Chapter

The Enteric Glial Network Acts in the Maintenance of Intestinal Homeostasis and in Intestinal Disorders

Juliana de Mattos Coelho-Aguiar, Carla Pires Veríssimo, Deiziane Viana da Silva Costa, Beatriz Bastos de Moraes Thomasi, Ana Carina Bon Frauches, Fabiana Pereira Ribeiro, Ana Lucia Tavares Gomes, Gerly Anne de Castro Brito and Vivaldo Moura-Neto

Abstract

The enteric nervous system (ENS), also known as second brain, innervates our gastrointestinal tract controlling its functions, such as motility, fluid secretion, nutrient absorption, and even involvement in the control of immunity and inflammatory processes. In the gut, the gliocytes are known as enteric glial cells (EGCs). Enteric glial cells form a network that permeates the entire gut. Enteric glia express the cell surface hemichannel of connexin-43 (Cx43) necessary for the propagation of Ca2 + responses, necessary to maintain their functions. In this chapter, besides the development of ENS and its glial cells and the similarities with the astrocytes in the central nervous system, we approached the important role of the glial network in the control of gut homeostasis, in the interaction with the immune system, and its participation in pathological conditions. EGCs are even capable of replacing lost neurons. Thus the enteric glia is a multifunctional cell, which through its multiple interactions maintains the integrity of the ENS allowing it to be resistant to the different and constant aggressions suffered by the digestive system.

Keywords: enteric glial cells, glial network, gut homeostasis, gut inflammation, enteric neurodegeneration

1. Introduction

The enteric nervous system (ENS), also known as second brain, innervates our gastrointestinal tract from the esophagus to the rectum including the pancreas and gallbladder, controlling its functions. The ENS develops from the neural crest cells (NCCs). At the vagal (at the level of somites 1–7) and sacral (posterior to somite 28) regions of the anteroposterior axis, some of NCCs, the enteric neural crest cells (ENCCs), enter the rudimental digestive system, proliferate, and migrate to colonize the primitive gut [1].

The differentiation of enteric neurons starts prior to the enteric glial cells (EGCs). Behind the migratory wavefront, the first neurons arise at E10–E10.5 in the foregut level. Genes of multipotent ENCCs, such as Sox10, FoxD3, and P75, are downregulated, and cells begin to express specific neuronal markers, such as βIIItubulin, RET, HuC/D, and peripherin. Subsequently, at E11.5, the glial differentiation takes place, and the ENCCs downregulate RET expression, while markers such as Sox10, FoxD3, and P75 continue to be expressed. Additionally, other genes that are known to be specifically expressed in EGCs appear, including S100B, glial fibrillary acidic protein (GFAP), and proteolipid protein 1 (PLP1). The development of the mature ENS network is not complete at birth, and the neuronal differentiation is extended to up to 2 weeks after birth (for a detailed description of enteric neurons and glial cells development, [1]).

Initially, ENCCs migrate as intersections of narrow chains of cells. Later on, as development progresses further, these cells aggregate into numerous ganglia that are connected by neuronal projections and EGCs (**Figure 1**). The role of bone morphogenetic proteins (BMPs)-2 and (BMPs)-4 in the neural cell adhesion molecule (NCAM) regulation that are differentially expressed by the cells to form these ganglion-like aggregates is already known [2–6]. The growth factor endothelin3 (EDN3) is important to keep ENS progenitors cells in a proliferative state. It inhibits reversibly the commitment and differentiation of these cells, and in this way it is involved in the correct migration of enteric neural crest cells to colonize the gut [4]. Lack of EDN3 leads to aganglionosis of the distal bowel [5]. It is well known that thyroid hormone 3,5,3′-triiodothyronine (T3) plays an important role in CNS development, and also appears to play a role in the development of the ENS. In vitro, T3 inhibits cell proliferation and stimulated neurite growth of differentiating murine enteric neural crest cells [6]. But, interestingly, this work also showed that spheres of neonate mice ENS progenitor cells increased EDN3 expression by more than 3-fold after T3 treatment, demonstrating a likely crosstalk between these signalling pathways [6]. In the adult mammalian, the ENS is organized into the myenteric and submucosal ganglionated plexuses composed of neurons and EGCs and non-ganglionated plexuses, composed of EGCs that tightly follow neuronal projections that reach all regions of the intestines, including the mucosa. The myenteric plexus (or Auerbach's plexus) is located between the outer longitudinal and circular muscle layers, and the submucosal plexus (or Meissner's plexus) lies in the submucosal region (between the mucosa and the muscular layers) [1].

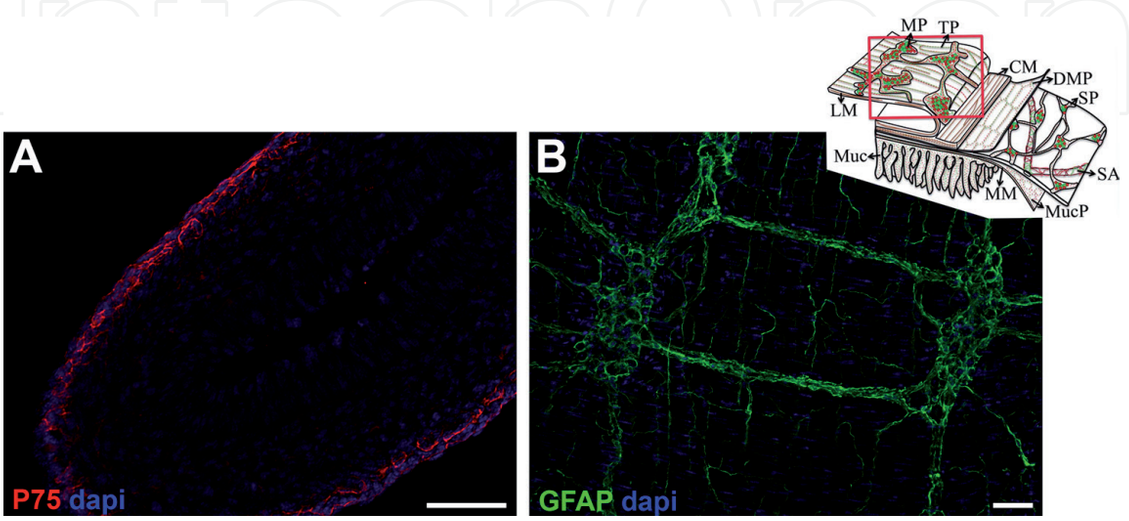

Figure 1.
(A) Transverse section of mouse embryo gut at embryonic day (E)14.5 stained for the glial marker P75. The cells are not yet organized in ganglia. (B) Longitudinal muscle with the adherent myenteric plexus (LMMP) of adult mouse colon. The enteric glial network is evidenced by GFAP staining. Scale bars: 50 μm.

EGCs are distributed across all layers of the intestine and are currently classified into four different subtypes based on their location and morphology. Intraganglionic EGCs (type I) present numerous short and irregular processes and resemble the protoplasmic astrocytes of the central nervous system (CNS); the interganglionic EGCs surround neuronal projections that connect multiple ganglia (type II); the mucosal EGCs (type III) are found around neuronal projections located in the mucosal region and present long and branched processes; and intramuscular EGCs (type IV) are bipolar and elongated and accompany the nerve fibers that cross the muscle layers [7, 8]. In fact, their wide distribution reflects on their performance in different physiological aspects of the gastrointestinal (GI) tract. Indeed, EGCs were shown to participate in the homeostasis of the intestinal epithelial barrier (IEB), to coordinate the GI motility taking part in neurotransmission, and also to modulate inflammation and immune responses.

2. Enteric glia: a unique glial cell type - similarities and differences with astrocytes

In the first studies about enteric glia, the ultrastructure of the glial cell of myenteric plexus was described as a small cell body with many processes. It was suggested that the star-like morphology, as well as the anatomical relation to neurons, resembles astrocytes from the CNS rather than Schwann cells [9]. Jessen et al. [10] showed that intraganglionic EGCs express the characteristic marker of an astrocytic cell, glial fibrillary acidic protein (GFAP), corroborating the assumption that ENS glial cells are analogous to CNS astrocytes [11, 12], although they have different embryological origins.

EGCs and astrocytes exhibit molecular similarities in their electrophysiological properties [13] and express the same group of proteins, including the GFAP [14], and the S100β-linked binding pathway [15]. However, not all properties of the EGCs are similar to astrocytes. They have different embryological origins, for example, astrocytes coming from neuroepithelium and EGCs from neural crest. During the embryonic stages, neuregulin signaling via the ErbB3 receptor is critical for the development of the EGCs, whereas astrocytes do not require such signaling [16]. Unlike astrocytes, EGCs do not express the protein of the aldehyde dehydrogenase 1 L1 (Aldh1L1) [8] but express the transcription factor Sox10 [17] and the protein PLP1, implicated in myelin production and most commonly found in oligodendrocytes and Schwann cells. In fact genic signature of EGCs seems to be more similar to that of oligodendrocytes and Schwann cells than to astrocytes [18].

Similar to astrocytes, EGCs interact with and modulate the performance of different cell types, as we will see throughout this chapter. In addition to interacting with neurons, EGCs establish multidirectional communication with other cell types, such as intestinal mucosal epithelial, muscle, mesenchymal, and immune cells [19].

3. Formation of the gut glial network and the communication through connexin-43 (Cx43) hemichannels

Yet during development, EGCs begin to form a network of interconnected cells that permeate the entire gut (**Figure 1**).

Gabella noted that a striking feature of EGC is the presence of numerous intramembrane particles on its surface [20], and a small part are gap junctions. These intramembrane particles are believed to be hemichannels. It has recently been found that, as astrocytes, enteric glial hemichannels are connexin-43 (Cx43)

compounds [21]. Cx43 hemichannels are Ca^{2+}–permeable channels that are also controlled by Ca^{2+} [21].

Like astrocytes, activated EGCs have excitability mediated by transitory intracellular Ca^{2+} elevations, considered central to many functions. Most of the enteric glial receptors for neuroactive compounds are G-protein-coupled receptors, and most of these leads to activation of downstream effectors that elevate intracellular Ca^{2+}. As mentioned by Gulbransen in his book (2014, p. 28) [22], being able to detect the increase in Ca^{2+} levels was essential to establish that neuron-glial communication occurs in ENS and to identify involved mediators. The study realized by McClain et al. [21] also showed the role of Cx43 hemichannels in the propagation of "calcium waves" through the enteric glia network and in the regulation of GI motility [21]. It was shown that glial "calcium waves" activated by extracellular ATP or ADP were disrupted by glial specific loss of Cx43 and result in aberrant ENS network activity and GI dysmotility.

Cx43 expression in EGCs is also related to inflammatory process. Neuronal loss is one of the intestinal inflammation characteristics caused by purinergic receptor activation [23]. Recently, inhibition or genetic ablation of Cx43 in EGCs prevented inflammation-induced neuronal death [24]. This is interesting because it shows that ATP released by EGCs, through Cx43 hemichannels, is involved in both inflammation and motility [21], as mentioned above.

It is possible that Cx43 expression in EGCs is also related to regulation of the intestinal epithelium barrier (IEB). Animals with ablated Cx43 in EGCs also exhibited an increased fluid content in stools [21]; this may imply a role of Cx43 in regulating the IEB, since EGCs have protective effects on enterocytes. A co-culture study showed that EGCs induced in enterocytes an increase in transcription of genes involved in cell-to-cell and cell-to-matrix adhesion and also an increase in cell adhesion [25]. Some of the glia-derived factors, for example, ATP and prostaglandins, could be released through the Cx43 hemichannels [26]. The other effects may come from cell-to-cell contact. EGCs and enterocytes express Cx43 [27], so they may perhaps be joined by Cx43 gap junctions. In fact, the membrane potential of differentiating enterocytes becomes more positive exclusively when they migrate away from the crypt-villous junction [28], possibly due to gap junctions with EGCs (they have higher membrane potential) in this region [29].

In addition to the Cx43 hemichannels, EGCs also have sodium, potassium, and aquaporin-4 channels, whose presence and subtypes vary among their subtypes. Aquaporin channels, for example, are expressed in EGCs within the plexus, but not in extraganglionic EGCs [22].

Even in autism, it has been speculated that inadequate Cx43 expression in EGC could affect GI motility, which is in fact altered in some patients. Some monogenetic autism spectrum disorders are caused by mutations in genes that encode transcriptional or epigenetic factors, for example, methylCpG2-binding protein (MeCP2) in Rett syndrome or TCF4 in Pitt-Hopkins syndrome. These mutations could affect the transcription machinery required for proper expression of Cx43 in EGCs [30].

Thus, EGCs act largely through the release of different molecules, which can happen through the Cx43 hemichannels.

4. Functions of EGCs in gut homeostasis: released factors by EGCs play a role in intestinal epithelial barrier, neurotransmission, and gliotransmission

As already mentioned, EGCs are located throughout intestinal layers and interact with different cell types within the gut. Thus, this cell type is expected

to play a number of important roles in the coordination of gut functions. In fact, studies using genetic tools to abrogate GFAP expressing cells resulted in disruption in epithelial integrity, extensive intestinal necrosis, and inflammation, followed by degeneration of enteric neurons [31, 32], evidencing the importance of EGCs for intestinal homeostasis.

EGCs exert their function through the release of important molecules. In the intestine, glial-derived neurotrophic factor (GDNF) is released by EGCs and acts as an anti-apoptotic factor to epithelial cells, neurons, and EGCs [33–36]. GDNF inhibits epithelial cell apoptosis by the activation of GFRα1–GFRα3 receptors and RET co-receptor and the activation of mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase/serine–threonine kinase (PI3K/AKT) signaling pathways [37, 38]. GDNF also inhibits apoptosis of EGCs in an autocrine manner [34]. Mu opioid receptor activation by morphine in EGCs decreases their GDNF synthesis, with consequent IEB disruption [39]. Moreover, GDNF has been shown to increase the integrity of the IEB via ZO-1 upregulation [38].

Among neuroactive molecules, ATP is the most well-characterized molecule released from EGCs. ATP is released through the opening of Cx 43 hemichannels [21, 40]. More specifically, the released ATP modifies adjacent glia, triggering intercellular Ca^{2+} waves and influencing adjacent neurons. The ATP released by EGCs may induce neuronal cell death, as discussed above (topic 2). Nitric oxide (NO), a key inhibitory neurotransmitter in the ENS and a factor that drives oxidative stress in disease, is also produced by EGCs [41, 42]. This molecule is produced by the enzyme inducible nitric oxide synthase (iNOS). Under pathological stimuli, EGCs express iNOS and produce large amounts of NO, which may be protective or deleterious depending on the circumstances. Some evidence, however, suggest that EGCs may constitutively express iNOS and that NO plays a physiological role by modulating epithelial ion transport [41].

The data above have suggested that EGCs play an essential role in neuronal support and neurotransmission. Indeed, EGCs actively participate in neurotransmission. Intraganglionic EGCs provide enteric neurons with essential precursors for the synthesis of neurotransmitters such as NO [43, 44], glutamate, and γ-aminobutyric acid (GABA) [45]. In health, EGCs provide antioxidants, like reduced glutathione [46, 47], and growth factors (e.g., GDNF) [48] to neurons. In addition, EGCs support neurotransmission by regulating the bioavailability of neuroactive substances in the extracellular environment. EGC enzymes are essential for the removal of neuroactive compounds surrounding enteric neurons. Moreover, glial potassium channels maintain neurotransmission and prevent the death of excitotoxic neurons by regulating and buffering potassium [13, 49].

A poorly understood question is how EGCs interpret and process the signals they receive from enteric neurons to eventually play their presumptive roles in neurons and GI function.

Little is known about EGCs activating ligands, but the Ca^{2+} transients probably trigger different modes of gliotransmission, such as Ca^{2+}–dependent exocytosis or factor release through the Cx43 hemichannels [50].

Boesmans et al. [51] demonstrated that enteric neurons can communicate with adjacent EGCs, releasing purines through their panexin channels. In fact, ATP and purines are the most ubiquitous signaling molecules involved in the enteric transmission of neurons to glia in vitro [52, 53] and in situ [21, 23, 54–56], but EGCs also have other receptors that allow glia to initiate responses to neurotransmitters released by neurons and other neuroactive substances, including receptors to norepinephrine, glutamate, thrombin, lipid signaling molecules, serotonin, bradykinin, histamine, and endothelin [22].

As mentioned before, it is already known that in vitro propagation of Ca^{2+} responses between EGCs depends on ATP release through hemichannels [40]. And

later it was seen that substances released by Cx43 hemichannels mediate intercellular communication between EGCs [21].

How other populations of EGCs outside the enteric ganglia interact with enteric neurons is currently unknown.

5. EGC plasticity

The ENS has a significant ability to adapt to microenvironmental influences throughout life, either by inflammatory bowel diseases or by changes in eating habits [57]. The mechanisms of cellular communication involved in the plasticity of EGCs are not yet fully understood. Understanding how EGCs act and especially how they perform the role of progenitor cell and differentiate into neuron is of paramount importance for a better understanding of how the ENS performs its complex functions.

It has already been shown that numerous neural crest-derived stem cells are found in different locations in the adult organism, including the intestine [58]. When isolated by flow cytometry for p75 markers [59] or integrin-α2 [60], or also through dissociation for in vitro cultivation of neurospheres [61, 62], EGCs can give rise to a large number of other cells including glial cells, neurons, and even myofibroblasts. Following transplantation of these cells into intestinal explants, EGCs differentiate into glial and neuronal cells [61, 63]. These data underscore the plastic potential of EGCs that, when transplanted into the CNS, are able to function as oligodendrocytes and astrocytes [26]. It is noteworthy that under different physiological conditions and after injury [60], EGCs proliferate and differentiate into neuron only upon specific injury situations [64, 65]. Liu and colleagues have shown that it is possible to induce neurogenesis in the myenteric plexus in vivo by activating the serotonin receptor upon administration of the 5-hydroxytryptamine 4 (5-HT 4) agonist [66]. Indeed, other studies in postnatal bowel suggest that serotonin also promotes ENS repair and neurogenesis via 5-HT4 receptor [67, 68]. Moreover, studies have shown that mouse and human EGCs undergo neurogenesis after colitis [65, 69].

Under chemical injury with benzalkonium chloride detergent (BAC), it was possible to observe neurogenesis in vivo. About 3 months after injury, EGCs adjacent to the aganglionic area give rise to sox10-positive glial cells expressing the neuronal marker HuC/D [64]. It has been proposed that interruption of contact between cells (ganglion structure dissociation) may initiate neurogenesis from precursor cells expressing EGCs markers (sox10, p75, S100β, GFAP). These studies suggest that tissue dissociation to establish cell culture, as well as that observed in chemical injury, could activate the neurogenic potential of EGCs.

A recent work, however, suggested that constitutive neurogenesis occurs in the gut [70], contrasting with data obtained by other groups that suggest that intestinal neurons are not easily replaced under healthy conditions [71–73]. Moreover, this study highlighted a population of nestin-positive adult progenitor cells that give rise to new neurons, different from that of GFAP-positive EGCs. These data contrast with previous works that had shown nestin and GFAP co-expression by EGCs [60]. Furthermore, nestin-expressing intestinal NSCs cells give rise to neurosphere-derived neurons and glia in vitro. Besides these cells can differentiate into glial, neuronal, and mesenchymal lineages in vitro and also generate neurons in vivo [74].

EGCs do not produce extracellular matrix (ECM). However, their processes contact basal lamina proteins including heparan sulfate proteoglycan, type IV collagen, and laminin [11, 75, 76]. This suggests that the microenvironment is also a factor of great relevance for the function of EGCs and neurons. Recent data demonstrate

that EGCs in vitro, in absence of appropriate substrates were stimulated to initiate neuronal differentiation. Therefore, it seems that the contact of adult EGCs with laminin plays a crucial role in inhibiting their potential for neuronal differentiation (Veríssimo et al., 2009).

6. Implications of EGCs in pathological conditions

The importance of the correct neuron-glial communication is evidenced in a situation of intestinal inflammation and neurodegeneration, when EGCs act as a direct mediator of neuronal cell death.

6.1 EGCs in inflammatory bowel diseases (IBD)

Chronic inflammation in the GI tract can cause important changes in the ENS, as demonstrated by several studies in patients with IBD, such as ulcerative colitis (UC) and Crohn's disease (CD) [77, 78]. Both UC and CD are characterized by inflammation, which is accompanied by the release of a range of pro-inflammatory cytokines, following intestinal dysmotility [79, 80].

An increase in GDNF and GFAP immunolabeling was observed in EGCs in inflamed colonic mucosa of patients with UC, CD, and *Clostridioides difficile* (*C. difficile*) infectious colitis [78]. In addition, S100B upregulation has also been identified in a variety of diseases, such as UC [42, 81, 82], celiac disease [83], and intestinal mucositis induced by antineoplastic drugs [84, 85]. Increased expression of S100B was also found in the intestine of humans with *C. difficile* infection (CDI), in animal model of CDI, and in mouse ileal loop injected with *C. difficile* toxin A (TcdA) (unpublished data).

In intestinal injury, reactive gliosis is a response of EGCs to protect the neuronal network during intestinal inflammation [86]. However, depending on the degree of inflammation, this event may cause damage to neurons and to EGCs themselves due to a deregulated response of these cells to the virulence factors of pathogenic bacteria or pro-inflammatory mediators released by immune cells, neurons, or EGCs. This dual effects may have an important effect in the instability of the release of protective and dangerous factors, such as GDNF, an anti-apoptotic factor, and S100B, a pro-inflammatory cytokine, by EGCs [34, 35].

6.1.1 Ulcerative colitis and Crohn's disease

A strong upregulation in levels of GDNF was reported in the intestinal crypts and in the myenteric and submucosal plexuses in patients with CD. In UC, GDNF immunoreactivity was reported to be less pronounced than CD. However, no alteration in GFR-1 was evidenced in patients with CD and UC. GFR-1 is a receptor for GDNF binding that is predominantly found at the basolateral parts of the human colonic epithelium [37].

The EGCs regulate the epithelial barrier function and inflammation through the release of S-nitrosoglutathione (GSNO), a potent nitric oxide donor. Interestingly, EGCs are the main source of GSNO within the intestine. It has been shown that the levels of GSNO are reduced in CD and UC [35]. GSNO regulates the intestinal permeability by stimulating in enterocytes the upregulation of proteins of tight junctions, such as occludins and ZO-1, and inhibiting the increase of phosphorylated myosin light chain (PMLC), as well as improving the location of these proteins [87].

EGCs from human colonic tissues with Crohn's disease have reduced 15-hydroxyeicosatetraenoic (15-HETE) synthesis. As GSNO, 15-HETE controls

the paracellular permeability of the IEB by inhibiting adenosine monophosphate-activated protein kinase (AMPK) and regulating ZO-1 expression [88].

NO, such as tumor necrosis factor-α (*TNF-α) and PGE2, plays an important role in the pathogenesis of ulcerative colitis and is secreted by EGCs.* S100B can induce increased NO release, as well as TNF-α and PGE2, by murine and human EGCs via S100B/TLR4 [42, 82].

Losses in 61% of the enteric neurons and 38% of the EGCs have been reported during ulcerative colitis in human [77]. In fact, activation of EGCs during colitis induced by dinitrobenzene sulfonic acid (DNBS) in mice had been shown to be the central mechanism in the development of enteric neuropathy, since the gating of glial Cx43 hemichannels by nitric oxide and subsequent ATP release are required for enteric neuron death [24].

6.1.2 Colitis by Clostridioides difficile

C. difficile is an obligate anaerobic, spore-forming Gram-positive bacillus that can colonize, germinate, and proliferate in the human gut after antibiotic use [89, 90]. The incidence of *C. difficile* infection (CDI) across the world has increased with 107,760 admissions per year [91, 92]. The clinical disease ranges from mild diarrhea to toxic megacolon, colonic perforation, and death [93].

The major virulence factors of *C. difficile* are toxins A and B (TcdA and TcdB). TcdA and TcdB stimulate the release of a variety of mediators such as interleukin (IL)-1β, IL-17, IL-23, TNF-α, CXC motif chemokine ligand 4 (CXCL4), CXCL2, and inhibitory macrophage migration factor (MIF) in several cells, such as epithelial cells, immune cells, and enteric neurons [94–97]. In contrast to those cells, EGCs challenged with TcdA and TcdB do not release detectable levels of IL-1β, interferon-gamma (INF-γ), and TNF-α [98].

The first studies on changes in the ENS evoked by *C. difficile* toxins showed that TcdA and TcdB excite enteric neurons stimulating the release of substance P and vasoactive intestinal peptide (VIP) via noradrenergic transmission inhibition and IL-1β pathway, respectively, resulting in neutrophil recruitment and secretory diarrhea [99–101].

A recent study demonstrated increased cell population expressing both HuC/D and SOX2 in inflamed colonic tissues in patients with CDI [65]. So, these EGCs are important for generating new neurons after intestinal injury.

A study of 447 and 444 patients with *C. difficile*-associated diarrhea acquired in the community and hospital, respectively, showed GI dysmotility in these patients after 1 year of the last diarrhea episode. Among the dysmotilities are IBD, gastro-esophageal reflux disease, constipation, and dyspepsia [102]. These dysmotilities have been shown to be related to ENS changes. Deregulated activation of EGCs during inflammation may alter their regulatory role in motility (discussed in topic 3), causing intestinal dysmotility.

It was demonstrated that TcdB stimulates morphological alteration and apoptosis in EGCs *in vitro* [98, 103]. TcdB-induced apoptosis of EGCs involves the NADPH oxidase/ROS/JNK/caspase-3 signaling pathway independently of the mitochondrial pathway [103]. In addition, TcdB induces senescence in EGCs. Cell senescence is characterized by alterations in the cell cycle, changes in metabolism, morphology, and gene expression that together may contribute to persistent inflammation [104].

6.1.3 Inflammation by other causes

It has been demonstrated that EGCs are involved in decreased infection foci and IL-8 secretion and in the inhibition of alterations in IEB resistance in infection by

Shigella flexneri in rabbit ileal loop model. Similar findings were found in human colonic mucosa explants infected with *S. flexneri*. GSNO released by EGCs showed to be a mediator responsible for protecting epithelial cells from *S. flexneri*-induced effects [105].

Giardia duodenalis (also known as *G. lamblia* or *G. intestinalis*) is a protozoan parasite capable of causing sporadic or epidemic diarrheal illness. Giardia duodenalis-induced infection is one of the most common human parasitic diseases worldwide [106]. Studies have shown a reduction in EGCs from the submucosal plexus of the mouse duodenum and jejunum during infection induced by assemblages A and B of *G. duodenalis*. However, only assemblages B of *G. duodenalis were observed to* induce a reduction in those cells from the duodenal myenteric plexus. Surprisingly, mice infected with *G. duodenalis* did not exhibit diarrhea and any alterations in GI transit time [107].

Antineoplastic drugs, such as 5-FU, irinotecan, and oxaliplatin, have been currently used to treat several types of cancer, including breast and colorectal cancer. Mucositis and diarrhea are common side effects of these antineoplastic drugs [108]. Many cells are stimulated to release inflammatory mediators during intestinal mucositis, and persistent GI over-contractility has also been demonstrated, even after inflammation has resolved, suggesting that chemotherapy might affect gut neuronal and EGC function [109].

During intestinal mucositis induced by oxaliplatin, reduced GFAP and increased S100B protein expression were evidenced, as well as reduced co-localization of GFAP and S100B in ileal myenteric plexus of mice [110].

In fact, increased S100B release by EGCs has been shown to be a mediator in charge of causing neuronal death, as well as reactive gliosis, epithelial damage, and inflammatory response (release of IL-6, TNFα, and NO) during 5-FU-induced intestinal mucositis via S100B/RAGE/NFκB [84].

As we will deepen later, EGCs can be stimulated by immune cells during intestinal inflammation. A recent study showed that mediators released by mast cells cause reactive gliosis and neuronal death together with the intestinal mucositis induced by irinotecan [85].

Figure 2 shows a schematic highlighting how EGCs are affected by and participates on intestinal inflammation.

6.2 EGCs in neurodegenerative diseases

Due to its great interaction with neurons and modulation of neuronal responses, it is possible to imagine that EGCs play a central role in neurodegenerative diseases. Indeed, the role of EGCs seems to be compromised in many neurodegenerative diseases, and this is true for both CNS and ENS.

Enteric neurodegeneration is a common marker for a group of diseases classically known as enteric neuropathies. The changes found present as alterations in enteric smooth cells and/or compromised functioning of the ENS—often impacting in GI motility [111]. The neuropathies chronic intestinal pseudo-obstruction (CIPO) and slow transit constipation (STC) are characterized by neurodegeneration affecting the lower GI tract. Moreover, it has already been shown that enteric glia is implicated in Parkinson's disease (PD), and participation in Alzheimer's disease (AD) is speculated [111].

CIPO is a condition characterized by failure of GI motility without apparent mechanical lesion [112]. Histological patterns show different classes of the disease depending on the cell type involvement (enteric neurons, smooth muscle cells, and interstitial cells of Cajal). Enteric neuron degeneration promotes intestinal neuromuscular disorders [111, 113]. In chronic idiopathic intestinal pseudo-obstruction

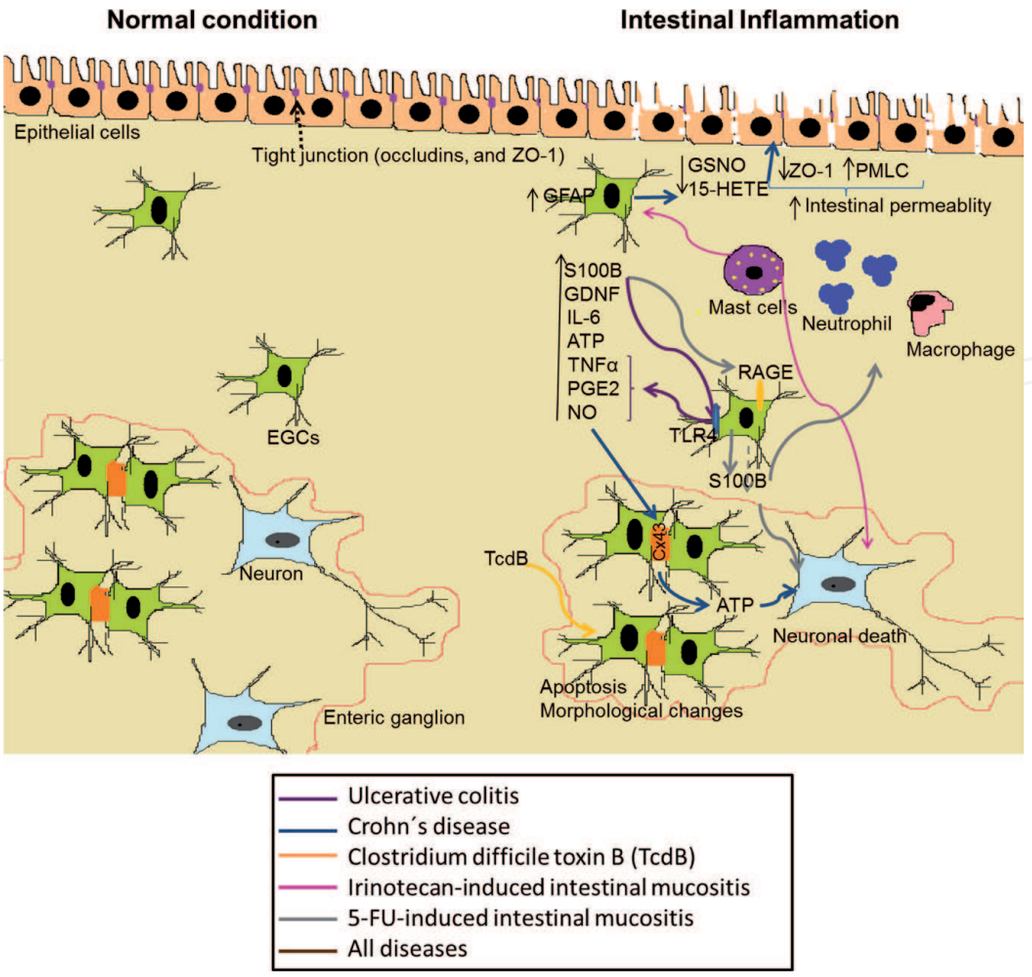

Figure 2.
Mediator release by EGCs during intestinal inflammation and their role in the pathogenesis of intestinal inflammatory diseases. During intestinal inflammation promoted by ulcerative colitis, Crohn's disease, colitis induced by C. difficile*, irinotecan- and 5-FU-induced intestinal mucositis, and enteric glial factors (S100B, GDNF and GFAP) are upregulated. EGCs are stimulated to secrete S100B, GDNF, IL-6, ATP, TNF-α, PGE2, and NO. In addition, EGCs produce reduced levels of GSNO and 15-HETE during Crohn's disease, resulting in increase of intestinal permeability by ZO-1 downregulation and PMLC upregulation. NO release by EGCs promotes Cx43 opening with consequent ATP release by EGCs, resulting in neuronal death in Crohn's disease. In 5-FU-induced intestinal mucositis, S100B released by EGCs via RAGE receptor activation drives reactive gliosis, neuronal death, and immune cell activation, whereas mediators released by mast cells induce reactive gliosis and neuronal death during irinotecan-induced intestinal mucositis . In ulcerative colitis, S100B activates TLR4, resulting in TNFα, PGE2, and NO by EGCs.* C. difficile *Toxin B (TcdB) induces EGCs apoptosis and morphological changes.*

(CIIP), EGC infection by JC virus (polyomavirus) has been described suggesting a role of enteric glia in this enteric neuropathy [114].

Constipation is a common functional GI disorder characterized by infrequent bowel motions and/or incomplete defecation [115]. Studies on the neuronal subtypes involved in the STC pathogenesis are still very uncertain. It was pointed that excessive production of NO in the colonic myenteric plexus of STC patients would inhibit propulsive contraction. Results about other neurotransmitters as VIP, substance P, and serotonin were contradictory [113]. Besides the decrease of enteric neurons and interstitial cells of Cajal, STC also presents a significant decrease of EGCs [116], and some discussion has emerged about constipation being a neuro-gliopathy [79]. Several reports showed that different conditions presenting constipation have a feature: loss of EGCs, and it points to a pathophysiological meaning since the EGC directly regulates enteric neurons and interstitial cells of Cajal through neurotrophic factors [116, 117] and ATP signaling [79, 118].

6.2.1 Parkinson's disease

In the last years, the literature has shown that some pathological conditions, such as PD, classically described to compromise the CNS are now recognized as multicentric neurodegenerative processes since they affect different systems such as the ENS [119–122]. A number of non-motor symptoms in PD have been identified, and many of them manifest early, even before the clinical stage of the disease (characterized by emergence of the classic motor features) when the diagnosis can be made [123]. They found lesions in autopsies of patients by identifying the presence of intraneuronal inclusions called Lewy bodies/neuritis, which are described as protein agglomerates where α-synuclein is the main constituent. The areas primarily affected were olfactory structures, the dorsal motor nucleus of the vagus nerve and the ENS [124, 125]. According to Braak's hypothesis, there could occur a migration of the ENS lesion via the vagus nerve to the CNS [124]. Indeed, Lewy neurites are detectable in the presymptomatic stage of PD along the autonomic pathways and in the GI tract [126]. Besides, analysis of human colon biopsies obtained 2 to 5 years before PD onset showed the presence of pathologic α-synuclein in neurodegeneration sites, suggesting that colonic α-synuclein staining can be considered a biomarker of premotor PD symptoms [127].

GI symptoms are the most debilitating PD non-motor features and are present in almost every patient at some stage of the disorder [124, 128, 129]. The symptoms commonly reported by patients are weight loss, dysphagia, decreased frequency of intestinal peristalsis, and difficulty in defecation [130]. Recent evidences indicate that PD pathological alterations in the gut involve EGCs and probably impairment of their critical role in GI physiology. In fact, colonic biopsies of PD patients showed an increased expression of GFAP both at the transcript and protein levels [131, 132] as well as a reduction in GFAP phosphorylation. These features strongly suggest that reactive gliosis may be associated with degenerative diseases [131].

In PD colon biopsies the upregulation of GFAP was accompanied by an increase in the expression of pro-inflammatory cytokines, mainly TNF-α, IFN-γ, IL-1β, and IL-6 [132]. These data suggest a link between glial dysfunction and enteric inflammation in the colon of PD patients.

Alterations in IEB have been observed in patients and animal models of PD [133, 134]. Modifications in protein levels and protein distribution that compose the barrier (e.g., occludin) were documented [128]. In agreement with this, PD patients show fecal biomarkers of inflammation as calprotectin and also increased intestinal permeability as alpha-1-antitrypsin [134]. It is known that the IEB is strongly regulated by EGCs [37–39]. Since EGCs is sensitized in PD patients and modulates all these processes, it is speculated that IEB could be impaired by altered glial signaling which could contribute to the inflammatory process.

Intestinal dysmotility is the symptom that affects directly patient's quality of life and is shared among PD patients. Constipation is the most common non-motor symptom manifested in both prodromal and clinic phases of PD [135–137]. Recently, constipation was included as a criterion for prodromal PD diagnostic, and discussion about the validation of constipation as a risk factor for the development of PD has been recurrent [138]. As already discussed, impairments in EGCs activity produce constipation due to a loss in the neural control of gut motility [21].

However, despite the evidence, there is still no direct demonstration of how enteric glia is involved in PD, either in the cause of the disease or its consequences.

In this way, the suggestion that PD could onset in the gut emerges from the identification of activated EGCs, local inflammation, impaired IEB, aggregation of α-synuclein in neurons, and GI disorders in a window prior to the appearance of classic motor deficits. Recently, Seguella et al. suggest that EGCs could be the

"missing link" that connects the ENS to the CNS [139]. The authors called attention to enteric glial cell-mediated inflammatory response, which could reach the CNS by the gut-brain axis and lead to neuronal cell death and disruption of synaptic interactions [139, 140]. Thus, EGCs would function as an "entrance door" to noxious stimuli from the intestinal lumen that could damage the CNS. However, the mechanisms by which the pro-inflammatory glial mediators rise to the CNS still remains to be clarified [139].

6.2.2 Alzheimer's disease

Alzheimer's disease (AD) is the most common neurodegenerative disorder affecting people in the world. The neurodegeneration causes a progressive cognitive decline and loss of working memory [141]. Among the non-cognitive symptoms of AD are the GI symptoms which point to a role of ENS in AD [142]. In fact, the brain biomarker of AD, the extracellular plaques containing β-amyloid, has already been described in the intestinal submucosa of patients [143] which is in agreement with the expression of amyloid precursor protein in enteric neurons and also EGCs [144]. The discussion of the peripheral immune response has been widely debated as the pathogenic pattern of AD that contributes to central neurodegeneration [145, 146]. In this context, some discussion has been raised about EGC possibly acting as a peripheric coordinator of immune differentiation of T cells [139] since EGCs express the major histocompatibility complex II and T-cell costimulatory molecules [147–149]. As mentioned above, EGCs are able to respond to an inflammatory environment contributing to the process, activate enteric neurodegenerative mechanisms, and immunomodulate the IEB. All these features could contribute to an inflammatory peripheral state and sensibilization of CNS through the blood–brain barrier [139]. It is still speculative to relate these glial interactions to AD, but there are indications of an immunomodulatory relevance of this cell in the GIT, as will be discussed again below.

6.3 Interactions between EGCs and the immune system

Recently, insightful and essential findings have shed light in the field of neuroimmunology, especially with the development of high solution technological approaches to underlie neuroimmune communications. It has been proposed that the immune and the nervous systems interact in health and disease and are expected to function alongside to promote tissue homeostasis [150]. More specifically, neuroimmune interactions have been suggested by understanding the relative anatomical positioning of cell types and their dynamics within the tissue in homeostasis and response to insults. Moreover, the expression of corresponding ligands and receptors by immune and nervous cells, for instance, may determine physiological interactions between the two systems. However, efforts to identify mechanisms to decipher how immune and neural cells interact in a steady-state environment and respond to genetic and epigenetic cues are still a challenge to be addressed.

Because the GI tract is the connection between the external with the internal environments of the body, the ENS is continuously exposed and expected to interact with the extrinsic (dietary and microbiota-derived metabolites) and intrinsic (immune system and stromal cells) environments of the gut. The strategical anatomical positioning of ENS and immune cells throughout the GI wall and their physiological features are crucial to defeat pathogens and maintain the intestinal homeostasis. Emerging studies have identified two distinct types of tissue-resident macrophages within the intestinal wall that are closely associated with ENS cells [150]. Lamina propria macrophages (LpMs) preferentially display pro-inflammatory phenotype and are the most abundant

cell group located just beneath the intestinal epithelium. These cells, together with neuronal processes and mucosal EGCs, form tight physical and functional barriers that protect the intestines against pathogens, although the mechanisms that underlie those interactions are still to be further explored [150].

At the level of the myenteric plexus, muscularis macrophages (MMs) are closely associated with neuron cell bodies and fibers and EGCs and present a tissue-protective phenotype. Similar to microglia in the CNS, MMs can phagocyte neuronal debris during homeostasis [70]. Another population of gut self-maintaining macrophages (gMacs) was described to be fundamental for ENS homeostasis since the genetic depletion of those macrophages led to a loss of enteric neurons resulting in reduced intestinal function [151]. Moreover, enteric neurons and innate lymphoid cells type 2 (ILC2) functionally integrate to initiate type 2 immune responses. The integration between neuron-ILC2 units is necessary for cytokine production and inflammation repair upon worm infection [152, 153].

EGCs also appear to participate in immune responses, but so far, its impact on immune cells is still relatively unexplored under homeostasis. However, an exciting study has recently discussed that GDNF secreted by EGCs activates IL-22-producing ILC3 via Ret signaling [154]. Interestingly, Ret signaling regulates Peyer's patches organogenesis, underlining the prospective role of EGCs in orchestrating innate immune functions in the gut [155]. Furthermore, experiments performed in the submucosal plexus (SMP) from patients with functional dyspepsia (FD) showed that morphological alterations both in EGCs and neurons are due to increased numbers of eosinophil and mast cell within ganglionic structures [156]. This also suggests that EGCs and the immune system work together to maintain the intestinal homeostasis.

It is known that EGCs protect T lymphocytes from cell death by upregulating the expression of IL-7 after exposure to pro-inflammatory cytokines such as IL-1β and TNFα [157]. Moreover, EGCs were suggested to have immunosuppressive characteristics in CD by inhibiting T-cell proliferation [158]). Nonetheless, the cellular and molecular mechanisms that govern the role of EGCs in intestinal pathologies remain unclear. EGCs express MHCII [148] that is upregulated under inflammatory conditions [149, 159], conferring an immunological feature to these cells in a pathological environment. Moreover, EGCs can secrete and respond to IL-1β, IL-6, and IL-10 and nitric oxide in vitro, as already mentioned, suggesting another property of these cells in the mediation of inflammatory responses [24, 160, 161]. Although, it is plausible that EGCs have an important function in modulating neuroimmune interactions, understanding their specific contributions to the maintenance of the gut homeostasis would be useful to decipher their roles in inflammatory disorders.

This would possibly suggest an immune protective role of EGCs to maintain the mucosal barrier. However, those studies failed in showing direct evidence that EGCs are necessary for intestinal barrier function. On the other hand, studies in which EGCs were disrupted but not entirely ablated did not show any noticeable signs of inflammation. In contrast, disruption in EGC homeostasis culminated in changes in mucosal function as well as in neurochemical coding, leading to alterations in enteric neurons and consequently in motor activity [162–164]. Thus, taken together, the immunological roles of EGCs protecting the intestinal environment from damage remain contentious by using genetic tools to ablate/disturb these cells.

7. Conclusion

As we could notice in this chapter, there are still many unexplained aspects of the EGCs physiology. Although we have already found interesting studies that show their

relation with neurons or alterations in cases of inflammation, the exact mechanisms by which EGCs activates neurons to control GI motility are still unknown. Little is known about their interaction with the immune system, for example, or their participation in neurodegenerative diseases that affect both ENS and CNS. Recently, Seguella et al. suggest that EGCs could be the "missing link" that connects the ENS to the CNS [139]. EGCs in the context of disease could be an important target for diagnosis and therapy of many intestinal and neurological disorders.

Taken together, these evidence show the importance of EGCs for the maintenance of intestinal homeostasis and that disturbance of glial functions could alter GI physiology through the modulation of neurotransmission and of the responses of the different cellular types or even activation of cellular signals to enter the neuronal differentiation processes in specific situations.

Author details

Juliana de Mattos Coelho-Aguiar[1,2*], Carla Pires Veríssimo[2], Deiziane Viana da Silva Costa[3], Beatriz Bastos de Moraes Thomasi[4], Ana Carina Bon Frauches[5], Fabiana Pereira Ribeiro[2], Ana Lucia Tavares Gomes[4], Gerly Anne de Castro Brito[3] and Vivaldo Moura-Neto[1,2]

1 Instituto Estadual do Cérebro Paulo Niemeyer, Secretaria de Estado de Saúde do Rio de Janeiro, Rio de Janeiro, Brazil

2 Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

3 Departamento de Morfologia, Faculdade de Medicina, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil

4 Departamento de Neurobiologia, Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil

5 The Francis Crick Institute, London, United Kingdom

*Address all correspondence to: jumcoelho@gmail.com

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