Role of NF-κB in immune and inflammatory responses in the gut

Summary
NF-κB is a pleiotropic transcription factor with key functions in the intestinal immune system. NF-κB family members control transcriptional activity of various promoters of proinflammatory cytokines, cell surface receptors, transcription factors, and adhesion molecules that are involved in intestinal inflammation. The perpetuated activation of NF-κB in patients with active inflammatory bowel disease suggests that regulation of NF-κB activity is a very attractive target for therapeutic intervention. Such strategies include antioxidants, proteasome inhibitors, inhibition of NF-κB by adenoviral IκB expression vectors, and antisense DNA targeting of NF-κB. These approaches will hopefully permit the design of new treatment strategies for chronic intestinal inflammation.

Introduction
Much recent research has focused on the regulation of cytokine gene expression by transcription factors in the mucosal immune system. In this review we will discuss the role of the transcription factor nuclear factor κB (NF-κB) in immune and inflammatory responses in the gut.

Members of the NF-κB family of transcription factors
Nuclear factor κB (NF-κB) designates a group of transcription factors defined in part by their ability to bind a specific DNA sequence first identified in the enhancer of transcription factors defined in part by their ability to bind nuclear factor κB. Members of the NF-κB family of proteins includes IκBα (MAD-3, pp40), IκBβ, IκBγ/p105, Bcl-3, IκBε/p100, and IκBβ.14–17 These proteins are characterised by multiple7–9 repeated sequences of 33 amino acids, termed SWI6/ankyrin repeats, which seem to be responsible for the interaction with the Rel domain of NF-κB. IκB proteins are organised as tripartite molecules consisting of (i) an N-terminal domain required for proteolytic degradation, (ii) a central domain with ankyrin repeats required for interaction with NF-κB, and (iii) a C-terminal domain (called PEST domain) which is essential for sequestration of NF-κB in the cytoplasm. The precursor proteins of p50 and p52, termed p105/IκBβ and p100/IκBε, contain in addition to the Rel homology domain ankyrin repeats and thus are structurally and functionally related to the IκB family. For instance, the precursor of p50 (p105) contains at its N-terminal domain p50 and in its C-terminal half seven ankyrin repeats.18–20 This protein sequesters p65, c-Rel and proteolytically released p50. Although the p50 releasing protease of p105 has not yet been identified, an ATP dependent ubiquitin system for p105 has been proposed.

IκB proteins exert multiple functions including prevention of nuclear translocation of NF-κB. The inhibition is based on the interaction between the C-terminal ankyrin repeats of IκB and the Rel homology domain of NF-κB. The IκBα and IκBβ proteins preferentially inhibit NF-κB complexes containing the p65 and c-Rel subunits. Interestingly, some IκB proteins have been found in the nucleus. This fact suggests that these proteins do not necessarily reside as an anchor in the cytoplasm to fulfill their function. In fact, the IκB-like protein Bcl-3 can function as

Figure 1 Members of the NF-κB/IκB families. After activation of the cell IκB is degraded and NF-κB can translocate to the nucleus.
The transcriptional coactivator after association with p52 on the DNA although Bcl-3 has been shown to inhibit p50 containing complexes. Furthermore, it has been proposed that IκB proteins are able to strip off DNA bound NF-κB.

Transcriptional regulation of NF-κB and IκB genes
p105 and p100 are constitutively expressed but their mRNA levels are increased in response to signals activating NF-κB, such as treatment with PMA, interleukin 1 (IL-1), and tumour necrosis factor (TNF). There is some evidence for autoregulation of NF-κB activity as the promoters of NF-κB are able to down-regulate NF-κB genes. Transcriptional regulation of NF-κB.

NF-κB activation also requires the p105 and p100 proteins. p105 and p100 are constitutively expressed but their mRNA levels are increased in response to signals activating NF-κB. NF-κB is ubiquitinated and degraded via the proteasome pathway. Finally, NF-κB translocates into the nucleus and binds to its target DNA sequences.

NF-κB has been recently suggested for IL-18 (fig 2). Members of the TNF receptor (TNFR) superfamily interact via their cytoplasmic tails with TRAF proteins which serve as adaptor proteins to recruit NIK, a specific NF-κB inducing kinase. In addition to the TNF signalling pathway, the IL-1 and IL-18 initiated signalling pathways lead to the activation of NIK. However, in the case of IL-1 NIK is activated through TRAF6 and IRAK (serine-threonine kinase) and a similar activation mechanism of NF-κB has been recently suggested for IL-18 (fig 2). NIK is classified as MAP kinase kinase kinase (MAP3k) and was identified as TRAF2 interacting protein. A serine-threonine kinase previously known as CHUK was shown to associate with NIK and IκBα in mammalian cells. Based on its property to phosphorylate IκBα CHUK was named IKKα (IκB kinase α). At the next step of the cascade IKKα associates with IκBα and phosphorylates the latter at serine 32 and serine 36. The modified IκBα is then specifically degraded via the ubiquitin/proteasome pathway and the active NF-κB dimer can translocate into the nucleus and bind to its cognate target sequence.

Figure 3 Targeting of the NF-κB activation pathway in intestinal inflammation. While alkylating agents and antioxidants may block protein kinases, antisense DNA can inhibit translation of p65. In addition, corticosteroids lead to blockade of p65 and adenosine expression vectors could deliver genes whose products inactivate NF-κB.

Signalling transduction pathways leading to NF-κB activation
Several unrelated stimuli like phorbol ester, TNF, IL-1, IL-18, LPS, and ultraviolet light have been shown to activate NF-κB, which is in agreement with the pleiotropic roles of NF-κB in many different cell types and tissues. Activation of NF-κB by IL-1, TNF and IL-18 requires binding of cytokines to their specific cell surface receptors (fig 2). For instance, TNF binds to its receptor and leads to activation of TNF receptor associated factor (TRAF) proteins via a receptor associated adaptor protein called TNF receptor associated death domain (TRADD). At the moment six members of the TRAF protein family are known (TRAF 1–6). TRAF2 is required for NF-κB activation via TNFR1 (75 kDa) and TNFR2 (55 kDa). In contrast, TRAF5 is also involved in NF-κB activation by other members of the TNF receptor family and NF-κB participates in NF-κB activation via IL-1. Furthermore, TRAF proteins interact directly with the cytoplasmic tails of two other TNFR family members (CD40 and CD30). TNFR1 mediated NF-κB activation also requires the serum-thioreonine kinase RIP, which is associated with TRAF proteins and interacts with the respective receptor coreceptor complex via TRADD. TNF signalling also activates JNK (stress activated protein kinase) and Fas associated protein with a death domain (FADD) which leads to apoptosis via a caspase-8 initiated cascade (fig 2).
promoter. Interestingly, such elements were also found upstream of the IL-3 gene and IL-8 expression is also regulated by NF-κB upstream of the IL-3 gene and IL-8 expression is also

Table 1 Genes regulated by NF-κB

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<thead>
<tr>
<th>Cytokines and growth factors</th>
<th>Interleukin 2</th>
<th>Interleukin 6</th>
<th>Interleukin 8</th>
<th>Interleukin 12p40</th>
<th>TNF-α</th>
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<td>Adhesion molecules</td>
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<td>Endothelial leucocyte adhesion molecule 1 (ELAM-1)</td>
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<td>Vascular cell adhesion molecule 1 (VCAM-1)</td>
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<td>Intercellular cell adhesion molecule 1 (ICAM-1)</td>
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<td>E-selectin</td>
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<td>Mucosal vascular addressin cell adhesion molecule (MAd-CAM-1)</td>
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<td>Cell surface receptors</td>
<td>T cell receptor β chain</td>
<td>T cell receptor α chain</td>
<td>β2-microglobulin</td>
<td>Interleukin-2R α chain</td>
<td>c-rel</td>
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<td>Transcription factors</td>
<td>c-myc</td>
<td>Interferon regulatory factor 1 (IRF-1)</td>
<td>IκBα</td>
<td>TAP-1 peptide transporter</td>
<td>LMP2 proteasome subunit</td>
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<td>Others</td>
<td>iNOS synthase</td>
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Lessons from NF-κB/IκB gene knockouts

Recently, the targeted disruption of various genes encoding NF-κB subunits has been described. These knockout mice revealed severe defects in immune function further supporting a key regulatory role for NF-κB in the immune system. Interestingly, the phenotype of these mice differed strikingly depending on the disruption of the respective NF-κB subunit. For instance, mice lacking the p50 subunit (NF-κB1) developed normally but had severe defects in immune cell function. B cells of these mice had an impaired ability to produce antibodies and to proliferate upon LPS challenge. Furthermore, p50 −/− mice were highly susceptible to bacterial infections with staphylococcus and listeria. If compared with p50, the phenotype of p65 (RelA) knockout mice was even more dramatic. These animals died during embryonic development, most likely because of extensive apoptosis of cells in the liver. Analysis of NF-κB regulated genes (GM-CSF, IκBα) revealed a loss of inducibility in the p65 knockout mice and cultured T cells from these mice showed strikingly reduced proliferative responses, underlining the functional importance of NF-κB p65 for appropriate immune function.

Mice lacking RelB developed normally until days 8–10. Subsequently, however, they showed a complex pathological phenotype, which is the result of multiple defects in the adult immune system. RelB −/− mice displayed a dramatic phenotype of each individual family member. IκBα knockout mice displayed constitutively high nuclear levels of NF-κB, giving rise to a dramatic phenotype of these animals. Mice lacking IκBα, although apparently normal at birth, died approximately seven days later. Their phenotype showed small spleens and thymuses, skin defects and increased granulopoiesis. In addition, upregulated expression of some NF-κB regulated genes (G-CSF and VCAM-1, as defined in table 1) was observed. Taken together, the different phenotype of knockout mice of the NF-κB/IκB families indicates the unique function of each individual family member and shows that there is no simple redundancy among these proteins. Furthermore, the generation of NF-κB/IκB deficient mice has provided strong evidence for a key role of NF-κB in controlling multiple steps of immune cell function such as apoptosis, cytokine production and chronic inflammation.

Role of NF-κB in the mucosal immune system

Dysregulated cytokine production and signalling mechanisms by epithelial cells, mucosal lymphocytes and macrophages have been implicated in the pathogenesis of both Crohn’s disease and ulcerative colitis, the two major forms of human inflammatory bowel disease (IBD). Over the past few years, various murine models of chronic intestinal inflammation resembling IBD have been established. These models have provided important clues as to the

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nature of such dysregulation and to its possible cytokine based treatment. Thus, in studies of several of the models most closely resembling Crohn’s disease it was found that production of large amounts of Th1-type cytokines (e.g. IFNγ and TNF) whose promoters are regulated by NF-κB is a major and essential feature of the inflammation. Finally, it has been shown that Th1 cytokine production in these models is triggered by macrophages via increased production of IL-12, a cytokine that plays a major role in driving T cell differentiation and whose expression is also at least partially triggered by NF-κB.

The above data encouraged studies on the identification of signalling pathways and transcription factors that govern cytokine gene transcription in IBD. Although some NF-κB family members are apparently important in preventing inflammatory responses (e.g. RelB), it was found that nuclear NF-κB levels are increased in patients with IBD. In particular, the p65 subunit was highly activated in epithelial cells and lamina propria macrophages from patients with active Crohn’s disease and ulcerative colitis. These findings are consistent with immunohistochemical data indicating increased expression of NF-κB p65 in active IBD and data from intestinal biopsy samples showing increased p65 in active Crohn’s disease. In addition, it was shown recently that a specific p65 antiseis oligonucleotide can block p65 expression and proinflammatory cytokine production by lamina propria macrophages in patients with active Crohn’s disease and ulcerative colitis. Furthermore, in a murine model of colitis p65 antiseis treatment led to an abrogation of chronic intestinal inflammation. In spite of these data on the role of NF-κB p65 in IBD, many additional questions have to be answered. In particular, there are few data concerning the role of other NF-κB/IκB family members in epithelial cells and T cells in the gut. In addition, the expression of IκB family members and their degradative mechanisms in IBD have only been partially characterised. Interestingly, recent data by Jobin and coworkers showed activation of NF-κB in epithelial cells in response to IL-1 and altered regulation of IκB degradation in native colonic epithelial cells. Such enhanced resistance of epithelial cells to IκB proteolysis suggested a potentially increased responsiveness to therapeutic blockade. Indeed, adenoviral mediated delivery of a mutant NF-κB repressing IκB protein resulted in inhibition of IL-8 production by intestinal epithelial cells. Furthermore, pharmacological inhibition of IκB degradation strongly reduced IL-8 secretion by intestinal epithelial cells. Finally, recent evidence suggests that NF-κB is important in regulating intercellular cell adhesion molecule (ICAM-1) expression in the intestine. Preliminary data from the same group also showed a beneficial therapeutic effect of proteasome inhibitors (that block NF-κB activation) in experimental colitis. Inhibition of NF-κB activity has been recently suggested as a major component of the anti-inflammatory activity of glucocorticoids that are frequently used for treatment of chronic intestinal inflammation in humans. Although activation of NF-κB p65 is not specific for patients with IBD, its perpetuated activation makes it a very attractive target for therapeutic intervention. Thus, downregulation of NF-κB activity emerges as a potential key event in the control of chronic intestinal inflammation in humans and strategies to inhibit NF-κB activity more specifically are desirable. Such strategies include antioxidants, proteasome inhibitors, inhibition of NF-κB by adenoviral IκB expression vectors, and antisense DNA targeting of NF-κB p65 (fig 3). Thus, the above data suggest that targeting of NF-κB may be a novel molecular approach for the treatment of patients with IBD that could lead to the design of new treatment strategies that have added specificity but reduced toxicity compared with standard immunosuppressive therapy.

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