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EDITORIAL REVIEW

NF-κB may determine whether epithelial cell–microbial interactions in the intestine are hostile or friendly

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The gastrointestinal tract contains the largest mass of lymphoid tissue in the body and is also the largest area that is constantly exposed to the environment. In the colon, the luminal environment consists of a large and highly complex microbial flora (approximately 10^{14} total bacteria). The colonic mucosa, which is continually exposed to this microbial flora, has to be capable of responding to pathogenic microorganisms but at the same time avoid responses to the resident bacteria. The factors that regulate the ability of the mucosa to mediate these functions are poorly understood, but epithelial cells appear to be of major importance in this process.

A single monolayer of epithelial cells separates the host from luminal bacteria and their products. This monolayer plays a critical role in mediating the host's innate and adaptive immunological responses to luminal pathogenic microorganisms [1]. Recently, there has been considerable interest in innate host responses mediated by intestinal epithelial cells to invasive bacteria or their secreted toxic products. These innate responses include the release of cytokines and chemokines, of which the potent polymorphonuclear chemoattractant IL-8 has been studied the most. In the current issue of the journal, Kim *et al*. [2] report that the previously demonstrated [3] secretion of IL-8 by intestinal epithelial cells in response to *Bacteroides fragilis* enterotoxin (BFT) is mediated by nuclear factor-κB (NF-κB). This and other studies suggest that NF-κB plays a key role in the regulation of innate immunity mediated by intestinal epithelial cells. Interestingly, recently reported studies also suggest that non-pathogenic bacteria are capable of down-regulating proinflammatory responses by epithelial cells. Before reviewing these studies further, a brief outline of the current understanding of the regulation of NF-κB activity [4, 5] would be helpful. This information is derived mostly from studies performed on non-epithelial cells, using proinflammatory cytokines as stimulants.

NF-κB encompasses inducible transcription factors that are important activators of genes involved in inflammatory and immunological responses. The active form of NF-κB consists of heterogeneous dimers that share a homologous region responsible for DNA binding, nuclear localization and cytoplasmic interaction with IκB proteins. The p60:p50 heterodimer is the predominant form of active NF-κB. It is stored in an inactive state in the cytoplasm bound to inhibitory proteins of the IκB family (of which IκBα is the best characterized to date). IκB masks NF-κB's nuclear localization signal, thereby retaining it in the cytoplasm [6]. Following stimulation (by proinflammatory cytokines such as IL-1, tumour necrosis factor-alpha (TNF-α) or bacterial lipopolysaccharide (LPS)), IκB is degraded to release active NF-κB dimer, which migrates to the nucleus to bind to its sequence recognition motif on promoters of target genes. Degradation of IκB occurs by proteolysis, following phosphorylation and ubiquitination. Inducible phosphorylation of IκB is mediated by IκB kinase (IKK) complex in which IKKβ plays a critical role. IκB phosphorylation allows recognition by a recently identified complex of proteins that attach ubiquitin polypeptides [7], leading to subsequent degradation by the 26S proteasome complex. Phosphorylation of IκBα occurs at serines at positions 32 and 36 and their substitution inhibits ubiquitination and degradation.

NF-κB has been shown to be an important signalling event in intestinal epithelial cells stimulated with IL-1, and altered degradation of IκBα in epithelial cells, compared with haematopoietic cells, has been demonstrated [8]. The study reported by Kim *et al*. [2] used mammalian expression vectors encoding mutant IκBα (with substitutions of serine residues at positions 32 and 36) and an expression vector encoding mutant IKKβ that did not express kinase activity. These mutant expression vectors block activation of NF-κB. Purified *B. fragilis* toxin induced expression of IL-8 mRNA transcripts and protein in intestinal epithelial cell lines. As expected, BFT also induced activation of IL-8 transcriptional reporter in epithelial cells, as well as that of NF-κB. BFT-induced activation of both these transcriptional reporters was inhibited in cells transfected with mutant IκBα or IKKβ, and thereby illustrates a central role of NF-κB in the regulation of IL-8 expression in these cells. Recently, other groups have also reported similar findings in epithelial cells infected with pathogenic bacteria.

Enteropathogenic *Escherichia coli* [9], enteroinvasive *E. coli* [10], invasive *Shigella flexneri* [11], and pathogenic *Salmonella* strains [10, 12] have been shown to induce the expression of IL-8 in intestinal epithelial cells via activation of NF-κB. Activation of NF-κB has also been demonstrated by adherent *Pseudomonas aeruginosa* in respiratory epithelial cells [13], implying that the relevant intracellular signalling pathways and transcription factors may be common to all mucosal epithelial cells. *In vivo*, the mucosal pathology induced by the pathogenic microorganisms is characterized by polymorphonuclear cell infiltration, and the key role of IL-8 in this process has been reported [14]. Although polymorphonuclear cell infiltration induces tissue damage, it has been shown to control the translocation of bacteria such as *S. flexneri*. The above studies suggest that because of its ability to regulate the expression of IL-8 (and also other proinflammatory

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cytokines), NF-κB is of major importance in the regulation of innate immune responses by mucosal epithelial cells in response to pathogenic microorganisms.

The mechanisms by which BFT induces activation of NF-κB in epithelial cells remain to be determined. This enterotoxin is a metalloprotease that is believed to mediate its cytotoxic activity via its effect on epithelial cell adhesion molecules [15, 16]. Studies have implicated enterotoxigenic *B. fragilis* (ETBF) in diarrhoeal illness in children [17–19]. However, ETBF and BFT are also present in stools of healthy children and adults [20]. The study by Kim *et al*. [2] and others [3] have demonstrated the ability of BFT secreted by ETBF to induce the expression of proinflammatory cytokines in intestinal epithelial cells and also to induce cell death [21, 22]. The reason for the capacity of ETBF to cause disease in some individuals but not others remains unknown but, in addition to host factors, other resident luminal bacteria may determine the outcome of host–BFT interactions.

In many of the above studies that demonstrated activation of NF-κB by pathogenic bacteria [9, 10, 12], exposure of epithelial cells to non-pathogenic strains of bacteria did not have the same effect. Indeed, a recent study has shown that non-pathogenic bacteria may down-regulate the expression of IL-8 induced by pathogenic strains [23]. Thus, non-pathogenic *Salmonella* strains inhibited epithelial cell expression of IL-8 induced by proinflammatory strains (or the proinflammatory cytokine TNF-α). The immunosuppressive effect of non-pathogenic *Salmonella* involved

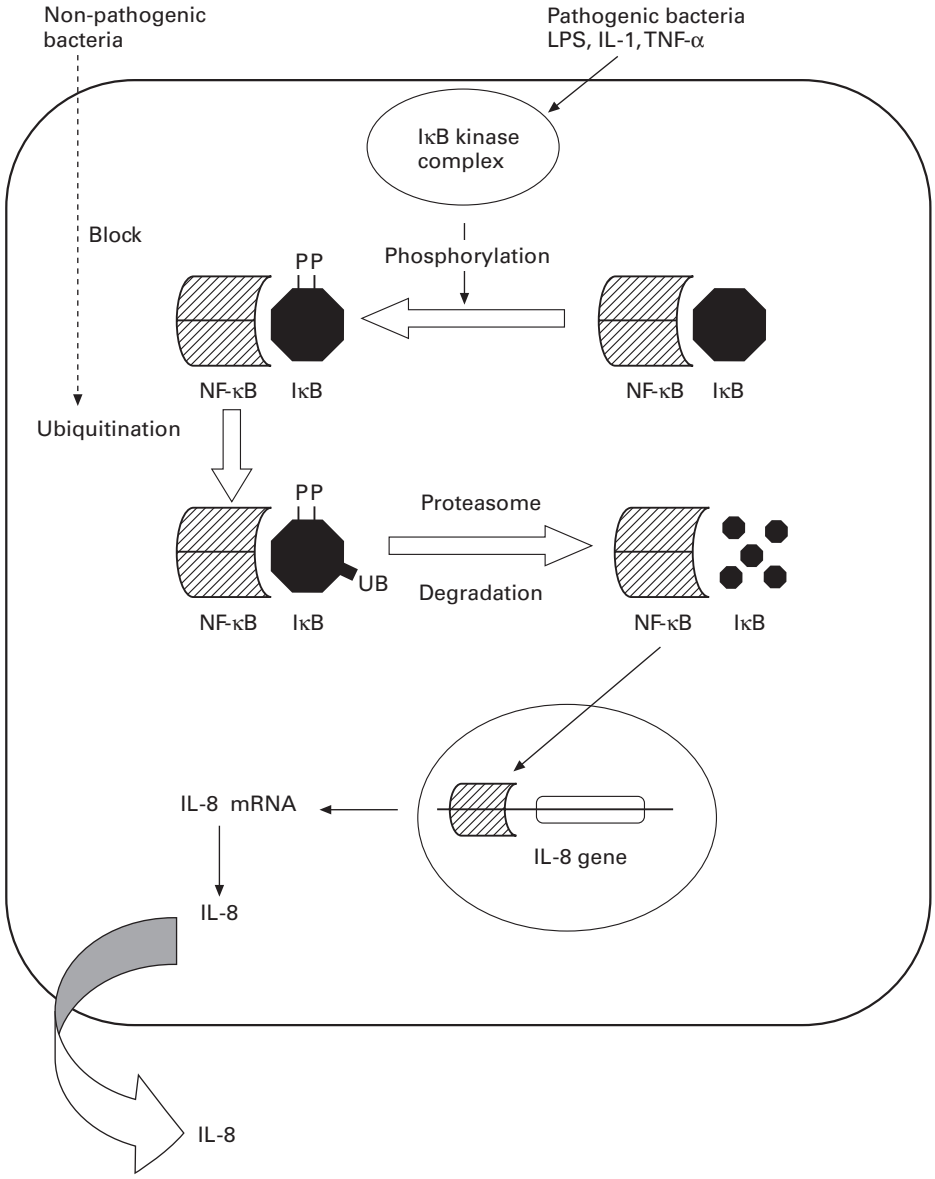

Fig. 1. A model of NF-κB activation in intestinal epithelial cells. Pathogenic bacteria and pro-inflammatory cytokines induce phosphorylation of IκB via the IκB kinase complex. Ubiquitin polypeptides are attached to phosphorylated IκB with its subsequent degradation by the 26S proteasome complex. The active NFκB dimer then migrates to the nucleus and binds to its sequence recognition motif on the promoter region of IL-8 gene, leading to transcription and translation of the chemokine. Non-pathogenic bacteria can inhibit IκB degradation by abrogating the polyubiquitination step, and thereby inhibit expression of IL-8 because NF-κB is retained in the cytoplasm.

inhibition of IκBα degradation by abrogation of the polyubiquitination step that occurs following phosphorylation (Fig. 1).

Such studies illustrate the potential mechanism by which the normal colonic mucosa avoids tissue-damaging inflammatory responses to the resident luminal microbial flora. Moreover, they demonstrate the potential capacity of non-pathogenic bacteria to modulate proinflammatory responses induced by pathogenic microorganisms. Indeed, there is recent evidence of clinical benefit from the use of such 'friendly' non-pathogenic bacteria in the treatment of inflammatory disease [24, 25].

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