The Journal of Physiology

TOPICAL REVIEW

Determinants of colonic barrier function in inflammatory bowel disease and potential therapeutics

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Abstract Intestinal barrier dysfunction is a main feature of the inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis. Leak flux diarrhoea and a facilitated uptake of noxious antigens are the two consequences resulting from an impaired epithelial barrier. Barrier perturbations in IBD comprise alterations in epithelial tight junctions (TJ), i.e. a reduced number of horizontal TJ strands and an altered TJ protein expression and subcellular distribution. Moreover, increased incidence of apoptotic events as well as erosions and ulcerations can add to that leakiness. These barrier defects are attributed to enhanced activity of pro-inflammatory cytokines like TNFα, INFγ, IL-1β and IL-13, which are highly expressed in the chronically inflamed intestine. Although the aetiology of IBD is far from being clear, chronic inflammation is believed to result from an inadequate immune response as a consequence of genetic predisposition as well as changes in, and altered responses to, the intestinal microbiota. On the other hand, an insufficient mucosal response to bacterial stimuli results in an insufficient immune response towards intestinal pathogens. However, detailed characterization of barrier defects offers the opportunity to consider and test therapeutic interventions. Beside cytokine antagonists, different plant compounds and probiotics have been shown to stabilize the barrier function by affecting TJ protein expression and distribution.

(Received 22 November 2011; accepted after revision 31 November 2011; first published online 4 January 2012)
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Abbreviations CD, Crohn's disease; DSS, dextran sulfate sodium; EMT, epithelial-mesenchymal-transition; HNF4α, hepatocyte nuclear factor 4 α; IBD, inflammatory bowel disease; IFNγ, interferon-γ; IL, interleukin; MLCK, myosin light chain kinase; SCFA, short chain fatty acid; TGFβ, transforming growth factor-β; TJ, tight junction; TNFα, tumour necrosis factor-α; UC, ulcerative colitis; ZO, zonula occludens.

Introduction

Patients with inflammatory bowel disease (IBD) including Crohn's disease (CD), ulcerative colitis (UC), and microscopic colitis suffer from inflammation-induced leak flux diarrhoea. This type of diarrhoea is caused by a passive loss of ions and water from the circulation into the intestinal lumen as a result of an impaired intestinal barrier. On the other hand, enhanced uptake of noxious antigens from the

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DOI: 10.1113/jphysiol.2011.224568

gut lumen enhances mucosal and systemic inflammatory processes as a permissive factor for IBD. Because of this *circulus vitiosus*, susceptibility of the barrier already increases with small alteration.

Numerous studies have been conducted to identify and characterize the mechanisms of barrier disruption in IBD and have highlighted the major role of the epithelial tight junction (TJ) in this respect. Having the multifactorial nature of IBD pathogenesis in mind, this review focuses on the role of cytokines, the relevance of genetic dispositions and the influence of the intestinal microbiota on intestinal barrier function with special attention on TJs.

Tight junctions – determinants of intestinal barrier function

The intestinal barrier is established by a polarized monolayer of epithelial columnar cells, which are connected by intercellular junctions. From these the TJ is the structural feature which helps to maintain a strict and regulated separation of the body against the luminal content of the gut. This separation is also necessary to prevent back leakage of absorbed ions and nutrients or to avoid the entry of luminal antigens and microorganisms. The TJ can be recognized in freeze fracture electron microscopy as a complex network of continuous strands built up by intramembranous particles (Staehelin, 1973). Structurally, the TJ is composed of four different types of integral membrane proteins: occludin (Furuse *et al*. 1993), the claudins (Tsukita *et al*. 2001), tricellulin (Ikenouchi *et al*. 2005) and junctional adhesion molecule (Martin-Padura *et al*. 1998). Claudins in particular play a critical role in barrier function. In over-expression, silencing or knock-out approaches several of the 27 claudins so far described in mammals were shown to have sealing or pore-forming properties within the gastrointestinal tract (Van Itallie & Anderson, 2006). While claudin-1, -3, -4, -5 and -8 have sealing functions (Van Itallie *et al*. 2001; Furuse *et al*. 2002; Amasheh *et al*. 2005; Amasheh *et al*. 2009b; Milatz *et al*. 2010), others such as claudin-2, -10b or -15 act as paracellular channels and promote a charge-selective passage of small ions (Amasheh *et al*. 2002; Gunzel *et al*. 2009; Tamura *et al*. 2011). In addition, claudin-2 was shown to act as a paracellular water channel (Rosenthal *et al*. 2010). Composition, structure and permeability of the TJ are tissue-specific, and strictly regulated by physiological as well as pathophysiological stimuli including inflammatory regulators.

Barrier defects in IBD

The importance of an intact epithelial TJ becomes evident in IBD. Clinical symptoms of IBD result from intestinal inflammation and subsequent epithelial dysfunction including impaired absorptive functions and barrier defects (leak flux diarrhoea). Investigations on sigmoid colon biopsies from patients with mild CD revealed impaired TJ complexity, characterized by a decreased number of TJ strands, reduced depth of the main TJ meshwork and more strand breaks (Fig. 1). In addition, expression of sealing claudin-3, -5 and -8 and occludin was diminished, while pore-forming claudin-2 was up-regulated. Furthermore, claudin-5 and -8 were distributed off the TJ (Zeissig *et al*. 2007). Similar changes were observed in UC, including down-regulation of occludin, claudin-1 and -4 and up-regulation of the pore-forming claudin-2 (Heller *et al*. 2005). Structurally, strand count and meshwork depth were reduced (Schmitz *et al*. 1999a; Heller *et al*. 2005).

In addition, leaks resulting from epithelial apoptosis (Gitter *et al*. 2000; Bojarski *et al*. 2001) were found to be increased in CD and UC (Heller *et al*. 2005; Zeissig *et al*. 2007). Consequently, microerosions due to arrested restitution caused by the Th2 cytokine interleukin-13 (IL-13) are an early event in UC (Heller *et al*. 2005). Recently, epithelial-mesenchymal-transition (EMT) has been identified as an important barrier feature in coeliac disease and could be equally important in IBD. EMT can change cell polarization within the epithelium, thereby intensifying endocytotic antigen uptake from the lumen with induction of a parallel subcellular redistribution of TJ proteins (Schumann *et al*. 2012).

Thus, gross lesions (erosions), apoptotic leaks, changes in TJ structure or composition as well as altered endo-/transcytosis are important barrier pathomechanisms, which enable noxious antigens derived for example from food or microorganisms to penetrate the mucosal barrier to a significant extent. Enhanced endocytosis in IBD could also be a mechanism by which bacterial translocation is enhanced in IBD. Moreover, our group has gained evidence that changes in tricellulin contribute to enhanced macromolecular passage in UC (Krug *et al*. 2010), as this TJ protein normally restricts macromolecular permeability in tricellular TJs by tightening the central tube (Krug *et al*. 2009). The main aspects of barrier dysfunction in IBD are summarized in Table 1.

Role of inflammatory cytokines in barrier disturbance

Pro-inflammatory cytokines play a key role in the induction of barrier defects in IBD. Tumour necrosis factor-α (TNFα) and interferon-γ (IFNγ) are increased in CD (Th1 profile), while in UC the inflammatory response is characterized by an increase in TNFα and IL-13. Interestingly, in cell culture and animal models these cytokines were found to induce comparable barrier defects, as observed in CD or UC, including TJ changes,

Table 1. Aspects of barrier dysfunction in IBD

Inflammatory bowel diseases	Cytokine profile	Epithelial resistance (Ω cm^2)	Tight junction proteins	Other pathomechanisms	References
Crohn's disease (sigmoid colon)	TNFα, IFNγ (Th1)	23 ± 3 (59% of control) (active, mild to moderate inflamed)	Claudin-2 ↑ Occludin ↓ Claudin-3 ↓ Claudin-5 ↓ and redistributed Claudin-8 ↓ and redistributed	Epithelial apoptosis ↑	Zeissig *et al*. 2007
Ulcerative colitis (sigmoid colon)	IL-13, TNFα (Th2)	20 ± 3 (21% of control) (active, mild to moderate inflamed)	Claudin-2 ↑ Occludin ↓ Claudin-1 ↓ Claudin-4 ↓ Tricellulin ↓	Epithelial apoptosis ↑ Ulcerations Microerosions	Heller *et al*. 2005; Schmitz *et al*. 1999a; Krug *et al*. 2010
Collagenous colitis (sigmoid colon) (microscopic colitis)	TNFα, IFNγ (Th1)	29 ± 2 (23% of control) (macroscopically normal)	Claudin-2 ↑ Claudin-4 ↓ Occludin ↓	Malabsorption	Burgel *et al*. 2002; Tagkalidis *et al*. 2007

Regulation: ↓ down, ↑ up.

apoptosis induction and enhanced bacterial translocation (John *et al*. 2011). Cytokines can affect TJs in two ways, first by expression regulation and second, and perhaps more importantly, by affecting the redistribution processes. For example, TNFα as well as IL-13 can increase claudin-2 protein expression in HT-29/B6 cells. In the case of TNFα, this was induced via the phosphatidylinositol-3-kinase pathway (Fig. 2) (Mankertz *et al*. 2009). Exposure of native rat colon to TNFα and IFNγ revealed up-regulation of pore-forming claudin-2 and down-regulation of barrier-forming claudin-1, -5 and -7 (Amasheh *et al*. 2009a). Beside pro-inflammatory cytokines, we recently found the transforming growth factor-β (TGFβ), which is assumed to be rather protective in IBD (Monteleone *et al*. 2008), to increase claudin-4 expression by stimulating claudin-4 promoter activity directly (Hering *et al*. 2011a). Myosin light chain kinase (MLCK) is one important regulatory element of TJ protein regulation which was found to be affected in the intestine of IBD patients (Blair *et al*. 2006). Phosphorylation of MLCK leads to reorganization of perijunctional F-actin, and consequently to redistribution of TJ proteins from the tight junction domain of the enterocyte towards intracellular compartments (Shen *et al*. 2006). Recently,

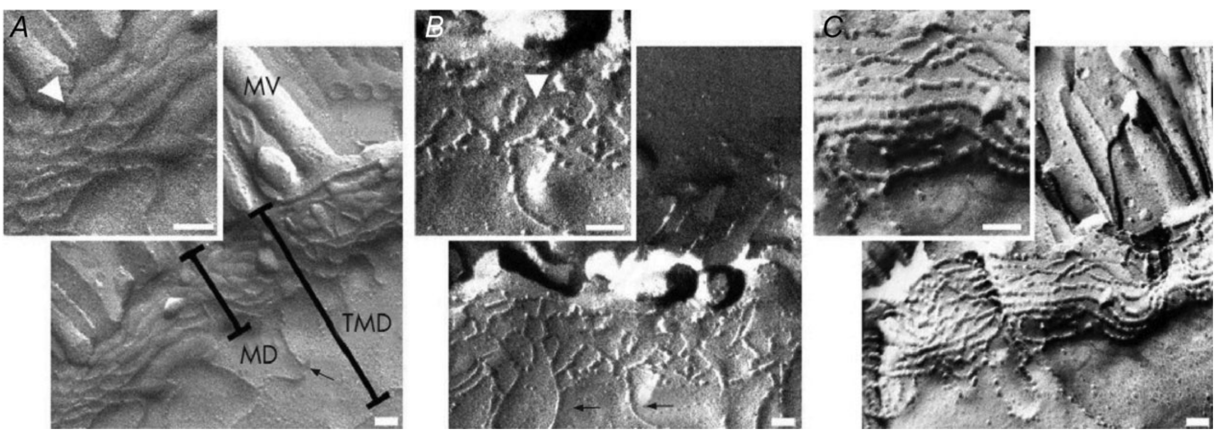

Figure 1. Freeze fracture electron microscopy of TJ strands from control (*A*) and mild to moderately inflamed Crohn's disease (*B* and *C*)
Controls show continuous TJ strands, while in Crohn's disease a reduced number of TJ strands, frequent strand breaks (arrowhead in *B*) and a discontinuous TJ network are obvious (*C*). Scale bars indicate 100 nm; MV, micro villi; MD, main TJ meshwork depth without aberrant strands; TMD, total TJ meshwork depth from the most apical to the most basal strand including aberrant strands. Reproduced from Zeissig *et al*. 2007.

MLCK-dependent zonula occludens-1 (ZO-1) exchange was suggested to be critical for this process (Yu *et al.* 2010). In addition, MLCK activation stimulates IL-13 synthesis, which in turn increases claudin-2 expression (Weber *et al.* 2010). TNFα and interleukin-1β enhance TJ permeability by stimulating MLCK gene expression via NFκB in Caco-2 cells (Ye & Ma, 2008; Al-Sadi *et al.* 2011). Structural and functional TJ regulation is also affected by MLCK-induced caveolin-1-dependent endocytosis of occludin (Marchiando *et al.* 2010). In contrast, IFNγ was found to induce TJ redistribution via a Rho/ROCK signalling-dependent macropinocytosis-like mechanism (Bruewer *et al.* 2005). As already mentioned, EMT has to be considered as a further mechanism, by which TJ proteins are re-distributed due to a loss of cell polarity. In coeliac disease TJ assembly and expression was affected by dysregulated cell polarity proteins Par-3 and PP-1 (Schumann *et al.* 2012). How far this also occurs in IBD patients and to what extent cytokines could regulate EMT might be elucidated in forthcoming studies.

Finally, TNFα and IL-13 can also enhance apoptotic events within the intestinal epithelium (Schmitz *et al.* 1999b; Heller *et al.* 2005), at which the contribution of apoptosis to leakiness might depend on the restitution capabilities of the harmed epithelium (Marchiando *et al.* 2011). Furthermore, IFNγ, TNFα as well as IL-13 were observed to increase bacterial translocation in cell culture approaches (Clark *et al.* 2003, 2005; Troeger *et al.* 2007).

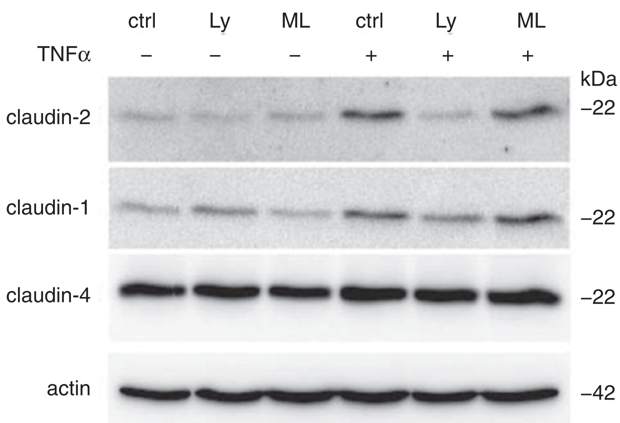

Figure 2. Involvement of phosphatidylinositol-3-kinase signalling (PI3K) in TNFα-induced claudin-2 up-regulation in HT-29/B6 cells

Western blot analyses represent expression level without treatment (ctrl), after TNFα treatment and after pre-incubation with PI3K inhibitor LY294002 (Ly) or MLCK inhibitor ML-7 (ML). Inhibition of PI3K prevents TNFα-induced claudin-2 up-regulation, while ML-7 has no influence. Reproduced from Mankertz *et al.* 2009 with kind permission from Springer Science+Business Media; copyright ©2009 Springer Verlag.

Aetiology of barrier dysfunction in IBD

However, despite extensive investigations and characterization of barrier determinants the aetiology of IBD is far from being clear. That is in part due to the fact that it seems to involve a multifactorial interplay of different factors. In particular, genetic disposition and changes to the intestinal microbiota are crucially linked to each other and may subsequently favour an inadequate immune response.

Genetic predisposition has been increasingly discussed as being of central importance over the last few years. For example, single-nucleotide polymorphisms (SNPs) in the genes encoding for PAR-3 and MAGI2, which both play a role in TJ assembly, were found to be associated with coeliac diseases and UC (Wapenaar *et al.* 2008). Furthermore, *HNF4α*, encoding the transcription factor hepatocyte nuclear factor 4α, was identified as susceptibility loci in a genome-wide association study on UC (Barrett *et al.* 2009). With regard to barrier function, this seems to be important as HNF4α is involved in transcriptional regulation of TJs, adherens junctions, and desmosome expression (Battle *et al.* 2006). Claudin-15 was identified as a direct target of HNF4α (Darsigny *et al.* 2009). In mice, conditional intestinal HNF4α deletion was associated with increased permeability, a more severe course of dextran sulfate sodium (DSS)-induced colitis (Ahn *et al.* 2008) and claudin-15 down-regulation (Darsigny *et al.* 2009), which was recently described to be linked to sodium deficiency and glucose malabsorption (Tamura *et al.* 2011). Moreover, claudin-2 gene expression is known to be controlled in a cooperative manner by caudal-related homeobox (Cdx) proteins, GATA-4 and HNF1α (Sakaguchi *et al.* 2002; Escaffit *et al.* 2005), which is positively regulated by HNF4α (Eeckhoute *et al.* 2004).

Mutations in the caspase-activated recruitment domain (*CARD15*) gene were assumed to be one risk factor for the development of CD. Beside other tissues, the *CARD15* gene product NOD2 is expressed in ileal crypts, where it senses bacterial components (Lala *et al.* 2003). *CARD15* mutations were found to be associated with an elevated mucosal permeability (Buhner *et al.* 2006; D'Incà *et al.* 2006). Although the mechanism has not been identified so far, *CARD15* gene expression was shown to be up-regulated by TNFα and IFNγ (Rosenstiel *et al.* 2003). Moreover, NOD2-signalling defects result in reduced expression of antimicrobial defensins (Wehkamp *et al.* 2004; Voss *et al.* 2006). This is assumed to be critical for the initiation of barrier defects (Rosenstiel *et al.* 2003; Wehkamp *et al.* 2004), as bacterial pathogens can gain direct access to the mucosal barrier and trigger inflammation or direct barrier disturbances, e.g. by induction of epithelial apoptosis (Nielsen *et al.* 2011), focal leaks (Troeger *et al.* 2007), and TJ disruption (Bucker *et al.* 2009; Hering *et al.* 2011b).

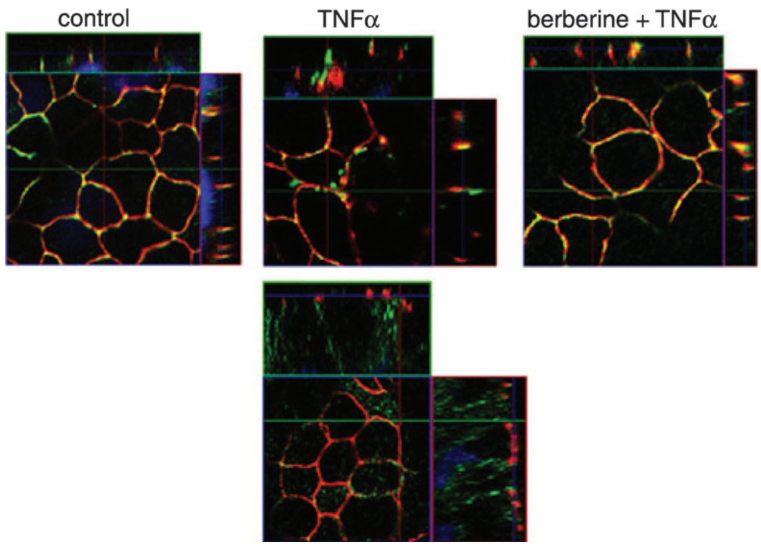

Figure 3. Berberine protects claudin-1 from TNFα-induced disassembly from the TJ
Immunofluorescent staining and subsequent confocal laser-scanning microscopy of HT-29/B6 cells shows claudin-1 in green and TJ marker ZO-1 in red. While claudin-1 is redistributed off the TJ to intracellular compartments after TNFα treatment, pre-treatment with berberine reveals colocalization of claudin-1 and ZO-1 (merge), indicated by yellow staining. Reproduced from Amasheh *et al*. 2010.

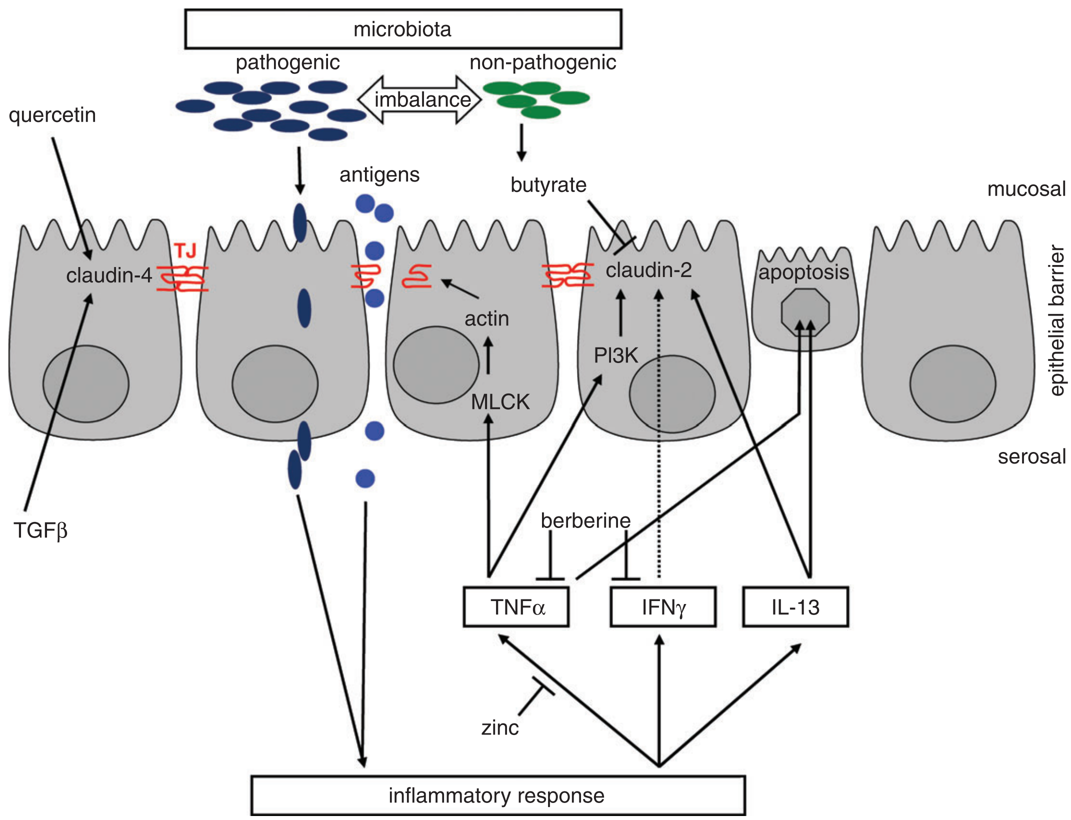

Figure 4. Main features of inflammatory barrier dysfunction comprise TJ alterations, e.g. subcellular redistribution, altered protein expression and composition as well as epithelial apoptosis
As key inductors, pro-inflammatory cytokines (TNFα, INFγ, IL-13) can trigger these changes by stimulating signalling cascades (e.g. MLCK, PI3K). Inflammation in turn is suggested to be induced or enhanced by the passage of luminal antigens or pathogens. Beside other factors, this can result from an imbalance or altered composition of the intestinal microbiota. With respect to therapeutic intervention, bacterial products (butyrate), food components (quercetin) or cytokines (TGFβ) are known to influence TJ protein expression but the inflammatory cascade can also be blocked, attenuating intestinal inflammation (berberine, zinc).

Barrier disturbances are also caused by alterations in the composition of gut microbiota, resulting in an imbalance of protective commensals and potential pathogens in IBD (Sepehri *et al.* 2007). This becomes evident from different mouse models. For example, IL-2- or IL-10-deficient mice, which suffer from spontaneous chronic intestinal inflammation (Elson *et al.* 2005), do not develop symptoms under germ-free conditions (Sellon *et al.* 1998; Dieleman *et al.* 2004). Similar effects were observed in DSS-treated mice, which had milder symptoms when kept germ-free (Hudcovic *et al.* 2001). As a further consequence of altered microbial complexity, production or availability of different bacteria-derived metabolites, e.g. the short chain fatty acid (SCFA) butyrate is reduced in IBD (Chapman *et al.* 1994). Butyrate not only serves as an energy source for the enterocytes, but also exerts anti-inflammatory properties (Segain *et al.* 2000; Tedelind *et al.* 2007). In addition, reduced production of butyrate may also lead to less activation of SCFA-coupled electroneutral NaCl absorption in the colon and thus limit the activity of a transport mechanism that reduces luminal fluid load in the colon (Krishnan *et al.* 1999). Furthermore, butyrate was recently shown to reduce bacterial translocation in a cell culture model (Lewis *et al.* 2010) and our group has obtained experimental evidence for a barrier-regulating function of claudin-2 (Plöger *et al.* 2010).

Therapeutic approaches

Although IBD cannot be cured so far, the increasing understanding of inherent pathomechanisms offers the possibility of specific therapeutic interventions, which can reduce symptoms by strengthening epithelial barrier function.

Counteracting the inflammatory action of cytokines by application of cytokine antagonist is one important option. TNFα antibody therapy revealed reduction of epithelial apoptosis to normal levels in CD patients (Zeissig *et al.* 2004). Mucosal healing was reported in UC patients as well (Afif *et al.* 2009). Although first investigations revealed no change in occludin, and claudin-1 and -4 after TNFα antibody treatment (Zeissig *et al.* 2004), effects on claudin-2 seem likely, perhaps after a longer period of therapy. Interestingly, the expression of TNFα and IL-1β was found to be inhibited by zinc. The zinc finger protein A20 is directly involved in the negative feedback regulation of NFκB signalling (Prasad *et al.* 2004). Zinc therapy decreased mucosal permeability in CD patients (Sturniolo *et al.* 2001). The impact of zinc on TJ composition and structure has not been studied so far in humans, but up-regulation of occludin and ZO-1 was reported in animal studies (Zhang & Guo, 2009).

Direct influence on TJ protein expression was observed from several plant components, such as the flavonoid quercetin or the isoquinoline alkaloid berberine. Quercetin found in different fruits or onions enhances barrier function in Caco-2 cells by up-regulating claudin-4 expression (Amasheh *et al.* 2008). Berberine as used in traditional medicine has anti-inflammatory effects in experimental colitis in rats (Zhou & Mineshita, 2000) and prevented barrier dysfunction induced by TNFα and INFγ in a cell culture model, e.g. claudin-1 distribution (Fig. 3) (Amasheh *et al.* 2010). Furthermore, several probiotic microorganisms are known to influence the expression of pro-inflammatory cytokines (Roselli *et al.* 2006) or to influence TJ expression and composition directly. For example, redistribution and reduction of ZO-1, occludin and claudin-1, -3, -4 and -5 could be prevented by the probiotic mixture VSL#3 in a murine model of colitis (Mennigen *et al.* 2009). *E. coli* Nissle 1917, the active compound of the preparation Mutaflor, is proven to be effective in maintaining remission in UC (Rembacken *et al.* 1999), enhances mucosal integrity (Ukena *et al.* 2007), and its effect on barrier-relevant TJ proteins is presently under investigation by our group with experimental evidence for a TJ effect (Hering *et al.* 2011c).

Conclusion

Disturbed barrier function is a key feature in IBD. The role of the microbiota and genetic disposition has been discussed to be pivotal for the aetiology of IBD but the formal pathogenesis remains to be elucidated. Misleading bacterial signalling results in a chronic inflammatory state, which can lead to barrier dysfunction represented, for example, by alterations in epithelial TJs. Genetic predisposition may influence the reactivity of the mucosal immune system and of the epithelium in IBD. In particular, the identification of single TJ components such as the claudins or tricellulin and our growing understanding of their functional nature and regulation have provided more insight into these mechanisms and will allow us to identify therapeutic interventions. The schematic diagram in Fig. 4 summarizes the main aspects of inflammatory barrier dysfunction, including our present understanding of the underlying pathomechanisms and influence of therapeutic components.

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