The intestinal epithelial barrier in health and disease

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Meir M, Flemming S, Burkard N, Wagner J, Germer C, Schlegel N. The glial cell-line derived neurotrophic factor: a novel regulator of intestinal barrier function in health and disease. Am J Physiol Gastrointest Liver Physiol 310: G1118–G1123, 2016. First published May 5, 2016; doi:10.1152/ajpgi.00125.2016.—Regulation of the intestinal epithelial barrier is a differentiated process, which is profoundly deranged in inflammatory bowel diseases. Recent data provide evidence that the glial cell line-derived neurotrophic factor (GDNF) is critically involved in intestinal epithelial wound healing and barrier maturation and exerts antiapoptotic effects under certain conditions. Furthermore, not only the enteric nervous system, but also enterocytes synthesize GDNF in significant amounts, which points to a potential para- or autocrine signaling loop between enterocytes. Apart from direct effects of GDNF on enterocytes, an immunomodulatory role of this protein has been previously assumed because of a significant reduction of inflammation in a model of chronic inflammatory bowel disease after application of GDNF. In this review we summarize the current knowledge of GDNF on intestinal epithelial barrier regulation and discuss the novel role for GDNF as a regulator of intestinal barrier functions in health and disease.

differentiation; enterocyte; GDNF; intestinal barrier; junction

The Intestinal Epithelial Barrier in Health and Disease

The intestinal wall is covered by a monolayer of different, polarized, and highly specialized cells including enterocytes, as well as the number of patients in whom this therapy fails (24, 41). Importantly, it was previously shown that complete regeneration of the intestinal mucosa, called “mucosal healing,” predicts long-term remission and clinical improvement of patients suffering from IBD (40).

There is evidence that the enteric nervous system (ENS) and factors secreted by the ENS play an important role in the regulation of IEB (38). The ENS consists of neuronal and glial cells that are arranged in two plexuses: the myenteric plexus and submucosal plexus. It is well known that neuronal effector proteins are involved in the regulation of IEB. Acetylcholine, vasoactive intestinal peptide, and nitric oxide influence mucosal blood flow, lymphocyte migration, mucus secretion, and resorption of nutrients (26, 39). Overall, it has been demonstrated that activation of enteric neurons results in a stabilization of IEB.

Recently, the neutrophic factor “glial cell line-derived neurotrophic factor” (GDNF), which is usually secreted by enteric glial cells, was identified to be a novel regulator of intestinal barrier functions. Additionally, a protective effect on the intestinal epithelial barrier was reported in an animal model of IBD that points to a potential role of GDNF in the pathogenesis of these complex diseases (59). In this review we aim to summarize and discuss the current knowledge on the effects of GDNF in intestinal barrier regulation and its potential role in the contribution to the pathogenesis of IBD.

The Intestinal Epithelial Barrier in Health and Disease

The intestinal wall is covered by a monolayer of different, polarized, and highly specialized cells including enterocytes,
goblet cells, Paneth cells, enteroendocrine cells, M cells, and neuroendocrine cells. There are two key events that are involved in the maintenance of the intestinal barrier.

One key factor in maintaining IEB is its ability for constant regeneration following mechanical, chemical, or inflammatory damage. This regeneration of the enterocyte monolayer depends on a constant regeneration from proliferative stem cells in the crypts. Under physiological conditions the epithelial monolayer is renewed every 5–7 days (44). This regeneration process begins with the migration of neighboring cells over the injured area, which is known as epithelial restitution (6). Additionally, the barrier depends on the proliferation and differentiation of new cells to compensate the loss of cells within the wounded areas. It is known that in IBD this regeneration of the epithelial wound is compromised. On the one hand inflammatory cytokines, like tumor necrosis factor (TNF)-α, increase the rate of cell shedding at the villi. This leads to gaps in the intestinal wall, which cannot be sealed by physiological restitution (31). On the other hand there is an increased rate of spontaneous apoptosis of enterocytes (30). Finally, it is known that the transmural inflammation in CD leads to a direct damage in the stem cell population and thus compromises the renewal of IEB (35).

The other key factor for the maintenance of the IEB is the integrity of the terminal bar. The terminal bar consists of different junctional proteins that seal the paracellular pathway. Tight junctions are formed by the transmembrane proteins occludin and different claudins, which are amended by junctional adhesion molecules (JAMs) and tricellulin. These proteins are linked to the actin cytoskeleton via a cytoplasmic plaque of zona occludens proteins (ZO-1, ZO-2, and ZO-3). Similarly, the adherens junctions are connected to the actin cytoskeleton. E-cadherin mediates mechanical stability and is aligned with actin filaments through intracellular components, such as p120 catenin, β-catenin, and α-catenin. Most basal are the desmosomes, which consist of the transmembrane proteins desmocollin 2 and desmoglein 2 in the intestinal barrier. These are linked to the intermediate filament system via desmoplakin, plakoglobin, and plakophilin.

Many sophisticated reviews have described the outstanding importance of tight junctions for IEB function (43, 48, 58). In brief, tight junctions can be regulated in three different ways: First, the composition of claudins can change IEB function. There are 24 different claudins that determine paracellular permeability based on charge and size of the molecules (17, 33, 56). There are “tight” claudins, i.e., claudin 1, 3, 5, and 8, and claudins that are described as more leaky, such as claudin 2, 10, and 15. It has been reported that in the human intestine the claudins 1, 2, 3, 4, 5, 15, and 20 are present in tight junctions (25, 34). In IBD, the cytokine IL-13 increases paracellular flux by an upregulation of the leaky claudin 2 (42), as well as a TNF-α-induced reduction of sealing claudins: claudins 3, 5, and 8 (67). Secondly, the distribution of tight junctions can be altered. In healthy small intestine tissue tight junctions are regularly distributed and build continuous strands at the cell borders. In the tissue of patients suffering from CD there are fewer tight junction strands and there are breaks within the strands (49). Thirdly, changes in contraction of the actin cytoskeleton can alter tight junction permeability. Important regulators of the actin cytoskeleton are the Rho GTPases RhoA, Rac1, and Cdc42. In our laboratory we demonstrated that activation of Rac1 and Cdc42 led to a stabilization of IEB, whereas a balanced activation of RhoA is needed, since activation, as well as inactivation, of RhoA led to destabilization of intestinal barrier function (47). This may be relevant in the pathogenesis of IBD, since Rac1-deficient mice develop a severe colitis after citrobacter infection (14) and Rac2 has been identified as one of the 100 gene loci that are associated with IBD (16). Another important regulator of the actin cytoskeleton is the myosin light chain kinase (MLCK). In IBD, TNF-α leads to an upregulation of MLCK (21). This leads to a perijunctional contraction of the actin cytoskeleton and, consequently, to barrier destabilization (11).

Whereas tight junctions seal the barrier, adherens junctions provide mechanical strength and contribute to tissue morphogenesis, migration, and the establishment of cell polarity (28). Recently, it was demonstrated that desmosomal integrity, i.e., desmoglein 2-mediated adhesion, is essential to maintain IEB function (46). Furthermore, a loss of desmoglein 2 was found in patients suffering from CD. The loss of desmosomal adhesion in IBD was paralleled by a reduction of claudin 1 and an upregulation of claudin 2 (51). Importantly, the activity of the p38 MAPK pathway is critically involved in desmosomal integrity, which makes it a potent regulator of intestinal epithelial integrity.

Effects of Enteric Glial Cells on Intestinal Epithelial Barrier Function

Enteric glial cells (EGC), as an independent cell population, have first been described by Gabella in 1971 (18). Immunostaining revealed that EGC express s100β and glial fibrillary acidic protein (GFAP). S100β is constitutively expressed by EGC and seems to play an important role in IBD. It has been demonstrated that immunostaining of s100 is increased in rectal specimen of patients suffering from UC compared with healthy controls and an upregulation of s100β is accompanied by enhanced mucosal NO levels similar to mucosa from Crohn patients (10, 57). EGC share more similarities with astrocytes in the central nervous system than with Schwann cells of the peripheral nervous system (15, 29). Like astrocytes, EGC seem to have an immunological function and transfer information to the enteric neurons. EGC interact with T lymphocytes and express major histocompatibility complex (MHC) class II. This expression of MHC class II is upregulated in patients suffering from CD (20, 32).

A potential influence of EGC on intestinal epithelial barrier integrity remained unclear for many years. Although most of the EGC are located in the submucosal and myenteric plexus, some of them are in close proximity of the intestinal crypts and even reach the basal membrane and the capillaries of the intestinal mucosa (23). Evidence for an influence of EGC on the IEB came from the observation that a toxic or autoimmune ablation of some enteric glial cells leads to a breakdown of intestinal barrier function in mice (9, 12). Interestingly, immune-mediated glial alteration in a transgenic mouse model by Cornet et al. (12) led to spontaneous fulminant jejunoileocolitis with a submucosal vasculitis similar to that seen in CD. Additionally, a coculture of EGC with intestinal epithelial cells increased IEB function (45). Furthermore, there is a reduced distribution of enteric glial cells in uninflamed colon segments of patients suffering from IB compared with control popula-
tions, suggesting that enteric glial cells could play a role in the pathogenesis of intestinal barrier defects in CD or UC (60). All these observations suggest a close interaction between EGCs and enterocytes. However, it is still not clear whether these effects of EGC are direct effects on enterocytes or indirect effects via activation or inactivation of enteric neurons. It has been speculated that a possible effect of EGC could be via stimulation of enteric neurons, since neuronal effectors such as acetylcholine, vasoactive intestinal peptide, and nitric oxide are known key regulators of IEB (26, 38). However, there is upcoming evidence showing that EGC directly regulate IEB.

GDNF Directly Promotes Barrier Maturation and Proliferation of Enterocytes and Exerts Antiapoptotic Effects

The main mediator secreted factor by EGC is GDNF. GDNF is a disulfide-linked homodimeric with a molecular weight of 30.4 kDa. It belongs to the cysteine-knot superfamily of growth factors that assume stable dimeric protein structures. GDNF signals through a multicomponent receptor system, composed of the RET (rearranged during transfection) receptor and one of the four GFRα (α1–α4) (GDNF family) receptors. Activation of the receptor complex in neurons can lead to modification of various signaling events, including p38 MAPK, ERK, protein kinase B (AKT), and PKA (2, 22, 54). GDNF has been intensively studied as a potential therapeutic agent for neurodegenerative diseases, such as Parkinson’s disease (37, 55, 64, 66). In models of neurodegenerative diseases GDNF has positive effects on proliferation of neurons and is necessary for the maintenance of neuronal morphology (7). Additionally, GDNF receptors RET and GFRα1–4 are expressed in CD4+ and CD8+ T cells, B cells, and monocytes, indicating a potential effect of GDNF on immune cells. Although it has been demonstrated that RET/GFRα signaling is dispensable for thymic T cell development (3), application of GDNF led to a decrease in TNF-α secretion in monocytes (59). Furthermore, GDNF directly influences the blood-brain barrier by upregulation of the tight junction protein claudin 5, which leads to barrier-stabilizing effects (50, 63).

In recent years it was consistently reported, that reduced levels of GDNF lead to morphological and functional abnormalities of IEB in mice comparable to the changes known in IBD (8, 61, 68). Conversely, it was shown that GND immunoreactivity was upregulated in experimental colitis and in specimen of patients with IBD, which was an effect predominately observed in enterocytes (52). In the latter study GDNF protected enterocytes from apoptosis via activation of p42 MAPK and AKT, which led to the suggestion that upregulation of GDNF in response to inflammation may be part of a rescue mechanism in IBD. On the other hand, none of the proinflammatory cytokines stimulated an upregulation of GDNF (52). A recent study demonstrated that both EGCs and enterocytes express GDNF in significant amounts under physiological conditions (36). However, the stimuli leading to GDNF expression in enterocytes still remain unclear. In this context it was shown that Toll-like receptor (TLR)-2 in EGC is required to secrete GDNF and, thereby, maintains the integrity of the ENS. TLR-2 is activated by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns molecules (DAMPs), which are closely linked to the pathogenesis of IBD (62). TLR-2-deficient mice showed profound alterations within the ENS with an increased susceptibility to inflammatory stimuli and a consecutive loss of intestinal barrier functions. The intestinal barrier was restored by the application of GDNF in this study. The underlying mechanism was attributed to a reconstitutive effect of GDNF on the ENS rather than on direct effects of GDNF on the intestinal epithelium (8).

Fig. 1. Effects of GDNF on intestinal barrier regulation are illustrated. GDNF is synthesized by EGC in TLR-2-dependent manner and by enterocytes. Enterocytes express the GDNF receptor GFRα and RET receptor (GR). GDNF has effects on proliferation of enterocytes and thereby promotes epithelial wound healing. GDNF promotes tight junction differentiation and has antiapoptotic in enterocytes. Additional barrier-protective effects can be assumed from the immunomodulatory effects by GDNF.
Based on these observations in EGC, it can be speculated that GDNF synthesis is also TLR-2 dependent in enterocytes. This, however, remains to be evaluated. In addition, it has been shown that TLR-2 is involved in regulation of tight junction integrity, as well as in cytokine production in enterocytes. Interestingly, TLR-2 is reduced in patients with IBD (1).

Zhang and coworkers (68) showed that application of recombinant adenoviral vectors encoding GDNF in a model of experimental colitis significantly reduced inflammation and loss of intestinal barrier functions. On the one hand, the underlying mechanism was attributed to anti-inflammatory effects, since GDNF blocked the secretion of various proinflammatory cytokines, such as TNF-α and interleukin-1β. On the other hand, the antiapoptotic effects of GDNF in enterocytes by activation of AKT, already described by Steinkamp and coworkers (52), were confirmed. Similarly, antiapoptotic effects of GDNF were observed in a model of acute intestinal ischemia. Additionally, GDNF prevented the hypoxia-induced downregulation of ZO1 (65). More evidence arguing for direct effects of GDNF on enterocytes derived from the observation that enterocytes express the RET receptor, as well as GDNF-susceptible receptors GFRα1 and GFRα2 (36, 52). A recent study demonstrated that GDNF has direct effects on enterocytes, especially under conditions of immature barrier functions, which are usually seen in the crypts of the intestine. In the mammalian intestinal crypt tight junction morphology is scattered (4, 19) and the permeability of different tracers suggests that intestinal villi have a huge number of narrow tight junctions, whereas junctions in the crypts are more leaky (27). These observations emphasize the need to investigate cells at different stages of differentiation to fully understand the function of the IEB. Under immature barrier conditions, GDNF led to a faster differentiation and maturation of enterocytes by affecting tight junction integrity. In an in vitro model the IEB tight junction proteins claudin 1, claudin 5, and occludin showed an augmented and more linear staining pattern following GDNF application, whereas overall concentrations of these proteins were not altered. This was shown to be mediated by the p38 MAPK pathway due to a significant dephosphorylation of p38 MAPK after the application of GDNF in vitro (36). The latter mechanism has been previously shown to stabilize the intestinal epithelial barrier (51). Additionally, GDNF induced a cAMP/PKA-dependent increase of proliferation and augmented epithelial wound healing. An important observation was that under mature barrier conditions, as they are usually seen in the villi, GDNF did not alter IEB function or proliferation (36).

**Outlook: Loss of GDNF and/or Compromised GDNF Signaling May Contribute to the Pathogenesis of IBD**

In summary, there is increasing evidence showing GDNF as an important regulator of intestinal epithelial barrier functions by promoting epithelial wound healing and tight junction differentiation in enterocytes and by inhibiting apoptosis in intestinal epithelium. Additional barrier-protective effects can be assumed from the immunomodulatory effects by GDNF. Apart from these effects, it is obvious that GDNF targets major regulatory mechanisms, which are consistently reported to be involved in the loss of intestinal barrier functions in IBD: 1) In enterocytes of patients with IBD the typical contraction of the cortical actin-myosin ring is present as a consequence of increased p38 MAPK phosphorylation (13, 21). 2) A constant increase of apoptosis and cell shedding is known in patients with IBD (30). 3) It is known that epithelial wound healing is significantly compromised in patients with IBD (53) (Fig. 1). All of these events significantly contributing to the loss of intestinal epithelial barrier functions in IBD could potentially be abrogated by GDNF. Therefore, it can be discussed whether changes in GDNF expression and/or defects in GDNF-mediated signaling events are critically involved in the pathogenesis of IBD. Furthermore, it should be evaluated whether GDNF could be used as therapeutic agent in patients with IBD.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

M.M. and N.S. analyzed data; M.M. and N.S. prepared figures; M.M., S.F., N.B., J.W., C.-T.G., and N.S. edited and revised manuscript; M.M., S.F., N.B., J.W., C.-T.G., and N.S. approved final version of manuscript.

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