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Enteric glia and neuroprotection: basic and clinical aspects

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De Giorgio R, Giancola F, Boschetti E, Abdo H, Lardeux B, Neunlist M. Enteric glia and neuroprotection: basic and clinical aspects. Am J Physiol Gastrointest Liver Physiol 303: G887–G893, 2012. First published August 9, 2012; doi:10.1152/ajpgi.00096.2012.—The enteric nervous system (ENS), a major regulatory system for gastrointestinal function, is composed of neurons and enteric glial cells (EGCs). Enteric glia have long been thought to provide only structural support to neurons. However, recent evidence indicates enteric glia-neuron cross talk significantly contributes to neuronal maintenance, survival, and function. Thus damage to EGCs may trigger neurodegenerative processes thought to play a role in gastrointestinal dysfunctions and symptoms. The purpose of this review is to provide an update on EGCs, particularly focusing on their possible neuroprotective features and the resultant enteric neuron abnormalities subsequent to EGC damage. These neuroprotective mechanisms may have pathogenetic relevance in a variety of functional and inflammatory gastrointestinal diseases. Basic and clinical (translational) studies support a neuroprotective role mediated by EGCs. Different models have been developed to test whether selective EGC damage/ablation has an impact on gut functions and the ENS. Preclinical data indicated that selective EGC alterations were associated with changes in gut physiology related to enteric neuron abnormalities. In humans, a substantial loss of EGCs was described in patients with various functional and/or inflammatory gastrointestinal diseases. However, whether EGC changes precede or follow neuronal degeneration and loss and how this damage occurs is not defined. Additional studies on EGC neuroprotective capacity are expected to improve knowledge of gut diseases and pave the way for targeted therapeutic strategies of underlying neuropathies.

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neuroprotective properties in gut physiology and in a variety of functional and inflammatory GI diseases.

The scope of this review is to provide a critical appraisal on EGCs particularly focusing on their neuroprotective features and the resultant enteric neuron abnormalities possibly associated with EGC injury. Specifically, we discussed the different in vivo and in vitro models that have been developed to test whether a selective EGC damage/ablation has an impact on neuronal survival and maintenance. In addition, the knowledge on EGC abnormalities in patients with functional and inflammatory GI disorders has been analyzed. However, whether EGC changes precede or follow neuronal degeneration and how this damage occurs remain unsettled. Finally, an innovative aspect that has been detailed concerns the evidence that viruses may target EGCs, likely leading to neuronal damage. This mechanism might have a pathogenetic relevance in patients with severe gut dysmotility.

**Enteric Glia: Structural and Functional Aspects**

EGCs originate from neural crest progenitors which migrate and colonize the GI tract. Upon colonization of the embryonic gut, neural-crest derived progenitors mature into neurons and glia via Hedgehog/Notch pathway (22, 34, 37). Recent data also suggest that EGCs may give rise to neurons in vitro and in vivo under specific conditions (21, 27, 29). Once developed, EGCs share morphological features and antigenic markers with the astrocytes of the central nervous system (CNS) (20). EGCs are small cells showing a starlike appearance with numerous 10-nm filaments mainly containing glial fibrillary acidic protein (GFAP) (37). A subtype of EGCs surrounds nerve processes in the mucosa, thereby establishing close contacts with the epithelial cells, while another population wraps around neuronal cell bodies in the ganglionated plexuses (20, 32, 37).

The EGCs outnumber neurons with a ratio ranging from 1.3 to 1.9 and from 5.9 to 7.0 in the human submucosal and myenteric plexuses, respectively (23), and surround the neuronal cell bodies tightly (i.e., the remaining extracellular space consists of narrow 20-nm gaps) (20). As in the blood-brain barrier, ECGs possess several flat endfeet (or cytoplasmic expansions) that form a blood-enteric barrier protecting the enteric neurons from potentially harmful extraganglionic substances (41). Compared with other peripheral glial cells (e.g., Schwann cells), EGCs differ in that they do not form basal laminae and they ensheathe nerve bundles and not individual axons (6).

Available immunohistochemical markers for EGC labeling in the adult gut include GFAP, S100β, and Sox8/9/10, the first two being the most frequently used (23, 37) (Fig. 1). In the gut, GFAP is considered a specific mature EGC marker (25), although it does not reliably distinguish between enteric and extrinsic glia, since GFAP is also expressed in nonmyelinating Schwann cells (26). S100β is a Ca²⁺ binding protein stored in the cytoplasm and/or in the nucleus of both nervous and nonnervous tissue where it exerts diffusible neurotrophic properties (52). Although there are several S100 isoforms in the nervous tissue, EGCs constitutively and specifically express only the S100β (37). The nuclear Sox transcription factor family (Sox8/9/10) represents one of the latest markers proposed for EGC identification (49). Sox8/9 expression has been demonstrated in neural crest cells and in immature glial cells, whereas Sox10 appears to be restricted to EGCs in the mature ENS (51). Thus, by using antibodies targeting Sox8/9/10, it is possible to label EGC nuclei and thereby perform precise and quantitative analysis of EGC density (21, 23).

EGCs have long been thought to be a scaffold for neurons with no other properties. This old view has been thoroughly changed by recent data showing that EGCs are involved in many crucial tasks, such as synthesis of neurotransmitter precursors, uptake and degradation of neuroligands (i.e., detoxification of glutamate and γ-aminobutyric acid), and expression of neurotransmitter receptors, thereby contributing to neurotransmission. Furthermore, EGCs exhibit immunological properties (16, 33, 37, 38), participate in epithelial barrier functions (11, 32, 43, 45, 46), and evoke neuroprotection (19, 28, 39), the latter being the focus of this review.

**EGC Neuroprotective Functions: Lessons from Enteric Glia Ablation Models**

Recent research has addressed the protective role of EGCs toward enteric neurons. This property has been tested by using EGC ablation strategies in vivo or in vitro.
**In vivo studies.** Since there is no specific stimulus able to selectively stimulate EGCs without activating other cell types, different in vivo and in vitro models have been developed to test the effect evoked by EGC damage. To date, the results obtained have indicated that enteric glia ablation is associated with changes in gut physiology related to enteric neuron abnormalities (4, 12, 14, 31).

Bush et al. (12) devised a Gfap-herpes simplex virus (HSV)-thymidine kinase (Tk) transgenic mouse model, which is characterized by the HSV-Tk gene controlled by the Gfap promoter leading to expression in EGCs as well as in the astrocytes of the brain and spinal cord. Since the viral TK protein is able to phosphorylate ganciclovir (GCV), the active GCV-triphosphate form inhibits cellular polymerases, thereby evoking EGC cytotoxicity and death. As a result, systemic treatment with GCV evoked a massive depletion of GFAP-immunoreactive EGCs. Notably, EGC ablation occurred exclusively in the ileum and was associated with striking effects, i.e., enteric neuronal loss and fulminant ileitis. The latter feature was never completely explained by Bush et al., although a combination of mechanisms including impaired intestinal motility (which may occur because of neuronal loss), altered epithelial cell barrier (directly related to EGC ablation), and luminal factors (e.g., bacterial overgrowth) could all have contributed to the severe inflammatory state observed in this transgenic model. Since there are a relatively limited number of publications regarding the Gfap-HSV-Tk transgenic mouse model, the question of whether changes in gut microbiota may represent a crucial factor for severe small bowel inflammation is still unclear. The inflammatory changes were reminiscent of early-stage Crohn’s disease (CD) or neonatal necrotizing enterocolitis. Nonetheless, why EGC damage affects only the ileum is another unclear aspect that necessitates further analysis.

Another similar transgenic model of glia damage has been developed by the specific immune-mediated targeting of EGCs (14). This model has been obtained by crossing two strains of transgenic mice (GFAP-HA × CL4-TCR), one expressing influenza virus hemagglutinin (HA) in astrocytes and EGCs, and the other with CD8+ T cells sensitized against HA and thus expressing the specific receptor. The resulting progeny showed a selective apoptotic depletion of EGCs mediated by a mechanism of intestinal inflammation involving a CD8+T cell-mediated cytotoxic autoimmune response. Compared with controls, the GFAP-HA × CL4-TCR mice exhibited a selective EGC depletion (34% and 72%) and apoptosis (4% and 14.5%) in the myenteric and submucosal plexus, respectively. These EGC abnormalities were associated with massive intestinal inflammation involving CD8+T cell-mediated cytokotoxic autoimmunity. This response led to extensive disruption of the mucosal integrity, submucosal edema, and vascular inflammation, eventually evolving into fulminant jejunoileocolitis. However, no sign of neurodegeneration in the ENS was observed in contrast to the previous model. The reason(s) why Gfap-HSV-Tk showed neurodegeneration while GFAP-HA × CL4-TCR mice lacked that aspect remain unclear. Likely, different methodological aspects, biological characteristics, and survival times (i.e., ~5 vs. 19 days of GFAP-HA × CL4-TCR and Gfap-HSV-Tk mice, respectively) might explain the different neurodegenerative profile observed in the two transgenic models. Overall, the EGC loss appears more severe in Bush’s model (12) compared with Cornet’s one (14) (i.e., an almost complete EGC loss vs. 34% loss of GFAP+ cells, respectively). On the basis of these findings, the authors suggested that immune-mediated EGC damage could contribute to the initiation and/or progression of human inflammatory bowel (IBD) diseases, especially CD.

Whether EGC abnormalities played a role in enteric neuron changes and accompanying GI motor abnormalities was the focus of another study by Aubé et al. (4). Using the same transgenic model, the authors evaluated jejunal muscle activity, GI transit, paracellular permeability, and neurochemical plasticity in subclasses of submucosal and myenteric neurons expressing VIP, choline acetyl-transferase (ChAT, a cholinergic marker), substance P (SP), and neuronal nitric oxide (NO) synthase (nNOS). The results showed that the EGC disruption obtained in this model was associated with a mild ganglionitis as indicated by CD3+ lymphocytes detected within myenteric ganglia. However, there was no sign of inflammation in the intestinal tissue. Although this inflammatory response in the myenteric ganglia was not associated with evident neuronal loss, changes in neurochemical code occurred in submucosal and myenteric plexi. Specifically, compared with controls, the ENS of transgenic mice showed a decrease in the proportions of SP- and VIP-positive submucosal neurons whereas those positive for ChAT were unchanged. On the other hand, the proportion of ChAT-positive myenteric neurons increased whereas nNOS-positive neurons decreased with no detectable changes to VIP- and SP-containing neurons. Taking these findings together, Aubé et al. hypothesized that a decrease in VIPergic submucosal neurons could explain the increased permeability whereas the decrease in nNOS expression in myenteric neurons could be involved in the delayed transit observed in this model. GI functions, i.e., intestinal permeability and motility, were affected by EGC loss. Together these findings suggest that glial cells exert an important role in regulating ENS plasticity and function.

Interestingly, in a knockout animal model of the mitochondrial transcription factor A gene (Tfam) severe gut dysmotility occurred and was associated with a concomitant loss of enteric neurons and EGCs. This model, reminiscent of human intestinal pseudo-obstruction, suggests a major role played by mitochondrial dysfunction in neuron and glia degeneration (47).

Toxic agents have also been used to interfere with EGC functions. In this respect, previous data regarding gliotoxin fluorocitrate (FC), a drug known to inhibit aconitase, the tricarboxylic acid enzyme, indicated a functional (metabolic) derangement of CNS astrocytes. On the basis of this background Nasser et al. (31) carried out experiments to demonstrate that chemically induced EGC ablation by FC alters GI contractility and motility in vitro and in vivo. The results showed that FC-mediated functional damage of EGCs led to altered intestinal motility both in vitro (a decrease in the baseline tone and stimulated contractility in mouse ileal strips) and in vivo (reduced GI, but not colonic, transit). In contrast to other EGC ablation models, this study did not show any evidence of inflammation and ENS abnormalities in FC treated animals.

**In vitro studies.** To determine a direct glia-mediated neuroprotective role, coculture models composed of EGCs and neuronal cell lines and/or primary culture of ENS were developed (1). Using these models, Abd el et al. (1) transfected a primary culture of rat ENS with an adenoviral vector contain-
ing the thymidine kinase gene of HSV-Tk under the control of the cytomegalovirus (CMV) promoter. Treatment of the primary culture of the ENS with GCV induced a significant disorganization and a decrease in EGCs as identified by GFAP and Sox10 immunolabeling. Concomitantly with EGC ablation, a significant increase in neuron-specific enolase release and a reduction of Hu immunoreactive neurons were observed under basal conditions. This suggests that EGCs are involved in the survival of enteric neurons. Furthermore, this study also showed that neuronal susceptibility to damaging factors such as oxidative stress (H$_2$O$_2$ or dopamine) was enhanced as a result of EGC loss (1). Concerning soluble factors released by EGCs, in vitro and ex vivo data have demonstrated that reduced glutathione (GSH), but not oxidized glutathione or S-nitrosoglutathione, can prevent enteric neuronal cell death induced by H$_2$O$_2$. Selective genetic inhibition of glutamate cysteine ligase (the key enzyme of glutathione synthesis) in EGCs increased neuronal susceptibility to death induced by oxidative stress (1, 40). Furthermore, EGC protective effects could also be due to their detoxification properties since they are able to take up glutamate or $\gamma$-aminobutyric acid from an extracellular milieu by means of $\gamma$-aminobutyric acid transporter 2 (19), respectively.

Besides GSH, glial cell line-derived neurotrophic factor (GDNF) has been shown to play a major role in enteric neuroprotection. In particular, the loss of nitricergic neurons as a result of streptozotocin-induced diabetes was attenuated in GFAP-GDNF transgenic mice compared with nontransgenic controls. GDNF has consistently been shown to prevent enteric neuronal apoptosis induced by hyperglycemia through a mechanism involving decreased PKB phosphorylation (3). GDNF has also been shown to protect enteric neurons in vitro and participate in Western diet-induced neuroprotection in the ENS. Interestingly, nutritional modulation of GDNF production in the gut could be used as an interesting neuroprotective strategy (10).

More recently, EGCs have been demonstrated to produce lipid-derived mediators, such as 15-deoxy-$\triangle$12,14-prostaglandin J2 (15d-PGJ2) (5), which exerts a crucial role in intestinal epithelial barrier maintenance. EGC-derived 15d-PGJ2 was also shown to exert neuroprotection in the ENS through an increase in nuclear erythroid 2-related factor 2 expression and leading to an increase in intracellular neuronal glutathione production (2). A summary of these EGC-mediated neuroprotective mechanisms has been illustrated in Fig. 2.

Taken together, these studies suggest that EGCs can activate a variety of mechanisms to halt the noxious effects of acute and chronic insults. By eliciting the protective phenotype, EGCs should be considered a key player for enteric neuron survival and maintenance.

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**Fig. 2.** Enteric neuroprotection mediated by EGCs involves different mechanisms, including the glutathione-dependent pathway. **A:** extracellular GSH. EGCs can exert neuroprotective effects through the synthesis and release of GSH which can regulate an antioxidant response (e.g., scavenging H$_2$O$_2$) in the neuronal extracellular environment. **B:** intracellular GSH. EGCs can also enhance GSH within neurons. This effect is partly mediated by the synthesis of lipocalin-type prostaglandin D synthase (L-PGDS) and release of 15-deoxy-$\Delta$12,14-prostaglandin J2 (15d-PGJ2), which increases nuclear erythroid 2-related factor 2 (Nrf2) and of catalytic subunit of glutamate cysteine ligase (GCLc) expression and thereby enhancing GSH levels in enteric neurons. Thus EGCs play a central role by acting directly and indirectly as regulators of GSH in enteric neurons. EGCs can also release GDNF, a well-known mediator of enteric neuroprotection, by reducing apoptosis mediated by phosphatidylinositol-3-kinase/serine/threonine-protein kinase (PI3K/Akt). EGCs can also hamper excitotoxicity by removing GABA and glutamate (Glu) from the extracellular milieu via different transporters (i.e., $\gamma$-aminobutyric acid transporter 2, GAT2; L-glutamate transporter, Glt1) (19, 42). Thereafter, glutamine synthetase (GS), largely expressed in EGCs, converts Glu into glutamine (Gln), which is, with cysteine, an important substrate for GSH via GCLc enzyme. Finally, GABA can be transformed to glutamate by the enzyme GABA transaminase.
EGC Abnormalities in the Clinical Setting

In humans, a substantial loss of EGCs has been described in patients with various functional (8) and/or inflammatory GI diseases (9, 13). However, it still remains largely unclear whether these changes are currently directly responsible for GI dysfunctions/symptoms or whether they are the consequences of the disease.

Slow-transit constipation (STC) is a term usually applied to define a subgroup of patients with markedly delayed gut transit refractory to various therapeutic measures; some selected cases of STC (the most severe ones with colonic inertia) can be referred to surgery for total colectomy. Bassotti et al. (9) investigated a cohort of 26 severe and intractable STC patients and found that, compared with controls, the histopathological analysis of colonic full-thickness specimens revealed a wide array of ENS abnormalities characterized by a quantitative loss of enteric neurons (in both myenteric and submucosal plexuses), ICCs, and S100β-labeled EGCs. Because of the well-known properties exerted by EGCs on enteric neurons (previously discussed in this review), the authors were the first to imply a pathogenetic role of glial cell alteration in STC. This exciting possibility led them to indicate the existence of a “gliopathy” (in addition to a “neuropathy”) playing a role in STC. Whether the actual impairment of EGCs is likely to have an impact on the enteric nerves, however, awaits further confirmatory evidence not only for STC, but also for other functional GI diseases. In particular, whether EGC changes precede neuronal degeneration and loss and how this damage could occur remain unsettled. Other results showing EGC abnormalities with or without ICC depletion have been reported by Bassotti’s group in patients with diverticular disease (7) and megacolon (either idiopathic or secondary to Chagas disease) (24). The effect of the EGC abnormalities on enteric neurons under these conditions is still debated.

Concerning IBD patients with ulcerative colitis (UC), Cirillo et al. (13) demonstrated that EGCs directly participate in chronic mucosal inflammation. In particular, in the rectal mucosa of UC patients there was an increased S100β mRNA expression, immunoreactivity, and secretion that led to enhanced NO production through the specific stimulation of inducible NO synthase. Since UC is characterized by abnormal mucosal NO production and associated proinflammatory cytokines, it is tempting to speculate that these mediators might affect enteric neuron function and thus gut physiology. Furthermore, data obtained with an ex vivo human model of a jejunal segment from a patient with UC demonstrate that the EGCs can be recruited to the site of inflammation and contribute to the inflammatory response. This is a life-threatening condition characterized by recurrent intestinal subocclusive episodes and radiological evidence of air-fluid levels with concurrent severe digestive symptoms (e.g., nausea, vomiting, abdominal pain). The results of this study showed that 8 of 10 patients with a neurogenic CIPO of idiopathic origin had JCV TAg DNA sequences and 7 of 10 had TAg protein detectable in the microdissected myenteric plexuses of ileal or colonic specimens. In contrast, only 3 of 31 control subjects had TAg DNA (but no TAg protein) expression. No other neurotropic viruses (e.g., BK virus, simian virus 40, HSV, CMV, varicella zoster virus, and Epstein-Barr virus) were identified in CIPO and control specimens. The JCV capsid protein VP1 colocalized with the TAg protein in CIPO, but not in the control specimens. Notably, JCV VP1 immunolabeling was identified in cells expressing the glial marker GFAP, thus indicating that the virus specifically infects the EGCs of the myenteric plexus in CIPO. The data from this study provided a conceptual basis for a neurotropic virus, i.e., JCV, targeting EGCs and thereby leading to ENS damage and dysfunction underlying a severe dysmotility similar to CIPO.

Interestingly, in vitro experiments showed that certain viruses such as the adenovirus had a preferential tropism for EGCs compared with neurons (1). Whether neurotropic viruses may actually lead to enteric neuron abnormalities remains an open issue; however, the data of Selgrad et al. (44) and Abdo et al. (1, 2) pave the way for additional studies aimed at establishing the pathogenetic role of neurotropic viruses in GI motility disorders.

Conclusions

Since the early Virchow’s concept that glia had an exclusive mechanical support function, multiple lines of evidence have shed light on EGCs as key players in gut physiology. The intimate relationship between EGCs and enteric nerves provides a morphofunctional basis for neuroglia cross talk resulting in the maintenance and survival of intrinsic reflex circuits controlling motor, secretory, and absorptive patterns of the GI tract. Hence, targeting the EGCs leads to neuronal damage and gut dysfunction, as emerged from glial ablation models.

Taken together, the data obtained in experimental models of glia ablation and those on human diseases indicate that the role of EGCs in enteric neuroprotection is only partially elucidated. Additional studies are necessary to establish whether EGC damage leads to neurodegeneration.
Along this line, although still at an early-stage, EGC and neuronal abnormalities have been reported in functional and inflammatory diseases. Cell cultures, transgenic models, animal models of GI infection/inflammation, and human gut biopsies obtained from patients with functional and inflammatory bowel diseases represent a wide array of methodological approaches providing important results on EGCs and their neuroprotective potential. Researchers in this exciting field of neurogastroenterology should translate basic knowledge on EGCs into clinically relevant information. Knowledge regarding EGCs and their neuroprotective capacity may open new perspectives in the treatment of the enteric neuropathies underlying GI diseases.

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

AUTHOR CONTRIBUTIONS

REFERENCES


