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PATHOBIOLOGY IN FOCUS

Starring roles for astroglia in barrier pathologies of gut and brain

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The gastrointestinal tract is a highly innervated organ and enteric neuropathy is emerging as a central feature of a wide range of gut diseases. Although most considerations of the enteric nervous system have focused on neuronal dysfunction, a large population of astrocyte-like glia populates gut muscle layers and the intestinal mucosa, and mounting new evidence points toward enteric glia as active participants in gut pathology. Similarly, in the central nervous system increasing evidence suggests that dysfunctions of astrocytes play central roles in disease mechanisms. On the basis of the premise that gut-brain disease paradigms may exist, we explore the possibility that enteric glia constitute a previously unrecognized disease target in pathologies associated with intestinal barrier dysfunction, notably inflammatory bowel disease, necrotizing enterocolitis, irritable bowel syndrome, diabetes, autoimmune disease and neurotrophic virus infection of the gut.

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THE ENTERIC NERVOUS SYSTEM AND ITS KNOWN DISEASES

Gastrointestinal tissues are innervated by a highly complex and extensive component of the peripheral nervous system known as the enteric nervous system (ENS).[1] Enteric neurons control several aspects of gut function, including motility, microvascular circulation, epithelial secretion of fluid, ions and bioactive peptides and intestinal barrier function. In addition to neurons, enteric glia represent an extensive component population of the ENS and show morphologic and functional similarities to CNS astrocytes.[2] Enteric glia have long been suggested to provide trophic and cytoprotective functions toward enteric neurons, and likely are involved in regulating neuronal activity as has been demonstrated for astrocytes.

An emerging concept in gastroenterology is that a wide range of diseases, for example motility disorders and inflammation, can be considered in part as enteric neuropathies. Although it often remains elusive to determine whether these neuropathies are the cause or effect of disease activity, ENS alterations may at least in part be symptomatic. Until recently, studies of enteric neuropathies have mainly focused on characterizing altered neuropeptide expression patterns and the involvement of enteric neurons[1] (Table 1). Scarce, but increasing data suggest that enteric glia are also major players in gut disease. Indeed, the main histopathologic observations made by the group of Bassotti *et al*[3] have demonstrated that motor disorders of the gut, such as slow transit constipation, diverticular disease and idiopathic megacolon, are associated with enteric glial abnormalities. Reinforcing these observations are *in vivo* animal experiments in which alterations of glial cell function result in reduced intestinal motility and a slowing of gastric emptying.[4,5]

Increasing evidence also suggests that enteric glia play a major role in gut pathologies associated with barrier dysfunction. Alterations in intestinal permeability are observed in a wide range of diseases ranging from high-grade inflammatory pathologies such as inflammatory bowel disease, celiac disease and enteric infection, to low-grade inflammatory diseases such as irritable bowel syndrome and diabetes. Indeed, all of these diseases present an increase in intestinal permeability that may be regarded as a contributing event in the onset of pathology. Therefore, regulation of intestinal barrier function by its microenvironment, and in

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Table 1 Alteration of neuronal and glial functions in digestive diseases

Disease	Enteric neurons	Enteric glial cells
Crohn's disease	Neurochemical plasticity and neuronal cell death	Decreased GFAP expression in non-inflamed mucosa; increased expression of MHC II
Ulcerative colitis	Neurochemical plasticity and neuronal cell death	Increased GFAP expression in inflamed mucosa; expression of MHC II
Necrotizing enterocolitis	Neuronal cell death	Decreased GFAP expression
Diabetes	Decreased nNOS expression and neuronal cell death	Not determined
Atresia	Reduced neuronal cell density	Reduced GFAP expression
Intestinal slow transit constipation	Neurochemical plasticity	Decreased numbers of S100β^+ glia

particular by the ENS, may be responsible for altered gut permeability in these disease settings.

Enteric glia form an extensive network of cells in the intestinal mucosa, where they lie in close proximity to intestinal epithelial cells and to submucosal blood vessels (Figure 1). Transgenic studies targeting enteric glia in mice have demonstrated that this cell population plays an important role not only in protecting enteric neurons, but also in maintaining the integrity of the gut mucosa and in regulating its permeability and turnover.[2,5–8] Conditional ablation of enteric glia utilizing different transgenic approaches consistently resulted in the development of a fulminant intestinal inflammation and a disease progression that began with a breakdown of mucosal and/or vascular integrity. It seems likely therefore that enteric glia regulate intestinal barrier function at several levels. Here, we propose a paradigm that intestinal barrier regulation by enteric glia shows functional analogy to astrocytic regulation of blood–brain barrier function in the CNS. We also consider the potential pathologic consequences for enteric glial cell (EGC) dysfunction that may be evident in a number of enteric diseases characteristically associated with intestinal barrier failure.

GLIAL CROSS-TALK IN BARRIER REGULATION: A NEW GUT-BRAIN PARADIGM

The blood–brain barrier is a well-known, highly specialized endothelial interface essential for normal function of the brain and spinal cord.[9,10] Disruption of this barrier complicates many neurological diseases, including stroke and neuroinflammatory disorders. The CNS is also protected by less widely know barriers across epithelial cells in the choroid plexus and archanoid layer of the meninges. Functionally important comparisons can be made between barrier functions in the brain and across mucosal surfaces. Both tissue types maintain cellular homeostasis by regulating the movement of solutes and macromolecules across both paracellular and transcellular pathways. Paracellular permeability barriers are maintained by complex intercellular adherens and tight junctions between epithelial and/or endothelial cells.[11] Cellular and molecular interactions that regulate barrier functions are beginning to emerge, and several lines of evidence suggest that this regulation is likely to be multifactorial with a variety of cell types producing a range of molecules that may induce or compromise barrier functions.[9]

Within the CNS, the blood–brain barriers to solutes and inflammatory cells are at least in part regulated via interactions between astrocytes, pericytes and cerebral endothelial cells that form a tightly regulated and immune privileged neurovascular unit.[9,10] Astrocyte-end feet processes come into close contact with cerebral capillaries, and astroglial-derived soluble mediators and extracellular matrix components contribute to inducing and maintaining blood–brain barrier functions. Although the precise nature of these molecular mediators has not yet been fully elucidated, similar interactions appear to exist between astroglia and epithelial cells in the choroid plexus and arachnoid, forming a glial limitans. Various factors produced and released by astroglia can induce barrier properties in both endothelia or epithelia layers *in vitro*. It is interesting to note that *in vitro* studies suggest that during pathologic conditions astroglia also have the potential to release mediators that compromise the blood–brain barriers, leading to CNS permeability disorders.[9,10] Conversely, in the experimentally induced absence of astrocytes around injury sites *in vivo*, the traumatically damaged blood–brain barrier does not repair.[12] Thus, factors released by astroglia can have a substantial and varied potential to influence CNS barrier functions in health and disease.

Within peripheral nervous systems, glial cells also represent the most abundant non-neuronal cell type. However, until recently virtually nothing has been known about their ability to regulate vascular or epithelial integrity. To draw a parallel to astroglial-regulation of blood–brain barrier function, peripheral glia need to be able to promote mucosal integrity via direct cross-talk with an equivalent epithelial–myofibroblast barrier forming unit. EGC processes are in close proximity to gut epithelial cells and recent studies have shown that these cells secrete several mediators implicated in blood–brain barrier formation (Table 2). For example, EGC secrete glial-derived neurotrophic factor whose production is increased during intestinal inflammation and could act to protect intestinal epithelial cells from cytokine-induced

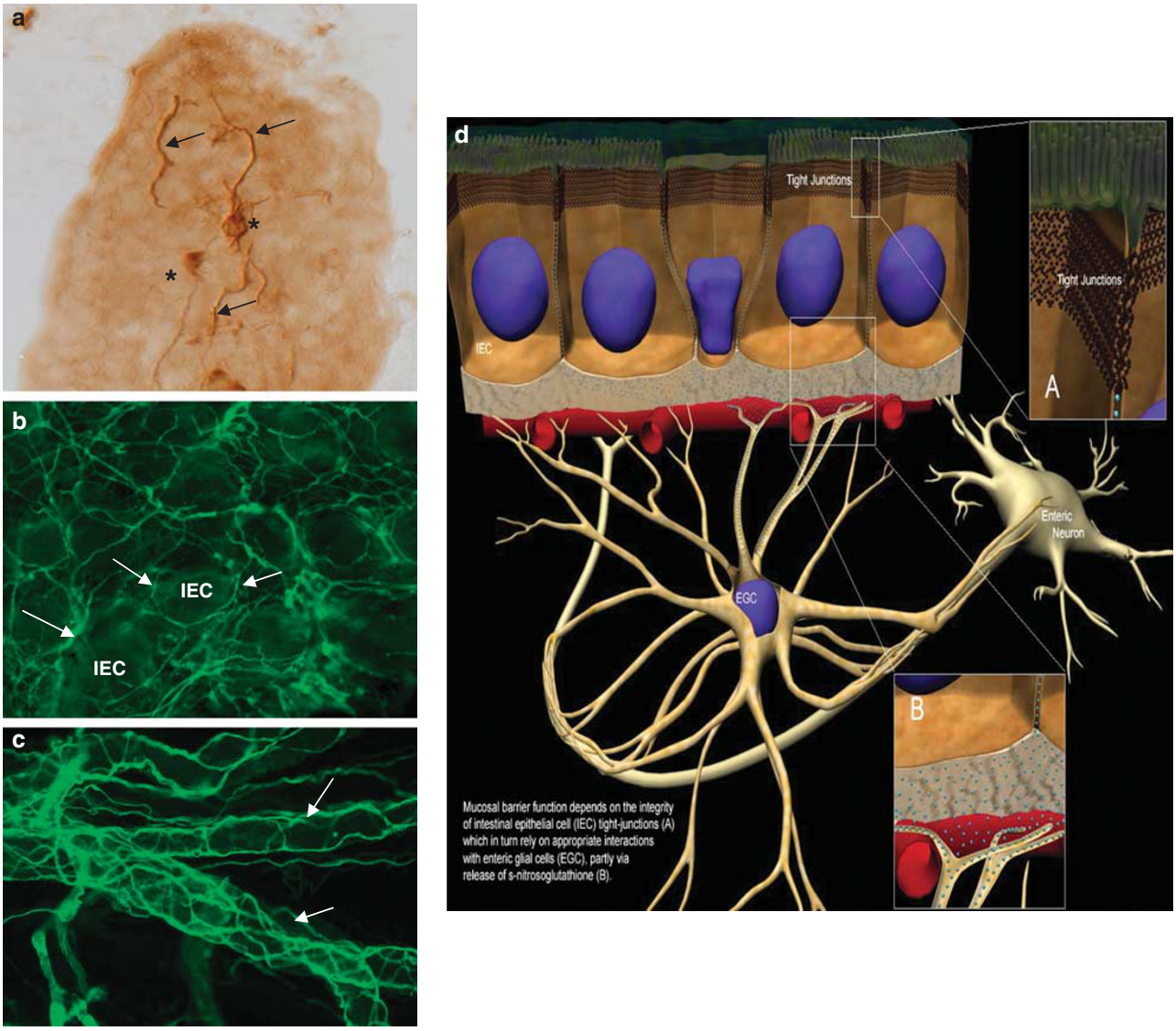

Figure 1 EGC in murine and human intestinal mucosa. (**a**) Immunohistochemistry showing GFAP expressing glia in a murine small intestinal villus. GFAP-positive glial cell bodies (*) and cell processes (arrows) are in close proximity to the intestinal epithelium (IEC), extending to the villous-tip. EGC processes are also abundant in human endoscopic biopsies. (**b**) S100-β immunofluorescent labeling of mucosal enteric glia (arrows) embracing intestinal epithelial crypt cages (IEC) and (**c**) submucosal blood vessels. (Magnification is $\times$400 and $\times$250 for **a**, **c** and **b**, respectively). (**d**) The mucosal-barrier function of intestinal epithelial cells depends upon the integrity of their tight junctions, (A) which in turn rely party on adequate concentrations of S-nitrosoglutathione (B) via EGC.

apoptosis.[13] Enteric glia are also prolific producers of transforming growth factor-β isoforms that promote intestinal barrier function.[8]

Enteric glia have also recently been shown to produce the nitric oxide metabolite S-nitrosoglutathione (GSNO), a novel potent inducer of intestinal barrier function in transgenic mice and in human colon.[2] S-nitrosylation of protein cysteine residues functions in a manner analogous to post-translational phosphorylation, and may drastically alter protein function.[14] In the case of GSNO-mediated regulation of epithelial barrier function, this appears in part to be influenced by altering the expression and/or S-nitrosylation of peri-junctional F-actin and the association of tight junction-associated proteins such as ZO-1 and occludin with the actin cytoskeleton. Many complex biological responses of nitric oxide have recently been attributed to S-nitrosothiols, and an insufficient expression of this class of molecule contributes to inflammatory disease pathogenesis in asthma[15] and amyotrophic lateral sclerosis.[16] It is therefore reasonable to ask the converse question of whether GSNO, which was found to derive from enteric glia and to promote barrier properties in intestinal epithelia, might similarly promote barrier functions in CNS endothelia. Several lines of evidence suggest that this might be the case. Astroglia *in vitro* generate GSH, and are likely to be able to generate and secrete GSNO where tissue levels of this S-nitrosothiol are normally high.[17] In addition,

Table 2 Putative factors involved in astroglial regulation of barrier functions across endothelium and/or epithelia

Barrier-inducing	Barrier-disrupting
Glucocorticoids	Proinflammatory cytokines (TNF-α, IL-1β, IL-6, MIP)
cAMP inducing mediators (VIP)	Purine nucleotides (ADP, ATP and AMP) and adenosine
Growth factors (TGFβ, basic FGF)	Free radicals and nitric oxide
Neurotrophins (GDNF)	Platelet-activating factor, leukotrienes and prostaglandins
Adrenomedullin and noradrenergic mediators	Arachidonic acid and phospolipase A2
Endothelin-1	Bradykinin
S-nitrosothiols (GSNO)	Histamine
Secreted extracellular matrix components	Glutamate
Collagen IV, fibronectin, laminin	Serotonin
Regulators of membrane P-glycoprotein and toll-like receptors	Complement-derived peptide C3a-desArg

GSNO delivered systemically has been reported to reduce blood–brain barrier permeability associated with various forms of CNS injury.[18] In this context, another noteworthy feature is that both the blood–brain barrier and intestinal epithelium express high levels of γ-glutamyl transpeptidase, an important bioactivator of GSNO-mediated S-nitrosylation signals. The main factors produced by astrocytes to induce barrier functions have remained elusive. Further studies will be required to follow-up on this interesting lead that GSNO may be an important player in CNS blood–brain barrier regulation.

A PATHOGENIC ROLE FOR GLIA IN DISEASES INVOLVING BARRIER DYSFUNCTION

Taken together, the findings discussed indicate that peripheral and central astroglia exhibit a widespread ability to regulate barrier functions of epithelia and/or endothelia (Figure 2). It is particularly interesting that the ability to induce barrier functions is in many cases interchangeable among astroglial-like cell types and target cells. For example, transplantation of enteric glia into the spinal cord accelerates normal repair processes of the vasculature at the site of injury and promotes the induction of a functional blood–brain barrier.[19] In addition, glia promote blood–brain barrier-like properties in peripheral sites such as blood-ocular barriers in the eye, the perineurium of peripheral nerves and the blood-myenteric plexus barrier in the gut.[20] *In vitro*, primary cell cultures of either astrocytes or enteric glia are able to induce barrier properties across endothelia and epithelia. Such findings strongly suggest that different subtypes of glia generate similar or related molecular mediators able to influence barrier properties and that the production of such factors may be severely perturbed during disease states.

In light of these findings, what are the likely pathologic outcomes of glial dysfunction? Transgenic deletion studies have clearly indicated that loss of glial cell function results in leaky gut and blood–brain barriers, leading in extreme cases to inflammation and necrosis. Elevated intestinal permeability is apparent in patients with Crohn's inflammatory bowel disease (originally diagnosed as a neuropathy), necrotizing enterocolitis, irritable bowel syndrome, diabetes, autoimmune disease and neurotrophic virus infection of the gut. Although, inflammation can certainly contribute to altered gut permeability, further studies are required to examine whether EGC populations are compromised in all of these patient populations. If this is indeed the case, what are the causative events responsible for glial cell disruption or alterations?

One intriguing hypothesis that has been suggested to occur in the CNS is that astroglia-expressing viral proteins are targets of autoimmune destruction. Complex genetic and environmental determinants are believed to trigger autoimmune disease, possibly via molecular mimicry between host and microbial antigens. Autoimmune diseases afflicting neurons of the ENS have been described in patients with Chagas disease-associated and paraneoplastic intestinal pseudo-obstruction.[1] Interestingly, these diseases show similarities to other T-cell-mediated paraneoplastic neurological diseases associated with the CNS. Although enteric glia have not yet been demonstrated as autoimmune targets in the gut, autoimmune antibodies that target CNS astrocytes are present in a disease setting resembling multiple sclerosis.[21]

Neurotrophic assault, for example by herpes- and measles-like viruses or by prions, could also represent cytopathic mechanisms targeting EGC. Alternatively, viral infection of enteric glia could lead to subsequent autoimmune targeting as has been shown in an animal model for multiple sclerosis, Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD).[22] Indeed, TMEV-IDD is caused by direct CNS infection by the virus, leading to activation of TMEV-specific T cells that breach the blood–brain barrier and target virally-infected glial antigen presenting cells. Enteric glia share this antigen-presenting capacity as demonstrated in transgenic mouse studies and as surface MHC-class II molecules on enteric glia are upregulated during Crohn's disease.[23] T-lymphocyte infiltration of neuronal plexi and enteric axonal abnormality and necrosis are typical in non-inflamed Crohn's disease tissues and are strongly predictive of disease recurrence. This begs the question whether enteric glia are involved in the pathology of Crohn's disease, and studies aimed at examining potential enteric glial autoantigens, antigen presentation, and their relative contribution

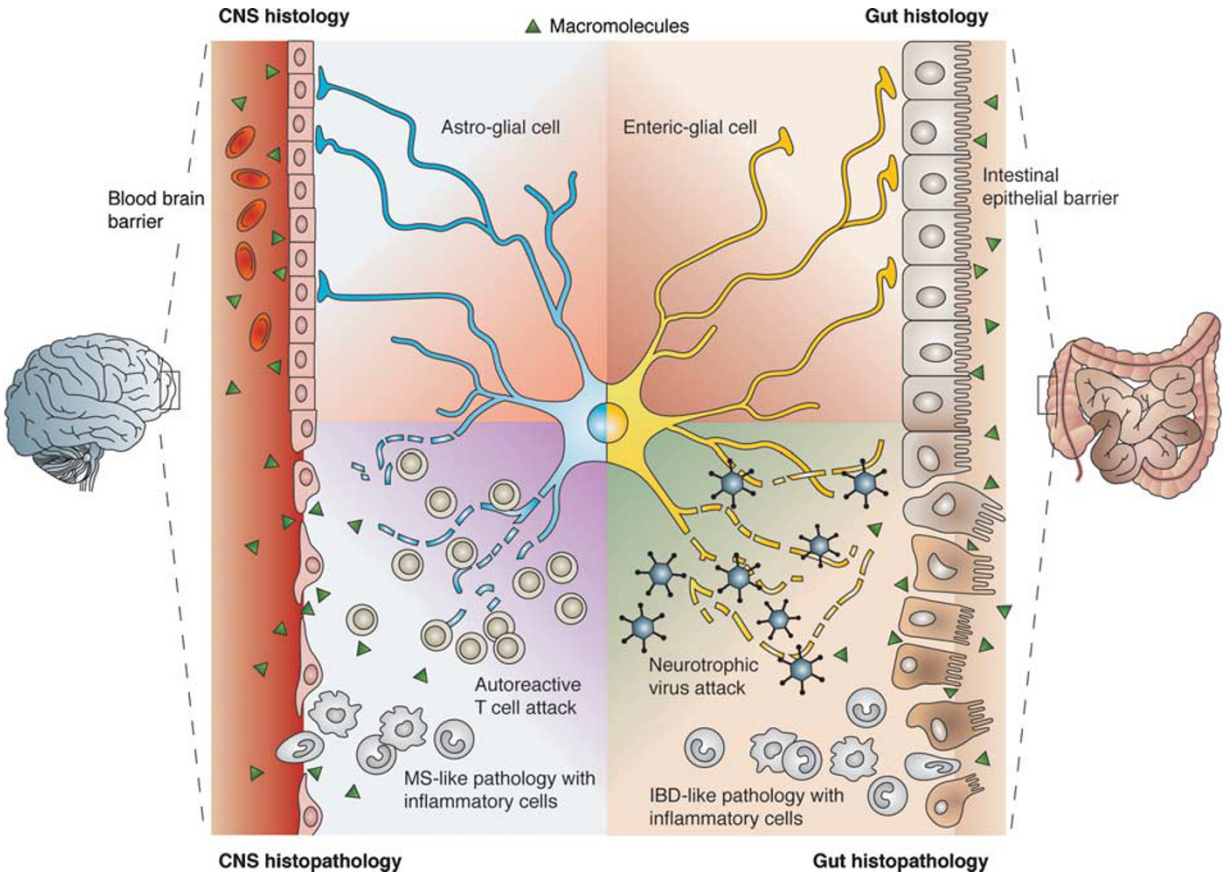

Figure 2 Gut-brain disease paradigm. Schematic illustration of astroglial regulation of barrier function in gut and CNS disease states. CNS morphology and pathology following disruption of blood–brain barrier (BBB) function are shown on the left. Gut morphology and pathology following disruption of intestinal epithelial cell (IEC) barrier function are shown on the right. Barrier dysfunction in both tissues is associated with an influx of polymorpholeukocytes, which contribute to disease activity. Increased barrier dysfunction is associated with a disruption of tight junction associated proteins expressed by BBB and IEC and is likely regulated by astroglial cell disruption, for example, by autoimmune reactions or neurotrophic virus infection.

in inducing intestinal and extra-intestinal neuropathy are therefore warranted.

It is also likely that enteric glia contribute to inflammatory permeability disorders in other ways. A notable astrocyte function lies in its ability to protect neurons and reconstitute blood–brain barrier function following inflammatory trauma to the CNS.[12] Astroglia respond to a wide range of pathological settings by exerting effector functions following activation by lipopolysaccharide, proinflammatory and immunoregulatory cytokines. This activation involves a stereotypical glial cell reaction (astrogliosis) that, on the one hand is designed to promote inflammatory and T-cell-mediated responses when there is a requirement to protect the CNS from infection. On the other hand, astroglia act to minimize the spread of leukocytes and inflammation into adjacent healthy tissue, and to enhance blood–brain barrier function. Presently, it is not known whether enteric glia share a similar capacity to regulate tissue inflammation, although recent studies suggest that they do. Enteric glia can certainly secrete various pro-inflammatory cytokines under inflammatory conditions and may proliferate in response to inflammatory mediators.

Lastly, it deserves mention that the cellular and molecular regulation of barrier function across epithelia are highly dependent on tight-junction integrity in many tissues including kidney, liver, lung, eyes, testis and skin, and that the regulation of these barriers in health and disease is not well understood. Given the pervasive presence in these tissues of resident populations of cells that express the astroglial marker glial fibrally acidic protein (GFAP),[6] it is tempting to speculate whether similar regulatory or disease scenarios might also operate in these peripheral tissues.

CONCLUSION

Enteric glia have recently been shown to induce barrier properties in intestinal epithelia via the regulated secretion of neuropeptides, growth factors and the S-nitrosothiol, GSNO. This novel function of regulating mucosal and vascular integrity, suggests that enteric glia may be important target cells in inflammatory and permeability disorders of the gastrointestinal tract, and that the molecules they produce, such as GSNO, warrant further investigation both in pathophysiological and potential therapeutic contexts. In addition, these findings provide evidence that astroglial-like cells in

both brain and gut contribute interchangeably to barrier functions, suggesting a previously unrecognized paradigm whereby cellular interactions previously thought to be unique to the blood–brain barrier, also regulate gut epithelial permeability.

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