The digestive neuronal–glial–epithelial unit: a new actor in gut health and disease

Michel Neunlist, Laurianne Van Landeghem, Maxime M. Mahé, Pascal Derkinderen, Stanislas Bruley des Varannes and Malvyne Rolli-Derkinderen

Abstract | The monolayer of columnar epithelial cells lining the gastrointestinal tract—the intestinal epithelial barrier (IEB)—is the largest exchange surface between the body and the external environment. The permeability of the IEB has a central role in the regulation of fluid and nutrient intake as well as in the control of the passage of pathogens. The functions of the IEB are highly regulated by luminal as well as internal components, such as bacteria or immune cells, respectively. Evidence indicates that two cell types of the enteric nervous system (ENS), namely enteric neurons and enteric glial cells, are potent modulators of IEB functions, giving rise to the novel concept of a digestive 'neuronal–glial–epithelial unit' akin to the neuronal–glial–endothelial unit in the brain. In this Review, we summarize findings demonstrating that the ENS is a key regulator of IEB function and is actively involved in pathologies associated with altered barrier function.

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Introduction

A growing body of evidence demonstrates that correct functioning of the intestinal epithelial barrier (IEB) is crucial to ensure health. The IEB has to fulfill two major tasks that can seem paradoxical—it must enable the absorption of nutrients whilst at the same time controlling the passage of pathogens or toxins. This multitasking ability is permitted by the structural organization and compartmentalization of the intestinal epithelium. Regulation of the IEB is highly modulated by components of its 'outer' microenvironment (microflora, for example) and 'inner' microenvironment (immune cells, fibroblasts or the enteric nervous system [ENS]).

The ENS is composed of two major cellular components: neurons and glial cells. The ENS coordinates major gastrointestinal functions (including motility, electrolyte secretion and vascular blood flow), and emerging data suggest that it is also able to regulate key functions involved in the maintenance of IEB homeostasis such as paracellular or transcellular permeability, intestinal epithelial cell proliferation and wound healing. In this Review, we summarize current studies characterizing neuronal and glial effects upon key functions involved in the maintenance and repair of the IEB. We also present evidence showing that the dysfunctions of the IEB observed in various diseases could be associated with ENS neuropathies. Finally, we suggest that targeting the ENS might be of future therapeutic interest in the treatment of diseases associated with IEB dysfunctions.

INSERM UMR913, Institut des Maladies de l'Appareil Digestif, Université de Nantes, CHU Hôtel Dieu, 1 place Alexis Ricordeau, 44093 Nantes, France (**M. Neunlist**, **L. Van Landeghem**, **M. M. Mahé**, **P. Derkinden**, **S. Bruley des Varannes**, **M. Rolli-Derkinderen**).

Correspondence to: M. Neunlist michel.neunlist@univ-nantes.fr

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The intestinal epithelial barrier

Physiological role of the IEB

The IEB is composed of a monolayer of intestinal epithelial cells organized into invaginations (termed crypts) and finger-like projections (called villi) in the small intestine, and into a succession of crypts alternating with a flat epithelial surface in the colon. Crypts represent the proliferation compartment whereas the villi (or the epithelial surface in the colon) are the differentiation compartment. All intestinal epithelial cells arise from intestinal stem cells that are located at the base of crypts. These cells give rise to rapidly dividing daughter cells or progenitors that migrate along the crypt–villus axis. Intestinal stem cells give rise to all intestinal epithelial lineages, that is, enterocytes, enteroendocrine cells and goblet cells, as well as Paneth cells in the small intestine.[1]

A key process involved in the maintenance of the IEB is its ability to repair following infectious or chemical insults or mechanical injury during peristalsis. Wound healing of the IEB involves a cascade of processes aimed at rapidly resealing the epithelial lining. During the early phases of repair, intestinal epithelial cells adjacent to the injured surface spread and migrate to cover the denuded area; this process has been termed epithelial restitution (reviewed elsewhere[2,3]). Later in the repair process, proliferation of intestinal epithelial cells occurs to compensate for loss, followed by maturation and differentiation of these cells.[4]

IEB functions are the result of an extrinsic as well as an intrinsic barrier. The extrinsic barrier arises as a result of the concomitant secretion of electrolytes, mucus and antimicrobial peptides by enterocytes, goblet cells and Paneth cells, respectively. Together these secretory products form a sterile mucus layer that is the first line of

defence against pathogens present within the gut lumen.[5] Besides this extrinsic rampart, the IEB also forms a physical barrier consisting of the lining of intestinal epithelial cells, which establish strong and elaborate contacts with the underlying extracellular matrix.

Molecular regulators of IEB functions

The structural organization of the IEB consists mainly of three junctional complexes linking adjacent intestinal epithelial cells (Figure 1).[6] Tight junctions are the most apical intercellular protein complex formed by transmembrane proteins, such as claudins, occludin and tricellulin, which are connected to the actin cytoskeleton via a cytoplasmic plaque including zona occludens (ZO-1, ZO-2 and ZO-3). The transmembrane receptor JAM (junctional adhesion molecule) is also found at tight junctions and is engaged in homophilic or heterophilic binding with other adhesion molecules such as integrins.[7,8] Adherent junctions are multiprotein complexes composed of the transmembrane protein E-cadherin and intracellular components such as p120 catenin, β-catenin and α-catenin, which link the adherent junction to the actin cytoskeleton. Adherent junctions are located directly beneath tight junctions and are necessary for tight junction assembly.[9] Finally, desmosomes are junctional complexes of transmembrane proteins (desmoglein and desmocollin) that connect keratin filaments of neighbouring intestinal epithelial cells via desmoplakin. These junctional complexes are located along the lateral membranes beneath adherent junctions. Tight junctions are responsible for the sealing of the intercellular space and regulate the paracellular passage of particles, whereas adherent junctions and desmosomes are strong adhesive bonds between intestinal epithelial cells that confer mechanical strength to the IEB.

Current views suggest that paracellular permeability is the result of two independent pathways. The first route is charge-selective and regulates the passage of small-sized solutes (less than 4 Å).[10–12] This first pathway is primarily regulated by tight-junction-associated claudin proteins, which create an electrostatic selectivity filter with characteristics that vary according to the members of the claudin family present within the tight junction.[13] The second pathway is used by large solutes and shows no charge discrimination.[11,14,15] This 'leak' pathway is thought to occur at contact points involving more than two intestinal epithelial cells[16] or to result from larger pores. Paracellular permeability is regulated by tight junction and adherent junction affiliation to the F-actin cytoskeleton, processes involving key enzymes such as myosin light chain kinase (MLCK),[17,18] RhoA and Rac1,[19,20] CDC42[21,22] and protein kinase C (PKC).[23] It is also modulated by post-translational changes of key tight-junction-associated proteins,[24] such as phosphorylation of ZO-1,[25–27] occludin[28] or claudins.[29] Paracellular permeability can also be regulated by endocytotic shuttling of occludin and claudins.[30] Long-term changes in paracellular permeability can be induced by transcriptional regulation of key molecular components of tight junctions.[31–33] Under physiological conditions paracellular flux of large molecules is very limited and passage of such molecules occurs mainly via transcellular routes regulated by endocytotic and transcytotic pathways.[34]

Key points

- Altered functioning of the intestinal epithelial barrier (IEB) has a central role in the aetiology of a wide range of diseases; efficient IEB healing is essential to maintain IEB homeostasis
- An anatomical unit comprised of enteric neurons, enteric glial cells and intestinal epithelial cells sets the basis for a functional digestive neuronal–glial–epithelial unit
- Enteric neuromediators as well as gliomediators can differentially modulate major IEB functions such as paracellular permeability, intestinal epithelial cell proliferation and wound healing
- Changes in the phenotype of enteric neurons and glial cells occur in various diseases but the involvement of the enteric nervous system (active or bystander) in these pathologies remains to be defined

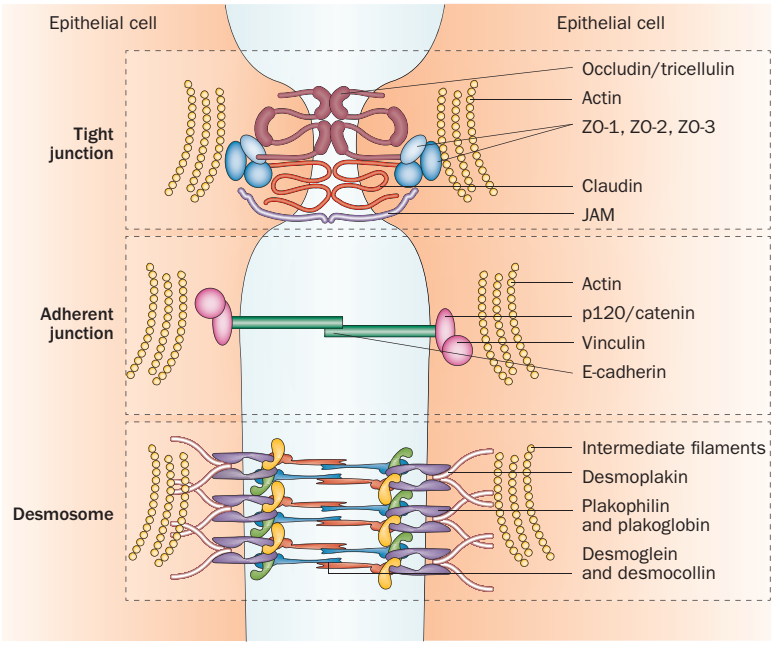

Figure 1 | Junctional complexes regulating epithelial cells interactions. Tight junctions, adherent junctions and desmosomes are the three main junctional complexes connecting adjacent epithelial cells. Tight junctions, which are the most apical protein complexes, seal the intercellular space and regulate intestinal epithelial barrier paracellular permeability, that is, the passage of molecules and/or particles between two epithelial cells. Adherent junctions and desmosomes anchor epithelial cells to one another and confer mechanical strength to the intestinal epithelial barrier. The protein components of these junctional complexes can be targeted by the enteric nervous system to regulate epithelial permeability, wound healing and mechanical strength. Abbreviations: JAM, junctional adhesion molecule; ZO, zona occludens.

Modifications of the IEB in health and disease

Important changes in barrier functions occur during key periods of life. In particular, the postnatal period is associated with important changes in paracellular permeability. After birth, individuals with high intestinal permeability are at risk of excessive passage of toxins and development of necrotizing enterocolitis.[35] However, high permeability also seems to be necessary for the development of oral tolerance and proper maturation

of the immune system.[36–38] In pigs, ileal paracellular permeability follows a bell-shaped curve with an initial increase followed by a decrease concomitant with the weaning period.[39] In addition, a mouse study has shown that major changes in tight junction mRNA expression occur during the postnatal period.[39]

In adult humans, increasing evidence suggests that altered IEB functions have a central role in the aetiology and/or pathophysiology of a wide range of digestive and extradigestive diseases,[38] such as type 1 diabetes mellitus,[40] rheumatic diseases[41] and autism.[42] Alterations of the IEB include increased paracellular and transcellular permeability, as well as reduced wound healing abilities leading to a 'leaky gut'. Therefore, therapeutic approaches aimed at enhancing and/or restoring IEB functions might be of major interest for the prevention of various chronic diseases.

Changes in IEB permeability

Increased permeability of the IEB is a common and key feature of several inflammatory digestive diseases. In patients with Crohn's disease, increased permeability has been observed in inflamed areas[43] and in noninflamed areas following a luminal stimulus.[44] In addition, an increase in intestinal permeability can occur in asymptomatic first-degree relatives of patients with Crohn's disease[45] and often precedes clinical relapse.[46–48] Similarly, increased small intestinal permeability occurs in patients with ulcerative colitis in remission as well as in first-degree relatives of these patients.[49] Increased transcellular permeability has also been reported in Crohn's disease[43] and ulcerative colitis.[50,51] Even if increased paracellular permeability is insufficient to induce colitis alone,[18] it could have a role in the initiation of inflammation, which might then be amplified and/or sustained owing to dysregulated immune responses in patients with IBD. In SAMP1/YitFc mice (a model of Crohn's-disease-like ileitis), the increased permeability associated with altered IEB function was shown to be the primary trigger initiating ileitis.[52,53] In *IL10*$^{-/-}$ mice, increased paracellular permeability was shown to precede the development of inflammation.[54] Interestingly, reducing paracellular permeability with inhibitors of the zonulin pathway led to a reduced inflammatory response in the *IL10*$^{-/-}$ model.[55] This finding further demonstrates the potential for targeting paracellular permeability for the treatment of IBD.

In patients with IBS, increased paracellular permeability was found to be positively correlated with visceral hypersensitivity.[56] In animal models of IBS, reducing paracellular permeability with an MLCK inhibitor[57] or using probiotic treatment[58] increased expression of tight junction proteins and reduced visceral hypersensitivity. Alternatively, modulating luminal protease content with a protease inhibitor in a mouse model of IBS has been proven to be of therapeutic interest, probably by preventing protease-induced barrier dysfunctions.[59]

Changes in IEB repair

Besides increased IEB permeability, other functions of the IEB are altered during IBD, such as defects in wound healing.[60] Efficient mucosal healing is an indicator of a good prognosis for the outcomes of Crohn's disease as it correlates with long-term remission and reduces relapse frequency and the need for surgery.[61–63] Therefore, approaches aimed at reinforcing or re-establishing IEB functions could be of interest both for the prevention of relapses, and for the treatment of IBD-associated barrier dysfunction. For instance, suppressing MLCK activity could prevent barrier dysfunction and promote mucosal wound healing.[64]

Regulation of IEB functions by the ENS

Organization of the ENS

Emerging data have identified the enteric nervous system (ENS) as a key regulator of IEB functions. The ENS is composed of >100 million enteric neurons and 400 million enteric glial cells, which are distributed along the digestive tract and organized into two major ganglionated plexi—the myenteric plexus (or Auerbach's plexus) and the submucosal plexus (or Meissner's plexus) (Figure 2).[65] Neurons of the myenteric plexus control the motor activity of the gut whereas those of the submucosal plexus regulate mucosal processes.[66] In contrast to the innervation of other organs, the ENS is capable of regulating digestive functions independently of the central nervous system (CNS). However, the CNS can modulate the activity of enteric neurons and thereby affect gastrointestinal functions.[67]

Enteric glial cells were identified in 1899 by Dogiel[68] as nucleated satellite cells in the proximity of enteric neurons. Although their contribution in maintaining gut homeostasis is increasingly acknowledged, their functions remain largely unexplored (reviewed elsewhere[69]). In the 1970s, electron microscopy revealed that enteric glial cell structures are more reminiscent of astrocytes of the CNS than Schwann cells of peripheral ganglia.[70,71] Enteric glial cells located within ganglia harbour short processes whereas those located along fibre tracts exhibit longer processes.[72] Immunohistochemical methods have shown that enteric glial cells express glial fibrillary acidic protein (GFAP)[73] and S100β,[74] which are two proteins that are also expressed by astrocytes of the CNS. In addition, mature enteric glial cells also express SOX10.[75,76] The entire myenteric population of enteric glial cells does not express all three markers at the same level, which could enable the definition of glial subpopulations, but whether each of these subtypes has an associated physiological role remains to be determined.[77] Existence of different glial subpopulations is also suggested by the existence of differences in morphology between enteric glial cells depending on whether they are mucosal, intraganglionic (myenteric or submucosal) or intramuscular.[69]

The digestive neuronal–glial–epithelial unit

Studies using neuronal retrograde tracer dye have demonstrated that the mucosa is highly innervated by submucosal and myenteric neurons (Figure 2). In the guinea pig small intestine and colon, each villus is innervated by 70–92 submucosal neurons.[78] A similar level of innervation has been reported in the human colon.[79]

Axons that innervate the intestinal mucosa contain a wide array of neuromediators such as acetylcholine, vasoactive intestinal peptide (VIP), substance P and neuropeptide Y. A predominant VIPergic innervation of the mucosa is observed in guinea pig small intestine[80] and colon.[81] In the human colon, the proportion of VIPergic submucosal neurons innervating the mucosa ranges from 40–80%.[82,83]

Regarding enteric glial cells, quantification of their density has not been reported to date. However, a dense network of S100β-positive enteric glial cells is observed along the crypt axis with a higher density at the base of the crypt compared with the villus[84,85] (Figure 2). The close proximity (in the range of 1 μm) between enteric glial cells, axons and intestinal epithelial cells revealed by electron microscopy sets the anatomical basis for paracrine communication between cells.[85,86] This anatomical unit associating enteric neurons, enteric glial cells and intestinal epithelial cells can be considered as a neuronal–glial–epithelial unit, which from an organizational point of view is reminiscent of the neuronal–glial–endothelial unit of the blood–brain barrier.[87]

Neuronal control of IEB functions

Effect on IEB permeability

Overall, activation of enteric neurons has been shown to result in the reinforcement of IEB functions. In particular, an *in vitro* study using a co-culture model of human submucosa and intestinal epithelial cell monolayers demonstrated that electrical stimulation of the ENS reduces paracellular permeability.[79] Interestingly, vagus nerve stimulation, which ultimately activates enteric neurons,[88] also exerts protective effects on IEB function. In particular, electrical or nutritional activation of the vagus nerve prevents alterations of paracellular permeability in models of septic shock,[89] colitis[90] or burn-induced IEB dysfunctions.[91] Similarly, sacral nerve stimulation has also been shown to reduce paracellular permeability in pigs.[92] However, earlier studies suggested that vagal stimulation increases intestinal epithelial permeability, resulting in the passage of serum proteins into the lumen, potentially owing to activation of paracellular pathways.[93] Whether vagal effects on the IEB result from its direct effect on intestinal epithelial cells or via modulation of intestinal inflammation,[94] or both, remains unknown.

Enteric neuromediators can exert different effects on IEB functions (Figure 3). Acetylcholine represents the prototypical neuromediator increasing both paracellular[95] and transcellular permeability.[96] The increased permeability observed in animal models of maternal separation is associated with an increase in choline acetyltransferase (ChAT) expression and can be prevented by muscarinic and nicotinic antagonists.[97] A bile-induced increase in paracellular permeability is also mediated by muscarinic and nicotinic pathways.[98] However, cholinergic pathways can also activate eosinophils and mast cells to mediate colonic mucosal barrier dysfunction in ulcerative colitis.[99] In addition to acetylcholine, other neuromediators, such as substance P, can also increase paracellular permeability. Indeed, perfusion of neurokinin A induces a rapid increase in paracellular permeability in rats.[100] In a clinical trial, treatment of patients who had diarrhoea-predominant IBS with a neurokinin antagonist was able to substantially reduce pain and/or discomfort, which are symptoms that are associated with increased permeability.[101]

VIP is increasingly being recognized as a key enteric neuromediator involved in the maintenance of IEB functions. Indeed, reduced paracellular permeability induced by electric stimulation of enteric neurons is prevented by VIP antagonists,[79] and VIP treatment induces a decrease in paracellular permeability in different intestinal epithelial cell lines.[79,95,102] VIP also prevents and/or reduces the increase in paracellular permeability induced either by neuromediators (such as substance P),[100] hypotonic solution,[103] inflammatory mediators, or by pathogens such as *Citrobacter rodentium*.[104] Besides its ability

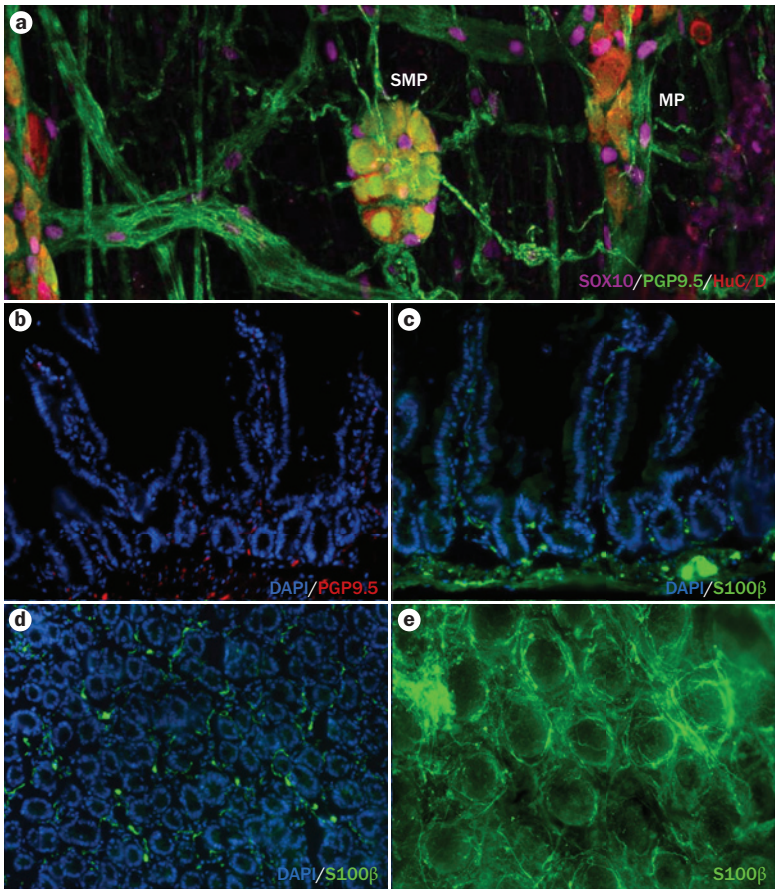

Figure 2 | The ENS forms a complex network in close proximity to the IEB. **a** | Immunohistological staining of whole thickness mouse colon for Sox10 glial marker (pink) and PGP9.5 (green) and HUC/D neuronal markers (red) reveals the complex tri-dimensional organization of the ENS (MP, myenteric plexus; SMP, submucosal plexus). **b** | Mouse small intestinal transverse section staining with PGP9.5 neuronal marker (red) and DAPI (blue) reveals enteric neurons mainly around the crypts. **c** | Mouse small intestinal transverse section staining with S100β glial marker (green) and DAPI (blue) show enteric glial cells at the base of the crypts as well as along the villi. **d** | Mouse small intestinal *en face* section staining with S100β glial marker (green) and DAPI (blue) reveals enteric glial cell organization around the crypts. **e** | Human colonic mucosa *en face* section staining with S100β marker demonstrates the enteric glial cell organization around the crypts. Abbreviations: ENS, enteric nervous system; IEB, intestinal epithelial barrier.

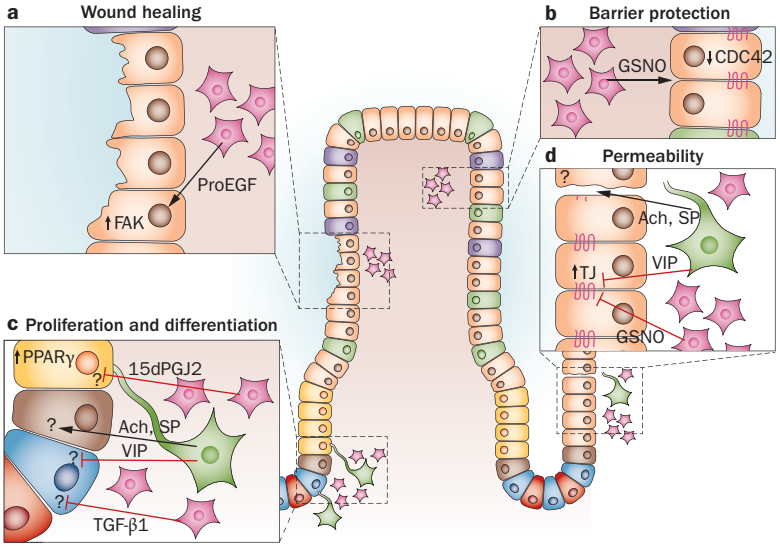

Figure 3 | Soluble factors produced by the ENS regulate IEB functions. Enteric neurons (green) and glial cells (pink) produce soluble factors that have differential effects on different intestinal epithelial cell types (enterocytes in light brown, intestinal stem cells in blue; Paneth cells in red, enteroendocrine cells in violet and goblet cells in light green), thereby regulating IEB proliferation, differentiation, healing, permeability and protection. **a** | Wound healing. Enteric glial cells can enhance wound healing via the release of proEGF, leading to increased activity and expression of FAK. **b** | Barrier protection. During infection by pathogens such as *Shigella flexneri*, enteric glial cells release GSNO leading to reduced CDC42 expression and enhanced intestinal barrier resistance. **c** | Proliferation and differentiation. Neurons and glial cells release mediators (such as VIP, or TGF-β1 and 15dPGJ2, respectively) that inhibit intestinal cell proliferation. Conversely, neuromediators (Ach and SP) can increase intestinal cell proliferation. **d** | Permeability. Enteric neuromediators can differentially regulate paracellular permeability—VIP reduces paracellular permeability, while Ach increases it. GSNO from enteric glial cells can also reduce paracellular permeability by increasing the expression of key tight junctions associated proteins such as ZO-1. Abbreviations: Ach, acetycholine; ENS, enteric nervous system; FAK, focal adhesion kinase; GSNO, S-nitrosoglutathione; IEB, intestinal epithelial barrier; PPARγ, peroxisome proliferator-activated receptor γ; SP, substance P; TJ, tight junction proteins; VIP, vasoactive intestinal peptide.

to regulate IEB paracellular permeability, VIP also exerts general protective effects in the intestine via immunomodulatory actions.[105]

Enteric neuromediators can affect paracellular permeability over short or long periods of time via distinct mechanisms. Short-term regulation of paracellular permeability mainly involves post-translational modification of key proteins or enzymes. In particular, in Chinese hamster ovary (CHO-m3) cells, acetylcholine stimulates the phosphorylation of myosin light chains (MLCs) and the formation of myosin-containing stress fibres,[106] although this activity has never been shown in intestinal epithelial cells. Acetylcholine also induces phospholipase A2 phosphorylation, and ultimately activates PKC, leading to increased transcellular permeability.[96] Conversely, VIP has been shown to reduce permeability by reducing MLCK activity.[104] Long-term modification of IEB permeability is associated with the regulation of expression of key tight junction proteins; for instance, VIP has been shown to increase the expression of ZO-1 mRNA and protein in intestinal epithelial cells.[79]

Altogether, enteric neurons have the ability to finely tune intestinal barrier functions via the release of mediators that enhance or reduce IEB permeability over short-term or long-term periods.

Effects on IEB repair and cell proliferation

Similarly to what was discussed for permeability, enteric neurons also have the ability to differentially modulate IEB proliferation and differentiation via the secretion of distinct neuromediators (Figure 3). The trophic effects of glucagon-like peptide 2 (GLP-2) on the intestinal mucosal epithelium have been shown to be mediated by the activation of enteric neurons *in vivo*; however, the neuromediators involved in this process are unknown.[107] In addition, enteric neurons exert direct antiproliferative effects on human intestinal epithelial cells via the liberation of VIP.[108] Conversely, other neuromediators, such as acetylcholine or substance P, stimulate intestinal epithelial cell proliferation.[109,110] A study has demonstrated that serotonergic neurons are able to enhance epithelial growth via the activation of 5-HT_{2A} receptors of cholinergic neurons.[111] Enteric neurons can also produce endocannabinoids, which have been shown to enhance colonic mucosal healing.[112]

The role of enteric neurons in the regulation of IEB repair mechanisms remains largely unknown *in vivo*, although indirect evidence suggests that they might have a positive effect on mucosal healing, given that vagotomy reduces gastric ulcer healing.[113] Whether such effects induced by sensory neuropeptides (such as substance P and calcitonin gene related peptide) reflect a direct impact of the ENS on IEB healing, or the ability of these neuropeptides to activate fibroblast and/or mast cells leading to increased mucosal healing, remains to be fully determined.[114,115]

Glial control of IEB functions

The study of enteric glial cells in gut physiology has been hampered for many years by the lack of tools and models to characterize their functions. In the early 2000s, the role of enteric glial cells in the control of IEB functions started being unravelled, mainly following the development of animal models of glial cell ablation.[116–118]

Effect on IEB permeability

In vivo, ablation of enteric glial cells has been shown to induce a fulminant jejuno-ileitis, which suggests that these cells are essential for the maintenance of IEB integrity. However, the relative contribution of the inflammatory response could not be distinguished from the direct contribution of enteric glial cell ablation to the IEB breakdown.[117,118] The development of other animal models has demonstrated that a moderate loss of enteric glial cells results in an increase in paracellular permeability even in the absence of gut inflammation[116] or prior to the development of intestinal inflammation.[119] However, no change in paracellular permeability was observed *in vivo* following treatment with a glial inhibitor, although a decrease in intestinal transit was reported.[120]

Savidge *et al.*[119] used a noncontact co-culture model of enteric glial cells and a confluent intestinal epithelial cell monolayer, to show that enteric glial cells increased IEB resistance and, concomitantly, reduced paracellular permeability. This effect was associated with upregulation of tight junction protein expression. Interestingly, *S*-nitrosoglutathione (GSNO)—but not reduced or oxidative glutathione—was able to reproduce the effects of enteric glial cells, suggesting the involvement of nitrosylation-dependent pathways in the control of paracellular permeability. GSNO was also able to reduce paracellular permeability in biopsy samples from patients with Crohn's disease[119] but not healthy controls, which suggests that dysregulation in GSNO pathways might have a role in Crohn's disease. The ability of enteric glial cells and GSNO to protect the IEB was further demonstrated in a study showing that enteric glial cells and GSNO prevented *Shigella flexneri*-induced increases in paracellular permeability *in vitro* and mucosal lesions *in vivo*;[121] the protective effects of the enteric glial cells partially resulted from the ability of GSNO to prevent bacterial invasion of intestinal epithelial cells. Indeed, enteric glial cells and GSNO were shown to substantially reduce the expression of small G proteins, such as CDC42, in intestinal epithelial cells. These proteins have a crucial role in the recruitment of the cytoskeleton during the invasion process of intestinal epithelial cells by *S. flexneri*. The effects of GSNO have a bell-shaped distribution, with high concentrations of GSNO demonstrating no protective effects. This observation might reflect the ability of GSNO to act as a nitric oxide (NO) donor, which, at high concentrations, is known to induce barrier damage.[122] Consistent with these findings, a study has shown that following treatment with lipopolysaccharide, the protective effects of enteric glial cells were enhanced by inhibition of inducible nitric oxide synthase.[123] Glial-derived NO has also been shown to regulate ion transport in intestinal epithelial cells.[124] Besides GSNO, other glial-derived mediators might also reinforce the IEB. In particular, glial-cell-derived neutrophic factor (GDNF), which is synthesized and secreted by enteric glial cells, helps maintain mucosal homeostasis during colitis, in part by preventing TNF-induced intestinal epithelial cell death.[125] GDNF can also restore IEB function *in vivo* in dextran sodium sulphate (DSS)-induced colitis,[126] and exerts direct protective effects on neurons,[127,128] thereby further enhancing the protective impact of the ENS on the IEB. Studies suggest that enteric glial cells could be a cellular mediator involved in the prevention of burn-induced IEB dysfunctions following vagal neurostimulation in mice.[91,129]

Effect on IEB repair and cell proliferation

The ability of enteric glial cells to regulate mucosal healing also contributes to its protective effects on the IEB. *In vivo* ablation of enteric glial cells markedly inhibited wound healing after mucosal injury induced either by diclofenac or following DSS treatment.[86] *In vitro*, enteric glial cells stimulated the repair of mechanically induced lesions in confluent monolayers of intestinal epithelial cells. These effects were associated with a massive increase in cell spreading of the intestinal epithelial cells surrounding the lesion.[86] Intestinal epithelial cell spreading and epithelial restitution were mediated, at least in part, by proEGF released by enteric glial cells. In addition, the effects of enteric glial cells on mucosal healing were mediated by an increase in intestinal epithelial cell focal adhesion kinase (FAK) expression and activity (which also has a major role in the regulation of intestinal epithelial cell motility).[86]

Another major role of enteric glial cells in the control of IEB homeostasis is the regulation of intestinal epithelial cell proliferation. Enteric glial cells exert drastic antiproliferative effects on intestinal epithelial cells. Co-culture models of proliferative intestinal epithelial cells with enteric glial cells revealed massive inhibition of intestinal epithelial cell proliferation, which was not associated with an increase in intestinal epithelial cell apoptosis but rather with an increase in intestinal epithelial cell surface area, thereby promoting cell–cell contact inhibition[85] and blockade at G0–G1 of the cell cycle.[85] Conversely, *in vivo* ablation of enteric glial cells leads to intestinal crypt hyperplasia.[116] The antiproliferative effects of enteric glial cells are mediated by various glial-derived mediators (for example, TGF-β1[85]) or lipid mediators (for example, 15dPGJ2[130]). In addition to their antiproliferative effects, enteric glial cells enhance intestinal epithelial cell differentiation and increase their adhesion to the matrix *in vitro*.[130,131] The antiproliferative effects and prodifferentiative effects of enteric glial cells are mediated in part by the activation of PPARγ pathways in intestinal epithelial cells, as enteric glial cells synthesize soluble ligands of PPARγ, such as omega 6 derivatives and 15dPGJ2.[130]

Enteric glia in IEB dysfunction: friends or foes?

Changes of enteric glial cell phenotype have been observed in various gastrointestinal disorders associated with barrier dysfunctions, such as IBD, coeliac disease[132] or necrotizing enterocolitis.[133] However, whether these changes have a bystander effect or whether enteric glial cells actively participate in the onset and/or progression of the disease remains to be defined.[134] The major change reported for enteric glial cells during intestinal diseases is either upregulation or downregulation of GFAP expression. Overall, increased expression of GFAP has been observed in inflammatory regions in patients with Crohn's disease and ulcerative colitis.[118,135] By contrast, for noninflamed areas, increased expression of GFAP is observed in ulcerative colitis whereas reduced expression is observed in Crohn's disease.[118] Increased expression of GFAP is a hallmark of reactive astrocytes in the CNS, and these cells have long been considered to have a deleterious role.[136] However, evidence now suggests that reactive astrocytes favour wound healing in the brain and spinal cord.[136] Whether such a paradigm also exists for enteric glial cells in the control of the IEB or neuronal functions is currently unknown. Ulcerative colitis seems to be the prototypical disease for reactive enteric glial cells. In this disease, increased expression of GFAP

is associated with increased production and release of S100β, which has been described to increase NO production, thereby conferring to enteric glial cells putative deleterious functional effects.[137,138] S100β expression is also increased in submucosal and myenteric plexi of inflamed areas in Crohn's disease.[139] These data are consistent with another study showing that enteric glial cells can also be a source of cytokines such as IL-6.[140] Conversely, increased GFAP expression in ulcerative colitis and Crohn's disease is also associated with increased GDNF expression,[135] whose protective effects on both intestinal epithelial cells and enteric neurons have been described above.[125,128] Besides phenotypical changes, inflammatory mediators can also effect enteric glial cell proliferation, although data supporting this idea remain controversial. An increase in enteric glial cell proliferation has been reported during 2,4,6-trinitrobenzene sulphonic acid (TNBS)-induced colitis *in vivo*.[141] *In vitro*, inflammatory mediators induced an increase in proliferation in human-derived enteric glial cells,[138] whereas no change was observed in rat enteric glial cells.[142] Altogether, the effect of reactive enteric glial cells on IEB function is still unclear and improved understanding could lead to the development of approaches aimed either at preventing or favouring the shift of the enteric glial cell phenotype towards reactive enteric glial cells in the treatment of IEB dysfunctions.

Modulation of neurons and glia

In a reciprocal manner, intestinal epithelial cells can affect neurons and enteric glial cell functions. Intestinal epithelial cells transduce luminal signals to enteric neurons via the release of 5-HT, and ultimately activate the enteric reflexes that control intestinal peristaltism[143] or mucosal secretion.[144] As well as these short-term effects, components of the IEB can exert long-term effects on neuronal functions. For example, ERBB2 expression in colonic epithelial cells is required for the postnatal survival of enteric neurons.[145] *In vitro*, intestinal epithelial cells can also regulate neuromediator expression and survival of enteric neurons via the secretion of soluble factors.[146] These effects on neuronal functions can also occur under pathological conditions. In response to an infectious or inflammatory insult, intestinal epithelial cells stimulate chemokine production by enteric neurons, ultimately leading to enhanced chemotactism of these cells.[147,148]

IEB-mediated control of ENS homeostasis also relies on the ability of the IEB to transduce, metabolize and/or transport nutrients that can ultimately affect the phenotype and function of the ENS. For example, short-chain fatty acids, such as butyrate, are produced by the microbiota and enhance excitability of neurons[149] via the release of 5-HT from enteric glial cells.[150] Butyrate also directly enhances neuronal synthesis of acetylcholine leading to enhanced gastrointestinal motility.[151] The potential effect of the IEB and/or nutrients on enteric glial cells still remains largely unknown, although a study has shown postnatal and diet-dependent changes in GFAP expression in these cells.[152]

A novel source of biomarkers

In view of the central role of the neuronal–glial–epithelial unit in health and disease, improved understanding of the reciprocal regulation between its three components, their phenotype and functions would represent a major advance in understanding digestive and extradigestive pathological processes. In this context, the neuronal–glial–epithelial unit represents a potential source of biomarkers of disease progression and/or response to treatment. Intestinal biopsies enable easy access to the neuronal–glial–epithelial unit during routine endoscopic procedures; in humans, biopsy samples are used to evaluate IEB functions[51] as well as analyse the ENS phenotype[153] and functions.[154] In this context, colonic biopsy samples demonstrated an increase in paracellular permeability that correlated with symptoms (such as visceral pain) and expression of ZO-1 junctional protein in patients with IBS.[56] Similarly, an assessment of neuropathological features of Parkinson's disease (Lewy neuritis) in the colon was performed on colonic biopsies—the density of these lesions correlated with nonmotor symptoms in these patients.[153] In addition, analysis of biopsy supernatants from patients with IBS has led to the identification of mediators involved in dysfunctions of the neuronal–glial–epithelial unit,[56,155,156] which might be of interest in the development of novel therapeutic targets for gastrointestinal disorders. In particular, biopsy supernantants were able to 'adaptively transfer' to healthy tissue *in vitro* or *in vivo* symptoms or gastrointestinal functional alterations observed in patients (such as increased intestinal paracellular permeability, increased visceral pain and increased neuronal excitability), enabling the identification of mediators involved (such as proteases).

Novel therapeutic targets

Considering the extent of the role of the ENS in regulation of the IEB, it might represent a novel therapeutic target for enhancing IEB resistance or barrier repair in various diseases. As well as conventional pharmacological-based approaches (using neuronal and glial mediators to reinforce the IEB), neurostimulation-based approaches (vagal, sacral or direct stimulation of the ENS) could be developed, provided that in the disease state the ENS retains its protective abilities towards the IEB. Nutritional targeting of the ENS is also an option—studies have shown that specific nutrients or bacterial-derived products exhibit the ability to modulate expression of neuronal or glial-derived mediators. Finally, the development of cell-therapy-based approaches using neuronal and glial cells could also represent an alternative for severe cases (such as ulcers) but this approach relies on the ability to isolate and cultivate ENS precursors and their ability to survive after engraftment and restore IEB functions.

Conclusions

Previously described as an anatomical unit, the digestive neuronal–glial–epithelial unit has now been revealed to be a functional entity with reciprocal regulation between

its cellular components. The ENS should be considered, along with the microflora, the immune system and fibroblasts, as a major actor in the maintenance of IEB homeostasis and integrity, at least under physiological conditions. Targeting the ENS to enhance its barrier protective effects represents a promising research avenue for the prevention and treatment of diseases associated with IEB dysfunctions. These pathologies include digestive diseases such as IBD and IBS, as well as extra-digestive diseases including obesity, asthma and even neurodegenerative diseases.

The importance of lesions observed within the neuronal–glial–epithelial unit in the pathophysiology of various diseases needs to be explored further. In this context, the development of novel endoscopic tools enabling the concomitant exploration of IEB dysfunctions and enteric neuropathies is important. These approaches should also enable easy assessment of the response of the neuronal–glial–epithelial unit to various therapeutic approaches within individual patients and therefore lead to the development of personalized medicine. Improved understanding of the genetic or epigenetic factors involved in neuronal–glial–epithelial unit dysfunctions might also be useful for identifying novel therapeutic targets. Finally, exciting findings concerning the digestive neuronal–glial–epithelial unit might be translated in the future to the neuronal–glial–endothelial unit of the brain, thus further reinforcing the similarities between our brain in the gut and the one in the skull.

Review criteria

Full-text articles were selected from PubMed using the following search terms: "intestinal barrier", "tight junctions", "enteric glia", "enteric nervous system", "glia and gut", "glia and intestine". Two abstracts were selected based on pertinent recent data presented at the 2012 Neurogastroenterology and Motility International Meeting in Bologna.

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