Inflammatory Mediators Contributing to Intestinal Epithelial Cell Apoptosis and Barrier Disruption in IBD

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Abstract

In Crohn's Disease (CD) and ulcerative colitis (UC), the major manifestations of inflammatory bowel disease (IBD), genetically predisposed individuals develop chronic intestinal inflammation in response to environmental stimuli, which are mainly derived from luminal flora. Intestinal responses to luminal flora breaching the intestinal barrier require cytokine-regulated activation of elements of innate and acquired immunity, leading to a targeted and contained inflammatory response. Recent population-based genetic analyses have identified polymorphisms in specific genes relevant to pathways critical for inflammatory signalling and cellular response to stress as carrying increased risk for the development of either CD or UC. Specifically, key mediators of apoptosis and autophagy are implicated in the genetic vulnerability to IBD. Patients with IBD have a compromise of their intestinal barrier integrity, as do their first-degree relatives even in the absence of clinical disease, underscoring the critical nature of barrier integrity in the prevention of aberrant immune responses to intestinal flora. Here we explore the relationships between two of the key proinflammatory cytokines mediating intestinal inflammation in IBD, TNF-α and IFNγ, and the mechanisms by which they regulate epithelial apoptosis and intestinal barrier. Specifically we review factors regulating the balance between pro- and antiapoptotic stimuli resulting from the activation of NF-κB and Akt-dependent signalling by proinflammatory cytokines, as well as the influence of oxygen tension and nutritional factors on these pathways.

Keywords: Apoptosis; Autophagy; Intestine; Epithelium; Barrier; Inflammatory bowel disease; Inflammation; Cytokines; TNF-α; IFNγ

IBD

IBD encompasses two independent chronic inflammatory conditions, UC and CD, both of which are characterized, to varying degrees, by recurrent episodes of cramping, lower abdominal pain, diarrhea, bloody stools, and chronic inflammatory changes. In CD, these findings are associated, over time, with a propensity for scarring, and fibrosis, resulting in the formation of strictures and fistulas [1]. While the pathophysiology of both UC and CD is clearly complex, recent genome-based studies have implicated common pathways, which offer promising new avenues for both the identification of underlying mechanisms and the development of novel targeted therapeutics. Although UC and CD are clearly multifactorial diseases [2], monozygotic twin studies demonstrate a genetic influence on the incidence of CD and, to a lesser extent, UC [3-6]. More recently, genome wide array studies of multiple populations have identified key roles for the regulation of cellular responses to stress and infection. Specifically, genetic polymorphisms in genes regulating bacterial sensing, as well as autophagy, apoptosis, and inflammatory signaling, have been linked to either CD or UC [7-10].

The Role of Tissue Responses to Intestinal Microflora

The appropriate acquisition and containment of luminal bacterial flora is essential for normal development of both intestinal morphology [11,12] and barrier [13,14], yet luminal flora are also critical triggers of inflammatory responses in experimental models of intestinal inflammation [15-17] and IBD [18,19]. Under baseline conditions, the immune responses of the healthy intestinal mucosa are tightly regulated to minimize inflammatory responses to the continuous presence of luminal flora and their products. The epithelial barrier minimizes access of bacteria to the mucosal tissue. Beneath the epithelium, resident innate immune cells sample antigens from luminal bacteria as well as the relatively low number of bacteria, which successfully breach the thick mucus layer and penetrate the epithelial barrier. Bacterial products then activate complex combinations of receptors (Pattern Recognition Receptors, PRRs), such as members of the membrane-associated Toll-like Receptor (TLR) [12] and cytosolic Nucleotide Oligomerization Domain (NOD) receptor (NLR) families, which constitute cellular mechanisms for recognition of molecular patterns consistent with non-self [20, 21]. Individual members of these receptor families are differentially localized, both within individual cells and along the axis of the intestinal crypt [22,23]. Their combined signaling responses to the presence of invading bacteria or their products determine whether the end result is effective bacterial killing and cell repair, or cell death, necessitating epithelial restitution and proliferation by the surrounding cells [23].

These findings may be relevant to IBD in that autophagy of bacterial products has been linked to both adaptive and innate immune responses. Autophagy is a process resulting from cellular stress that triggers a noninflammatory, lysosome-mediated cellular degradation

pathway. Autophagy can be induced by infection [24] or starvation [25,26]. Autophagic cellular remodeling is highly evolutionarily conserved across all nucleated cells and is critical for development, differentiation, and tissue remodeling. During this process, cytoplasmic contents are sequestered by formation of a double membrane bound structure called an autophagosome, which then fuses with a lysosome, allowing for organelle disassembly and macromolecule recycling [26,27]. Additionally, dismantling of organelles such as peroxisomes and damaged mitochondria by autophagy may prevent oxidative stress and cellular damage resulting from release of their contents. Furthermore, a role for the autophagic machinery has been described in the defense from intracellular bacteria and protozoa [24]. Autophagic processing may promote MHC Class II-mediated presentation of bacterial antigens present in the cytosol.

Specifically, polymorphisms in autophagy-related gene products Atg16L1 and IRGM have been linked to increased incidence of CD [10]. One potential role for autophagy in intestinal homeostasis and CD resides in the finding that Atg16L1 function is critical for macrophage-dependent inflammatory responses to LPS and gram-negative bacteria [28]. Additional findings also identify roles for autophagy in IEC-specific innate immune responses to intestinal flora. Study of Atg16L1 hypomorphs revealed a critical role for this gene product in epithelial antibacterial defense through Paneth cell secretory function [29]. Furthermore, epithelial-specific deletion of Atg7 also resulted in defects in NF-κB activation, suppressing LPS-dependent production of TNF-α and IL-1β in murine intestine [30].

The question of whether an autophagic cellular response leads to recovery or death of the individual cell remains complex. In many studies of programmed cell death, the appearance of autophagic cellular structures does correlate with death of that cell, However, it is not clear whether the relationship is causative [31]. Because studies of the role of autophagy in programmed cell death have often been performed in the presence of inhibitors of apoptosis, the true relationship between autophagy and apoptosis remains unclear. Furthermore, autophagic cell disassembly has been linked to both pro- and antiapoptotic mechanisms within the cell. It appears at least plausible that, at low levels of stimulus, autophagy precedes apoptosis as a critical cellular defense mechanism against stress or infection [32].

In the case of insurmountable cellular insult, autophagy may be followed by either apoptotic or necrotic cell death. Through apoptosis, cells can be dismantled and removed without triggering an inflammatory reaction [33,34]. In the absence of regulated cellular disassembly, cell death may proceed through necrosis with associated membrane rupture and release of proinflammatory cellular components, leading to further accentuation of the inflammatory response of the surrounding tissue [35,36]. Alternatively, in the presence of key cytokines or growth factors, stressed epithelial cells may undergo transformation to a more mesenchymal/fibroblastic phenotype leading to alterations in the epithelial structure and scarring [37].

IEC Barrier and Barrier Disruption as Hallmark of IBD

An intact epithelial layer is the critical first line defense against bacterial invasion and uncontrolled intestinal inflammation. The critical elements of the intestinal barrier begin with the elaboration of a rich, IgA-associated mucus layer, extending downward to the epithelium itself [38]. The mucus layer exists in two compartments with an outer fluid layer, which is richly associated with resident bacteria and their products. A more tightly packed, nearly sterile layer of mucus lines the epithelial surface itself, excluding the majority of luminal bacteria from direct contact with the apical surface of the epithelium [39]. Disruption of this mucin layer is associated with direct contact of bacteria with the epithelial surface [39] and increased severity of colitis in Muc2-/- mice [40].

The epithelial monolayer itself is sealed tightly by an array of intra- and intercellular protein-protein interactions, which result in the formation of intercellular junctions [41]. This array of junctions consists of the apically localized tight junctions (TJs) along with the adjacent adherens junctions (AJ). Together, TJs and AJs make up the apical junction complex whose components regulate both paracellular transport, through a series of size and charge selective pores, and cellular responses to cell-cell contact through catenin-dependent regulation of both cellular signaling and gene transcription [42]. Additional physical stability is provided to the epithelial monolayer by desmosomes, which also associate with junctional cadherins [43], such that disruption of desmosomes has both structural and signaling consequences within the epithelial cell [44].

The sum of compensatory responses of individual epithelial cells culminates in whole tissue responses leading to barrier disruption, restitution, epithelial-mesenchymal transition (EMT), or dysplasia. Diminished intestinal barrier integrity has been demonstrated both in patients with CD, and in their unaffected first degree relatives [45,46], implying that barrier compromise may be a key first hit rendering individuals vulnerable to a second environmental insult leading to clinical disease. The critical influence of intestinal barrier disruption on immune responses underlying IBD is a topic that has recently been thoroughly reviewed [38,47]. Here we intend to focus specifically on mediators regulating epithelial apoptosis and barrier integrity in response to intestinal tissue inflammation.

Inflammatory Cytokines Regulate Epithelial Barrier Integrity and Apoptosis

Apoptosis is a critical mechanism of noninflammatory removal of compromised cells and epithelial homeostasis, and it is not a surprise that excessive epithelial apoptosis disrupts epithelial barrier integrity, permitting increases in bacterial translocation. Proinflammatory tissue responses to invading bacteria are, in part, mediated through the production of TNF-α and IFNγ. TNF-α is recognized as a classic mediator driving inflammatory signaling and apoptosis, and has been specifically targeted with significant success in CD [1]. IFNγ has also been extensively studied in models of IBD, and gene polymorphisms in these mediators or their downstream receptors and signaling pathways have been linked to an increased propensity for IBD, with IFN-γ signaling linked specifically to UC [9] while polymorphisms in the TNF-α pathway are linked to CD [10]. These proinflammatory mediators have in turn been shown to further influence epithelial barrier integrity [48,49] with TNF-α specifically implicated in diminished barrier due to epithelial apoptosis [50].

Apoptotic Mechanisms Induced By Proinflammatory Cytokines

Intestinal epithelial apoptosis and barrier function are partially regulated by inflammatory cytokines that are secreted mainly by immune cells but also by IEC themselves in response to inflammatory

stimuli. The influence of such cytokines on epithelial permeability and the molecular composition of the apical junctional complex that controls permeability has been reviewed recently [51]. Especially, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) have been implicated in the induction of IEC apoptosis during IBD. Of note, it has been demonstrated that the mechanisms through which IFN-γ and TNF-α regulate the composition of TJs and AJs is independent or upstream of their ability to induce apoptosis in IEC because inhibition of apoptosis does not prevent the internalization of junctional molecules in response to treatment of T84 IECs with these cytokines [52]. The internalization of TJ components such as occludin and ZO-1 induced by TNF-α is rather dependent on caveolin-1 and requires myosin light chain kinase (MLCK) activation [53]. A link between TNF-induced TJ molecule endocytosis and IEC apoptosis has also not been demonstrated in this study. Instead, the major mechanisms through which TNF-α and IFN-γ induce apoptosis in IEC include the activation of NF-κB and JNK and consequently the induction of Fas ligand expression, activation of caspase 8 and downregulation of the antiapoptotic mitochondrial protein Bcl2 [54-58].

IEC survival in response to exogenous TNF-α, *in vitro*, and also DSS-induced colitis, *in vivo*, is promoted by the activity of the ErbB4 receptor tyrosine kinase [59]. ErbB4 activity is upregulated by TNF-α-dependent activation of TNF-α converting enzyme (TACE) during colonic inflammation. Downregulation of ErbB4 rendered IECs more vulnerable to TNF-induced apoptosis, which was also accompanied by decreased Akt phosphorylation. Thus, ErbB4 balances the proapoptotic effects of TNF-α during intestinal inflammation.

IFN-γ also activates multiple pathways contributing to the balance of pro and antiapoptotic signals in the inflamed intestine. IFNγ induces the expression of the TNF-α receptor, TNFRII, thus further sensitizing IEC to the effects of TNF-α [60]. Although IFNγ initially stimulates Akt-dependent activation of β-catenin-dependent proliferative responses, later consequences of IFNγ stimulation of this same pathway include transcriptional upregulation of Dickkopf-1 (Dkk1), an antagonist of the canonical Wingless-Int (Wnt)/β-catenin pathway with key effects on IEC homeostasis [61,62], as discussed below. Another possible mechanism by which IFNγ triggers IEC apoptosis is through the disruption of cell-cell contacts since it has been shown that activated caspases can cleave desmoglein-2 to destabilize IEC desmosomes [63].

In contrast, IFN-γ can also induce the expression of proteins that protect IEC against apoptosis comparable to TNF-induced expression of ErbB4. As we have recently shown, IFN-dependent upregulation and junctional localization of guanylate binding protein-1 (GBP-1) in crypt epithelium of individuals with IBD is a protective measure that counteracts the inflammatory and proapoptotic environment in IBD [64,65]. Proinflammatory signaling pathways that are activated by IFNγ and TNF-α have previously been reviewed [50,51,66]. In the following chapter, we will give an overview of effector molecules in signaling cascades that have specifically been implicated in modulating IEC apoptosis (Table 1).

NF-κB Signaling and NEMO

The NF-κB family is comprised of five transcription factors that are activated in response to infection and proinflammatory mediators. The canonical NF-κB signaling pathway has classically been regarded as a proinflammatory [67] although its true role in inflammation is actually more complicated. For example, activation of NF-κB induces the expression of proinflammatory cytokines, chemokines and adhesion molecules, which then trigger inflammatory responses [68]. However, NF-κB has also been shown to protect cells from TNF-induced apoptosis [69]. Moreover, recent studies show that inhibition of this pathway does not result in the expected anti-inflammatory phenotype in murine intestinal epithelium. Instead, signs of chronic inflammatory conditions have been reported following inhibition of NF-κB signaling suggesting that this pathway has a more complex role in regulating tissue homeostasis [54]. These Janus-like properties of NF-κB signaling make it a central turnstile in the regulation of immune responses that, if not properly controlled, can lead to severe chronic inflammatory conditions such as IBD.

Several mouse models investigate the role of NF-κB signaling in intestinal inflammation through the conditional, cell-specific knockout of genes encoding mediators, which are critical in regulating its activation. This approach makes it possible to examine the specific effects of NF-κB signaling in a single cell type. This is of special importance since local manipulation of NF-κB signaling in the entire colon by pharmacological inhibitors has been shown to be protective in murine models of chronic colitis [70]. These protective effects in whole animal models are likely to result from the inhibition of NF-κB signaling in immune cells rather than in other cell types because it has also been shown that treatment of macrophages with a peptide blocking the binding domain of the upstream activator of NF-κB, NF-κB-essential modulator (NEMO) reduced the activity of NF-κB and decreased the secretion of proinflammatory cytokines [71]. In contrast,

Effector molecule	Activated by	Mode of action	Effect on apoptosis
NEMO	TNF-α, IL-1β	NF-κB activation	↑
PUMA	NFκB/TNF-α	TNF-induced disturbance of IEC integrity	↑
GBP-1	IFN-γ	Stabilization of intercellular junctions	↓
ErbB4	TNF-α, TACE	Akt phosphorylation	↓
Dkk-1	IFN-γ	Antagonization of Wnt/β-catenin pathway	↑
GCC	enterotoxins	conversion of GTP to cGMP, Akt inhibition	↓
PHD	hypoxia	degradation of HIFs, hydroxylation of IKK-β	↑
HIFs	hypoxia	induction of a stabilizing gene expression profile	↓
sphingomyelin	sphingomyelinase	caspase activation, Bcl2 inactivation	↑
phosphatidylcholine		inhibition of proinflammatory cytokines	↓

Table 1: Summary of the described effector molecules and the mechanisms through which they regulate IEC apoptosis.

blockade of epithelial NF-κB activity through IEC-specific knockout of the regulatory subunit of NEMO mediating NF-κB activation resulted in spontaneous chronic colitis [72]. In this mouse model, NEMO-deficiency was characterized by apoptosis of colonic epithelial cells causing disruption of the intestinal barrier and bacterial translocation into the mucosa. The presence of bacteria then induced TLR activation and myeloid differentiation primary response protein 88 (MyD88)-dependent expression of proinflammatory cytokines leading to the recruitment of more immune cells, and finally to the development of chronic inflammation. These effects were eliminated when NEMO-deficient mice were bred onto a MyD88-deficient background [72]. This study elegantly underlines the importance of NF-κB signaling for the maintenance of intestinal epithelial barrier integrity (Figure 1). However, further investigations are needed to better characterize the interplay between IECs and immune cells and how cell-specific MyD88-dependent signaling regulates immune homeostasis following NF-κB activation in the colon.

PUMA

Additional targets of NF-κB have also been implicated in the regulation of intestinal epithelial cell homoeostasis. The p53-upregulated modulator of apoptosis (PUMA) is a target of the tumor suppressor p53 and activation of PUMA can induce apoptosis in various cell types including human colon cancer cells in response to several proapoptotic stimuli through a mitochondrial pathway [73]. Interestingly, PUMA is also activated by NF-κB in response to exogenous TNF-α, suggesting that PUMA may contribute to TNF-α-induced apoptosis in IEC [74]. Indeed, these authors demonstrated that TNF-α-induced apoptosis is reduced in PUMA-deficient mice. Moreover, in a model of ischemia-reperfusion-induced injury in the small intestine, apoptosis of IECs was markedly reduced in PUMA knockout mice compared to wild-type mice [75].

The role of PUMA in IBD has very recently been studied. Importantly, PUMA is upregulated in individuals with UC and the intensity of PUMA expression correlates with disease progression [76]. These results are consistent with findings in mouse models of chronic colitis in which PUMA expression was upregulated in IECs and required intact NF-κB signaling. Induction of chronic colitis by

either dextran sodium sulfate (DSS) or 2,4,6-trinitrobenzene sulfonic acid (TNBS) in PUMA-deficient mice resulted in a more moderate disease phenotype accompanied by reduced IEC apoptosis [76]. The authors also showed that treatment of mice with the anti-TNF-α mAb, infliximab, suppressed both PUMA expression and severity of inflammation in chronic murine colitis, suggesting that the main role of PUMA in the pathogenesis of IBD may reside in the mediation of TNF-induced disturbance of epithelial integrity caused by IEC apoptosis. Whether this is a general mechanism driving chronic inflammation in the gut needs to be determined using other models such as IL10-deficient mice or bacterial and viral infections.

It may appear a contradiction that NF-κB activation by TNF triggers PUMA expression and thus IEC apoptosis while NF-κB inhibition through depletion of NEMO also triggers IEC apoptosis. However, NF-κB has previously been shown to induce both pro- and antiapoptotic proteins [76]. It is tempting to speculate that the cell type, location and kinetics of the inflammatory response are of importance as to whether the pro- or antiapoptotic branch of NF-κB signaling is dominant. Clearly, the precise functions of NF-κB signaling and its effectors in IBD still appear elusive to date.

GBP-1

Experimental evidence also links NF-κB- and IFN-dependent signaling pathways in the feedback inhibition of proinflammatory cytokine-dependent apoptosis through induction of GBP-1 [77]. Human GBP-1 belongs to the family of large GTPases comprised of seven members that have been shown to be strongly induced by IFNs [78]. GBP-1 is known to inhibit endothelial apoptosis in response to serum depletion [79] and to be an activation marker of endothelial cells in inflammatory diseases [80]. We demonstrated recently that GBP-1 is also strongly expressed in IEC after IFN-γ treatment, and that GBP-1 is required to maintain epithelial barrier integrity under inflammatory conditions [65]. Interestingly, both in model cell culture systems, *in vitro*, and tissue biopsies of individuals with IBD, GBP-1 localized to tight junctions, pointing to a functional role of GBP-1 in junctional integrity and intestinal epithelial barrier function. Indeed, transepithelial resistance developed more slowly when GBP-1 was downregulated by siRNA. Moreover, IEC proved to be more sensitive to IFN-γ-induced apoptosis in the absence of GBP-1, and this was dependent on caspases since inhibition of caspases restored the ability of IEC to form stable barriers in the absence of GBP-1.

In corroboration of these findings, we could further show that induced expression of GBP-1 protected IEC from apoptosis in human and murine models of developing intestines [64]. In this study, expression of the type I IFN IFN-αA was induced by commensal *E. coli* and this increase in IFN-αA resulted in the expression of GBP-1 and protection of IEC from caspase-dependent apoptosis, thus providing evidence for the importance of commensal bacteria in the establishment of a tight epithelial barrier during the colonization of the developing intestine. These results clearly demonstrate that the induction of IEC apoptosis under inflammatory conditions is more complex than expected. Importantly, proinflammatory cytokines not only induce caspase-mediated apoptosis but also induce the expression of protective proteins, such as GBP-1, which serve to prevent a complete loss of barrier integrity during inflammation in the intestine.
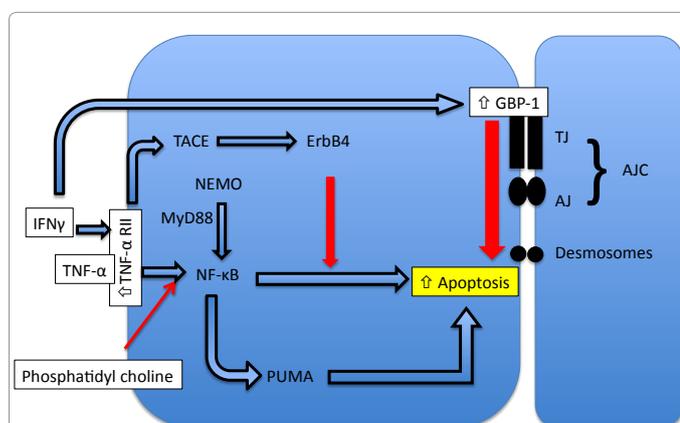

Figure 1: Proinflammatory cytokines activate NF-κB-dependent signaling pathways which balance positive and negative forces regulating apoptosis in IECs. Activating effects are indicated by blue arrows while inhibitory effects are indicated by red arrows.

Wnt/β-catenin signaling pathway and its antagonist Dkk1

The Wnt family of proteins consists of 19 secreted glycoproteins that play important roles in cellular homeostasis such as cell growth, differentiation, and apoptosis [81,82]. Secreted Wnt proteins can bind to receptor complexes consisting of one member of the Frizzled (Fzd) family and the co-receptor low-density lipoprotein receptor-like protein 5 or 6 (LRP5/6). This interaction leads to the transduction of intracellular signalling pathways [83]. Specifically, the canonical Wnt/β-catenin signalling pathway controls the amount of cytoplasmic β-catenin that can translocate into the nucleus where it then interacts with certain transcription factors and induces the expression of a variety of genes and their protein products [84]. In the absence of Wnt signalling, cytoplasmic β-catenin is phosphorylated and degraded by the ubiquitin/proteasome pathway. Binding of Wnt protein to its receptor frizzled activates dishevelled proteins that in turn inhibit GSK-3β, the kinase that phosphorylates β-catenin, leading to an accumulation of β-catenin and its nuclear translocation [84] (Figure 2).

Whether the Wnt pathway plays a role in the pathogenesis of IBD is not entirely clear but it has been shown that Wnt signals are required to maintain tissue homeostasis in the intestine [85]. Furthermore, several proteins of the Wnt pathways are upregulated in the mucosa of patients with UC, e. g. WNT2B, WNT5B, WNT7A, WNT11, frizzled (FZD) 3, FZD4, Dkk4 and dishevelled-2 (DVL2) while others are downregulated such as FZD1 and FZD5 [86]. Active Wnt/β-catenin signalling is required for intestinal epithelial homeostasis and regulates the balance of IEC proliferation and apoptosis [61,87,88]. Members of the dickkopf family bind to LRP5/6, competitively inhibiting the binding of Wnt proteins [89,90]. Specifically, Dkk1 has been shown to be a potent inducer of apoptosis in a variety of cell types including IEC [91]. Moreover, it has been shown that overexpression of Dkk1 leads to mucosal injury through inhibiting epithelial cell proliferation in the gut [92].

Nava and colleagues have recently shown that exposure of IEC to IFN-γ resulted first in β-catenin activation through Akt and subsequently

in the induction of Dkk1 expression. Dkk1, in turn, inhibited the Wnt pathway leading to increased IEC apoptosis [61] (Figure 2). Moreover, it has been shown that inactivation of the Wnt/β-catenin pathway can lead to apoptosis in colon cancer cells [93]. In contrast, disturbed Wnt signalling is also associated with the development of intestinal cancers [94]. Interestingly, by inducing apoptosis and inhibiting epithelial proliferation, Dkk1 is indeed important in the regulation of tissue morphology and homeostasis under inflammatory conditions, *in vivo*, as demonstrated recently [62], and may thus be a key component to balance Wnt signalling pathways in the intestine. Therefore, the exact spatio-temporal regulation of the Wnt pathway is likely to be crucial to ensure proper IEC homeostasis.

GCC

In addition to Wnt signalling, additional pathways regulating Akt activity have also been identified as relevant to IEC proliferation and homeostasis, although their specific roles in IBD remain to be determined. Guanylate cyclase C (GCC) belongs to a family of transmembrane enzyme-linked receptors expressed on the luminal surface of IEC [95]. These receptor-type enzymes are activated by the binding of endogenous ligands such as uroguanylin and guanylin, as well as by the binding of bacterial peptides such as heat stable enterotoxin. Upon ligand activation, GCC catalyzes the intracellular conversion of GTP to cGMP, which has been suggested to protect against apoptosis [96]. Indeed, in IEC this antiapoptotic effect was confirmed by data showing that GCC activation inhibited radiation-induced apoptosis in IECs by reducing the levels of cGMP [97]. Moreover, treatment of intestinal cancer cells with GCC agonists that increase cGMP levels reduced the rate of cell proliferation [98]. In agreement with this finding, GCC-deficient mice are more sensitive to tumor development in various cancer models likely via inhibition of Akt signalling in IEC [99], further implying a fundamental role of GCC and cGMP in IEC homeostasis and the progression of gastrointestinal cancer. GCC-deficient mice also showed increased IEC apoptosis [100]. Consistent with this, mice deficient for GCC or uroguanylin are prone to radiation-induced IEC apoptosis and these mice can be protected from IEC apoptosis by feeding cGMP before irradiation [97]. However, guanylin-deficient mice are characterized by only slightly enhanced IEC proliferation and migration [101].

In a very recent study, the influence of GCC activation and consequences of GCC or UGN loss on epithelial barrier functions were investigated. Intestinal permeability was increased in both GCC- and UGN-deficient mice under resting conditions in the jejunum but not in the ileum or colon [102]. However, after LPS challenge, only permeability in the ileum was increased, and this was more pronounced in the knockout mice. Thus, GCC signalling may play a context-dependent role in the regulation of epithelial homeostasis in the gut that seems to be regulated by the presence of its ligand UGN.

Interestingly, increased permeability in the absence of GCC was accompanied by increased IFNγ levels leading to MLCK and STAT1 activation, increased MLC phosphorylation, and reduced claudin-2 and JAM-A expression at tight junctions, *in vivo* and *in vitro* [102]. Activation of MLCK in response to proinflammatory cytokines is known to play an important role in the disruption of TJs and the intestinal epithelial barrier in response to proinflammatory cytokines [103,104]. In this respect, transgenic mice expressing constitutively active MLCK in IEC did not develop spontaneous intestinal disease but
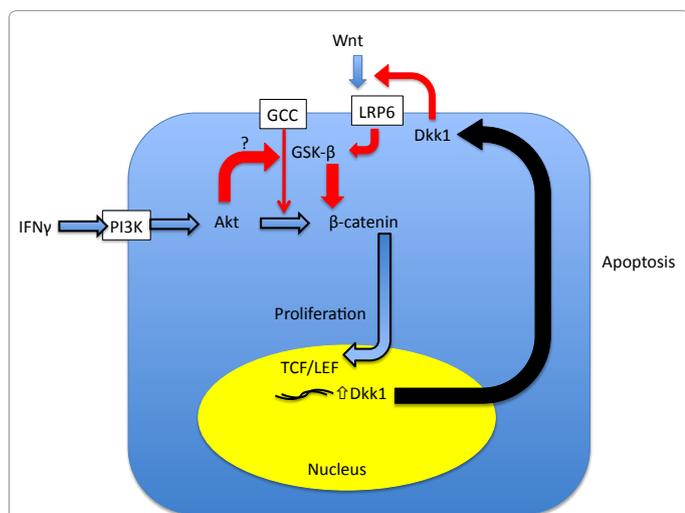

Figure 2: IFNγ-dependent activation of Akt triggers early proliferative responses followed by Dkk1-dependent feedback inhibition leading to IEC apoptosis. (Adapted from graphical abstract of Nava et al. [61]).

showed severe loss of barrier function accompanied by increased levels of IFNγ and TNF-α [103]. Thus, loss of GCC signalling may cause IEC barrier dysfunction via increased MLCK expression and activation. Although one study also exists that showed increased apoptosis after treatment with uroguanylin [105], accumulating evidence emerges that activation of GCC is vital in regulating IEC homeostasis, has a rather protective role against apoptosis, and inhibits tumor progression.

PHD1 and HIF-1α

The maintenance of cellular homeostasis is challenged in both tumors and inflamed tissues by relative tissue hypoxia. Specifically, intestinal inflammation is associated with tissue edema, microvascular inflammation and associated ischemia. These changes in perfusion along with the increased metabolic demands of stressed IECs and infiltrating inflammatory cells are associated with increased tissue hypoxia [106]. Alterations in cellular oxygen tension are detected by prolylhydroxylases (PHD), which control the stability of hypoxia-inducible factor (HIF)-1α. Proline residues in the subunit HIF-1α are hydroxylated in the presence of oxygen and as a consequence HIF-1α is ubiquitinated and degraded. Under conditions of hypoxia, PHDs are inhibited and cannot hydroxylate HIF-1α so that HIF-1α remains stable and can translocate into the nucleus where it binds to HIF-1β, activating a gene expression profile that promotes barrier stabilization [107-109]. Importantly, this HIF-1α-dependent barrier stabilization is protective in experimental colitis [106].

PHDs are a family of enzymes comprised of three members, PHD1-3, all of which are expressed in the intestine. Recently, PHDs have also been shown to be important regulators of apoptosis in response to hypoxia [110]. Robinson and colleagues showed that the mRNAs of PHD2 and PHD3 are expressed at similar levels in the intestinal mucosa whereas PHD1 is expressed to a much lower extent under resting conditions [111]. Although lowest in baseline expression in noninflamed tissue, PHD1 was upregulated in inflamed tissues from patients with IBD [112]. This upregulation of PHD1 appeared to be detrimental in that PHD1-deficient mice developed a milder colitis in response to DSS compared to WT and also relative to PHD3-deficient and PHD2-heterozygous mice [112]. Importantly, this protection under inflammatory conditions was associated with reduced levels of IEC apoptosis and thus an enhanced barrier function. Therefore, it is tempting to speculate that the increasing PHD1 levels under inflammatory conditions can contribute to the pathogenesis of IBD by inducing epithelial cell apoptosis and loss of barrier function.

In addition to regulating the protein stability of HIF-1α, PHDs disrupt the NFκB pathway, probably through hydroxylation of IKK-β leading to disturbed expression of antiapoptotic genes, altered tissue homeostasis in the intestinal mucosa, and heightened sensitivity to colitis [113,114]. Moreover, inhibition of PHD1 by dimethyloxalylglycine (DMOG) resulted in increased activity of both HIF-1α and NF-κB and protected against DSS-induced colitis through induction of antiapoptotic genes in IEC [115]. These data emphasize the importance of PHD1 in the progression of chronic colitis and argue for an inhibition of PHD1 as being an appropriate novel strategy for treating IBD.

Sphingomyelin

As mentioned above, the pathogenesis of IBD is not fully understood but there is accumulating evidence that nutritional factors
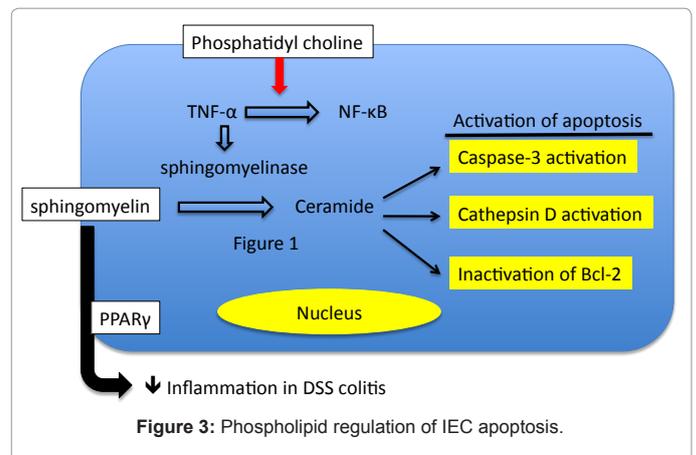

Figure 3: Phospholipid regulation of IEC apoptosis.

can influence both the initial vulnerability to the development of colitis as well as the duration of disease remission [116]. In this respect, specific phospholipids have garnered some recent attention. In particular, sphingolipids have been shown to be important mediators of inflammation [117]. For example, dietary sphingomyelin and its metabolite ceramide are involved in apoptotic signalling and thus play a role in the regulation of epithelial barrier function [118,119]. Sphingomyelin is hydrolized by sphingomyelinase in the small intestine or colon, and the resulting ceramide can induce apoptosis through activation of caspase-3 [120], inactivation of Bcl2 [121] or through activation of cathepsin D [122]. Of note, TNF-α can activate both sphingomyelinase and cathepsin D [123,124] as an alternative mechanism of TNF-induced apoptosis (Figure 3). Very recently, a link of dietary sphingomyelin to IEC apoptosis has been provided [119]. In this study, a sphingomyelin-enriched diet following DSS-induced colitis increased IEC apoptosis, which was accompanied by an increased activity of cathepsin D and caspases 3 and 9. These results implicate that at least during acute phases of colitis, a diet rich in sphingomyelin is detrimental. Almost in parallel, another study has been published demonstrating that dietary sphingomyelin ameliorated DSS-colitis in mice in a PPAR-γ dependent fashion [125]. However, in this study the direct effect of sphingomyelin on IEC apoptosis was not investigated. Although the reason for these contradictory results remains unclear, it may be that differences in overall diet, sphingomyelin dose and/or different mice strains have been factors. Thus the individual contributions of dietary sphingomyelin to epithelial integrity and intestinal inflammation warrant further investigation.

Another phospholipid, phosphatidylcholine, which is a key component of cell membranes and mucus, has been shown to restore intestinal epithelial barrier function under inflammatory conditions [126] and to inhibit TNF-α-induced upregulation of proinflammatory cytokines in IEC [127] (Figure 3). Since phosphatidylcholine content in the mucus of individuals with UC is significantly reduced compared to healthy individuals [128], a diet enriched in this phospholipid may be beneficial during acute phases of UC.

Concluding Remarks

Pharmacologic therapy for IBD has classically focused primarily on global attenuation of inflammatory responses or targeted modulation of adaptive immunity. However, it is becoming increasingly clear that a complex interplay exists among the signaling pathways induced by the

proinflammatory cytokines, TNF-α and IFNγ, which activate pathways leading to cell-specific and time-dependent changes in inflammatory tone as well as vulnerability to epithelial apoptosis. Furthermore, these cytokines can modulate barrier integrity independent of inflammatory responses. Genetic analyses have identified several key elements of cellular stress response as associated with IBD. Thus, individuals with CD and UC are ideally suited for personalized therapeutics specifically targeting their individual genetic predispositions as well as the downstream pathways leading to dysregulated chronic inflammatory responses. Clearly, nutritional management is also an important adjunct to pharmacologic intervention in managing a disease with such distinct contributions from aberrant responses to cellular stress. The combined identification of specific genetic predispositions and an improved understanding of the early barrier defects in innate immunity may continue to fuel the development of strategies for rational targeting of these pathways to allow for better maintenance of remission and potentially for the prevention of disease in identified families.

Acknowledgements

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