

IKK/NF- κ B signaling in intestinal epithelial cells controls immune homeostasis in the gut

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The intestinal epithelium regulates the interaction between commensal bacteria and the immune system not only by providing a physical barrier but also by expressing a number of immunoregulatory and antimicrobial peptides. Recent studies showed that inhibition of I κ B kinase (IKK)/nuclear factor- κ B (NF- κ B) signaling specifically in intestinal epithelial cells *in vivo* causes the spontaneous development of intestinal inflammation in mice. These findings identify IKK/NF- κ B signaling in intestinal epithelial cells as an important factor for the maintenance of epithelial integrity and immune homeostasis in the gut.

INTRODUCTION

Deregulation of immune responses in the gut causes inflammatory bowel disease (IBD). The gut epithelium has an important function in the maintenance of intestinal immune homeostasis by preventing the contact of luminal bacteria with immune cells through the formation of a physical barrier and the expression of antimicrobial peptides. Recent studies showed that I κ B kinase (IKK)/nuclear factor- κ B (NF- κ B) signaling in intestinal epithelial cells (IECs) has an important function in the maintenance of gut epithelial integrity and immune homeostasis. Inhibition of IKK/NF- κ B signaling specifically in IECs *in vivo*, achieved by conditional ablation of NEMO/IKK γ , causes spontaneous development of severe colon inflammation in mice. Mice with epithelial-restricted NEMO deficiency (NEMO^{IEC-KO}) exhibit increased apoptosis of IECs, disruption of the epithelial barrier, and subsequent translocation of bacteria into the mucosa, leading to chronic colitis. Genetic ablation of MyD88 or tumor necrosis factor receptor 1 prevents this disease development, suggesting that Toll-like receptor (TLR) signaling and tumor necrosis factor (TNF)-mediated responses are important for triggering colon inflammation in this model. Thus, epithelial IKK/NF- κ B signaling emerges as a critical regulator of immune homeostasis in the gut.

NF- κ B/IKK signaling in IBD

Human IBDs, including Crohn's disease and ulcerative colitis, are chronic diseases characterized by severe inflammation of the intestine.^{1,2} IBD patients suffer from chronic abdominal pain and diarrhea and have an elevated risk of developing colorectal

cancer. Despite recent advances in our understanding of IBD pathogenesis, important aspects concerning the immunopathology and genetic basis of IBD remain unclear. It is generally believed that chronic inflammation in IBD is the result of an abnormal immune response to the intestinal microflora. Numerous studies have demonstrated that immune system defects can cause IBD-like diseases in animal models, suggesting that the deregulation of immune responses has an important function in the pathogenesis of IBD. Although early studies focused mainly on the role of adaptive immunity and T lymphocytes, innate immunity was also recognized more recently as an important contributor to IBD. The gut epithelium has an important function in the maintenance of intestinal immune homeostasis by preventing the contact of luminal bacteria with immune cells through the formation of a physical barrier and the expression of antimicrobial peptides.^{3–5} However, the molecular mechanisms that control this function of gut epithelial cells are poorly understood.

The NF- κ B signaling pathway controls the expression of many genes with important functions in inflammation, immune responses, cell proliferation, survival, and apoptosis.^{6,7} In mammals, the NF- κ B/Rel family of transcription factors consists of five members: p65/relA, c-Rel, RelB, p50/p105 and p52/p100. NF- κ B/Rel proteins form dimers that are kept inactive by association with inhibitory proteins belonging to the I κ B family, which includes I κ B α , I κ B β , I κ B ϵ , and Bcl-3, and also the p105 and p100 precursors of p50 and p52, respectively. NF- κ B activation is induced by the IKK complex consisting of the IKK1 (IKK α) and IKK2 (IKK β) catalytic subunits and the NEMO/IKK γ

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regulatory protein.^{8–10} On activation by a variety of stimuli, including proinflammatory cytokines such as TNF and IL-1 β as well as bacterial components such as lipopolysaccharide, the IKK complex phosphorylates I κ B proteins at specific serine residues targeting them for polyubiquitination and proteasome-mediated degradation, thus releasing NF- κ B, which then accumulates in the nucleus and activates transcription of its target genes.^{11,12}

As a critical regulator of immune and inflammatory responses, the IKK/NF- κ B signaling pathway has been implicated in IBD pathophysiology. Given its prominent role in initiating inflammation, NF- κ B activation is associated with disease activity in IBD. Colonic biopsy samples as well as lamina propria macrophages from IBD patients show enhanced NF- κ B activity. Moreover, IBD patients display increased expression levels of p65/RelA, and a polymorphism in the promoter region of the *Nfkb1* gene, which encodes the p105 precursor of p50, has been associated with an increased risk of developing IBD.^{13–15} Consistent with these clinical observations pointing to a function for NF- κ B activation in promoting intestinal inflammation, several studies have shown that NF- κ B inhibition has beneficial effects in animal models of colitis. For instance, antisense oligonucleotides targeting p65 have been shown to protect mice against Dextran sulfate sodium-induced colitis.¹⁶ Although these experiments clearly showed that increased NF- κ B activity contributes to intestinal inflammation, the cell-specific function of NF- κ B in this disease remained unclear. As many cell types participate in the pathogenesis of IBD-like diseases, NF- κ B is likely to have diverse functions in different cells. In immune cells, elevated NF- κ B activity is likely to induce increased expression of proinflammatory mediators leading to more severe inflammation. On the other hand, NF- κ B activation on intestinal epithelial cells might be important to protect these cells from the toxicity of inflammatory mediators.

NF- κ B/IKK signaling in intestinal epithelial cells controls intestinal epithelial integrity and immune homeostasis in the gut

To investigate the function of IKK/NF- κ B signaling in the intestinal epithelium, we employed conditional gene targeting to ablate the different subunits of the IKK complex specifically in IECs.¹⁷ The two I κ B kinases are structurally similar but functionally distinct. IKK2 has been suggested to mediate proinflammatory signal-induced “canonical” NF- κ B activation, a function that also depends on NEMO/IKK γ .^{11,18,19} IKK1 is believed to be dispensable for canonical NF- κ B signaling but mediates NF- κ B activation through the “alternative” pathway by controlling p100 processing and also displays nuclear functions independent of NF- κ B.^{11,18,19} To assess the role of the “canonical” NF- κ B pathway in IECs, we generated mice with IEC-restricted NEMO deficiency (NEMO^{IEC-KO}) by crossing mice carrying loxP-flanked NEMO alleles²⁰ with villin-Cre transgenics²¹ expressing Cre recombinase in IECs. NEMO^{IEC-KO} mice were born normally but displayed rectal bleeding and severe diarrhea already a few weeks after birth. Endoscopic, macroscopic, and histological examination revealed widespread inflammation in the colon of NEMO^{IEC-KO} mice. The disease was already detected in young

mice before the age of weaning, indicating that the pathology develops early after birth. Colon inflammation in NEMO^{IEC-KO} mice was characterized by infiltration of innate immune cells such as dendritic cells, macrophages, and neutrophils during the early stages of the disease. T-cell infiltration was detected particularly in older animals, indicating that an innate immune response drives the early stages of the disease whereas adaptive immunity might participate later in the perpetuation and chronicity of colon inflammation. In addition to the infiltration of inflammatory cells, multiple cytokines and chemokines were highly expressed in the colon of NEMO^{IEC-KO} mice. The disease was confined to the colon, as the cecum and small intestine of these mice did not show pathological findings.

The spontaneous development of colon inflammation in NEMO^{IEC-KO} mice suggested that the canonical IKK/NF- κ B pathway has an important function in IECs, which is necessary for the maintenance of immune homeostasis in the gut. We hypothesized that epithelial IKK/NF- κ B signaling controls the maintenance of the intestinal epithelial barrier, which is important to prevent commensal bacteria from crossing the epithelium and coming into contact with mucosal immune cells. Immunohistochemical analysis of colonic cross-sections from young NEMO^{IEC-KO} mice revealed the focal presence of areas with apoptotic epithelial cells, indicating that NEMO-deficient epithelial cells die by programmed cell death, thus exposing the mucosa to intestinal bacteria. Indeed, commensal bacteria were detected within the colonic mucosa of these mice revealing a functional breach of the epithelial barrier. In addition to forming a physical barrier, the intestinal epithelium produces a number of antimicrobial peptides that are believed to have a function in controlling bacterial titers in the vicinity of the epithelium. The expression of mouse β -defensin 3 was down-regulated in the colon of NEMO^{IEC-KO} mice, suggesting that reduced antimicrobial peptide expression might be implicated in colitis development in this model, although at this stage there is no functional evidence directly supporting a causative association. Interestingly, a recent study reported that a genetic polymorphism in the human β -defensin 2 locus (homologous to mouse β -defensin 3) might be implicated in IBD.²² In this study, individuals with reduced β -defensin 2 gene copy numbers were found to have a significantly higher risk of developing colonic Crohn's disease. Thus, expression of this antimicrobial peptide might be important for the prevention of colitis in mice and humans.

The results obtained from the analysis of NEMO^{IEC-KO} mice suggested that canonical NF- κ B signaling in IECs is essential to protect the gut epithelium and maintain intestinal immune homeostasis. However, mice lacking IKK2, the I κ B kinase thought to be responsible for canonical NF- κ B signaling, specifically in IECs (IKK2^{IEC-KO} mice) did not develop spontaneous inflammation. We hypothesized that compensatory signaling through IKK1 could be responsible for the lack of disease in IKK2^{IEC-KO} mice compared with NEMO^{IEC-KO} mice, and generated mice lacking either IKK1 (IKK1^{IEC-KO}) or both IKK1 and IKK2 (IKK1/IKK2^{IEC-KO}) in IECs to test this hypothesis. Although IKK1^{IEC-KO} mice did not develop colitis, double IKK1/IKK2^{IEC-KO} mice developed severe colon inflammation

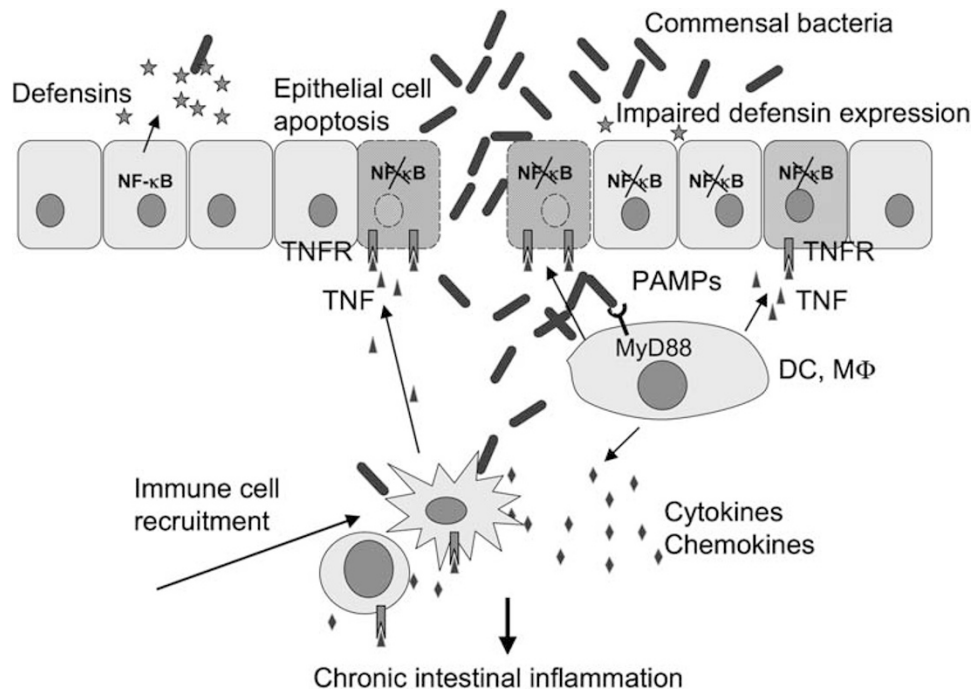


Figure 1 Schematic depicting the mechanisms leading to colon inflammation in mice with epithelial-specific inhibition of I κ B kinase (IKK)/nuclear factor- κ B (NF- κ B) signaling. DC, dendritic cell; M Φ , macrophage; PAMPs, pathogen-associated molecular patterns.

similarly to NEMO^{IEC-KO} mice.¹⁷ Taken together, these results demonstrate that the two I κ B kinases share a redundant function in mediating canonical NF- κ B signaling in IECs, which is essential to maintain intestinal epithelial integrity and normal immune homeostasis in the colon.

The data obtained from the analysis of IEC-specific inhibition of NF- κ B signaling point to a model in which increased apoptosis of IECs leads to a compromised epithelial barrier resulting in translocation of commensal bacteria into the mucosa. The recognition of bacteria by mucosal innate immune cells is expected to lead to increased expression of cytokines and chemokines and infiltration of immune cells resulting in colon inflammation. The increased expression of cytokines such as TNF in the vicinity of the NF- κ B-deficient epithelium will further contribute to increased apoptosis of IECs and the destruction of the epithelial barrier allowing more bacteria to invade the mucosa, leading to a vicious circle driving a chronic inflammatory response. This model would predict that recognition of commensal bacteria by immune cells through TLR-signaling would be essential for disease development, whereas expression of TNF would be likely to contribute to disease pathogenesis. Consistent with this notion, genetic experiments showed that NEMO^{IEC-KO} mice that are also deficient in tumor necrosis factor receptor 1 or MyD88, generated by crossbreeding with respective knockout mouse strains, do not develop colon inflammation, demonstrating that tumor necrosis factor receptor 1 and TLR/IL-1R signaling are both crucial for disease pathogenesis.

Collectively, these results demonstrate that IKK/NF- κ B signaling in IECs is essential for the protection of the colonic epithelium from apoptosis and maintenance of intestinal immune homeostasis. When IKK/NF- κ B signaling in IECs is impaired

below a certain threshold, achieved by ablation of NEMO or by the combined ablation of both I κ B kinases together, increased epithelial cell apoptosis compromises the epithelial barrier allowing commensal bacteria to invade the mucosa. The TLR-mediated recognition of bacteria by innate immune cells in the mucosa activates an immune response that leads to chronic inflammatory colitis (**Figure 1**). Disease initiation is likely to be triggered by stochastic events leading to local disruption of epithelial integrity and inflammation, which is then amplified with extension to the entire colon owing to the sensitivity of NEMO-deficient IECs to cytokine-induced death, in the absence of adequate antiapoptotic protection due to NF- κ B deficiency.⁷

Although this model is based on currently available experimental evidence, several aspects of the response leading to this disease remain to be investigated. The function of T lymphocytes in causing or regulating inflammation in the colon of NEMO^{IEC-KO} mice remains unclear. Furthermore, MyD88 is essential for signaling not only through TLRs but also through IL-1R. Given the increased expression of IL-1 β in the colons of these mice, it remains to be addressed whether MyD88 deficiency protects from disease development by inhibiting TLR signaling or IL-1R signaling. Along the same lines, the role of bacteria in inducing disease in this model remains to be directly addressed by the generation of germ-free mice. Moreover, the cell-specific function of individual chemokines and chemokines in driving the disease will need to be addressed experimentally using sophisticated genetic mouse models. Although tumor necrosis factor receptor 1 signaling is important for colitis development in NEMO^{IEC-KO} mice, it remains unclear whether TNF functions in IECs or in other cells to induce disease, and whether

apoptotic or proinflammatory signaling downstream intracellular signaling cascades are involved. These future experiments will elucidate the cellular and molecular mechanisms by which NF- κ B inhibition in IECs leads to spontaneous colitis in this mouse model. Such studies will also be relevant for human patients with NEMO mutations, which cause a range of complex pathologies most commonly characterized by ectodermal dysplasia with immunodeficiency.²³ Some of the patients carrying NEMO mutations were also reported to suffer from gastrointestinal complications including colitis,²⁴ suggesting that inhibition of IKK/NF- κ B signaling in IECs might also cause colitis in humans. Therefore, the elucidation of the mechanisms controlling the pathogenesis of colitis in NEMO^{IEC-KO} mice may also prove useful for a better understanding of colitis development in human NEMO patients and will hopefully lead to new therapeutic approaches.

DISCLOSURE

Manolis Pasparakis has received lecturing fees for giving lectures in Novartis and in Genentech.

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