and induction of the IκB/NF-κB pathway.

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system, although the exact mechanisms involved in signal transduction remain to be elucidated. The requirement of bacterial invasion for NF-κB activation is controversial, since noninvasive bacteria can also trigger NF-κB activation (31). However, it is evident that a variety of enteric microbial stimuli, including parasitic infection and nonviable cell wall polymers, can also induce NF-κB-dependent chemokine expression.

MANIPULATION OF INTESTINAL INFLAMMATION BY TARGETING THE IκB/NF-κB SYSTEM

The critical role of NF-κB in intestinal inflammation is eloquently illustrated by the profound inhibition of inflammatory responses following selective blockade of NF-κB activation. For example, local or systemic administration of p65 antisense oligonucleotides reversed chronic experimental colitis induced in mice by trinitrobenzene sulfonic acid (86). Likewise, administration of specific proteasome inhibitors markedly attenuated PG-P5-induced granulomatous colitis in rats, presumably by inhibition of NF-κB (25). In both studies, however, it was unclear which cell populations responded to NF-κB inhibition in vivo.

Pharmacological agents and molecular approaches have been used in vitro to investigate the role of NF-κB in cytokine-stimulated gene expression and signal transduction in IECs. Proteasome inhibitors effectively prevent cytokine-induced IL-8 and ICAM-1 gene expression by suppressing IκBα degradation and NF-κB activation in transformed IECs (55, 56). Of considerable importance, several anti-inflammatory drugs used in treatment of IBD mediate their effects in part through inhibition of the IκB/NF-κB pathway. Dexamethasone at pharmacologically relevant concentrations stimulates IκBα synthesis, stabilizes IκB mRNA, and apparently interferes with IκBα degradation in IEC-6 cells (58). Biopsy samples from IBD patients treated in vivo with corticosteroids revealed a decrease of NF-κB activity (3). Furthermore, the anti-inflammatory compound sulfasalazine used for IBD treatment blocks degradation of IκB mediated by both TNF-α and LPS and prevents NF-κB activation in transformed IECs (122). Of interest, mesalazine, an aminosalicylate, inhibits IL-1β-induced NF-κB activity by interfering with inducible p65 phosphorylation but not IκBα degradation (34). In addition, IL-10 has a dual mechanism of inhibiting NF-κB-mediated gene transcription by transiently suppressing IKK activity and interfering with NF-κB DNA binding by an undefined mechanism in monocytes (104).

To gain more specificity over the pharmacological approach, molecular interventions using dominant-negative versions of individual components of the IκB/NF-κB pathway delivered by adeno viral vectors were designed and tested in transformed and primary IECs. An adeno viral vector bearing a cytokine-resistant proteolysis IκBα mutated at serine-32 and serine-36 (AdSilκBaaAA) was effective in blocking gene expression of IL-1, IL-8, iNOS, and COX-2 by mRNA and protein analysis (60, 62). A dominant-negative TRAF-2 mutant was also effective in blocking TNF-α-induced gene expression of IL-8 and NF-κB nuclear translocation, to a much lesser extent than AdSilκBaAA (59, 62). This may suggest that targeting divergent adaptor proteins is less effective in blocking NF-κB than targeting convergent proteins (such as NIK and IKK). Strategies aimed at blocking NIK or IKKβ using adeno viral gene delivery should provide valuable answers regarding their efficacy as therapeutic targets, as suggested by preliminary results of NF-κB and IL-8 blockade by a NIK dominant-negative molecule (61).

Although adeno viral molecular approaches have been successful in inhibiting NF-κB in vitro, there is no evidence that similar strategies would be effective in vivo. For example, the use of gene therapy raises the concern of potential host immunological responses against viral vector proteins (39, 133). In addition, a method for the specific, efficient long-term delivery of an adeno viral vector into the intestinal epithelium or lamina propria would be needed to sustain exogenous expression. Interestingly, natural dietary products have recently been shown to be potent inhibitors of cytokine-mediated NF-κB activation and IL-8 expression through IKK inhibition, suggesting a potential therapeutic application in vivo (54) as a more practical approach compared with antisense oligonucleotide (86) and intranasal delivery systems (38).

The complexity of the NF-κB activation pathway provides various potential targets for selective therapeutic intervention in IECs. The development of specific and safe NF-κB inhibitors delivered either locally or systemically could create a useful arsenal to complement the more globally acting drugs currently used to manage intestinal inflammation. The biggest challenge in the therapeutic application of this class of antagonists will be to inhibit the deleterious side of NF-κB without impairing normal cell functions dependent on NF-κB.

SUMMARY AND PERSPECTIVE

Many aspects of cellular function are regulated by the IκB/NF-κB system. Biological processes such as immune and inflammatory responses, as well as cell growth and apoptosis, are in part regulated by NF-κB. The multiple signals leading to NF-κB activation converge on a series of adaptor proteins and kinases in a cascade that leads to IκB phosphorylation/degradation, nuclear translocation of NF-κB dimers, and transcriptional activation of NF-κB-dependent genes. Many research groups have demonstrated the critical role of NF-κB in proinflammatory gene expression by IECs after cytokine or microbial stimulation.

It appears that IκBα is relatively resistant to degradation due to decreased IKK complex activity in differentiated IEC lines and native epithelial cells and that differentiated IECs respond less efficiently to IL-1 stimulation and are more sensitive to Fas-mediated apoptosis. We postulate that this relative unresponsiveness allows IECs to exist in a quiescent state (Fig. 3A), while in constant contact with ubiquitous endogenous luminal bacteria and bacterial products, yet to be
capable of mounting a rapid response to microbial pathogens, resulting in chemotactic signals and adhesion molecules that recruit phagocytic effector cells to the injured mucosa (Fig. 3B).

Despite these rapidly accumulating findings, many questions remain unanswered. For example, the role that NF-κB plays in growth, differentiation, and apoptosis in IECs is currently unknown. Recently, it has been shown that NF-κB may control cell cycle regulation by modulating cyclin D1 expression (46). These biological processes are key determinants of intestinal development, repair, inflammation, and carcinogenesis. Therefore, understanding the contribution of NF-κB could lead to novel therapeutic approaches to these conditions. To design an efficient, nontoxic blocking strategy, more data are needed to describe the precise mechanisms of bacterial and cytokine induction of the signaling cascade through the IκB/NF-κB system. Equally important is the relative input and possible interaction of epithelial cells vs. lamina propria mononuclear cells in the development and persistence of intestinal inflammation. The generation of transgenic mice carrying an NF-κB inhibitor (such as mutated, nondegradable IκB) or expressing a constitutively active NF-κB (such as active IKK or NIK) regulated by an intestinal cell-specific promoter should help address the role and contribution of IECs in intestinal homeostasis and inflammation. Regulation of the IκB/NF-κB system in IECs represents a new and exciting era of research in intestinal inflammatory diseases and neoplasia that could potentially give rise to new targets for therapeutic intervention.
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