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

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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 Ca^{2+} 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 β III tubulin, 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 aganglionicosis 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].

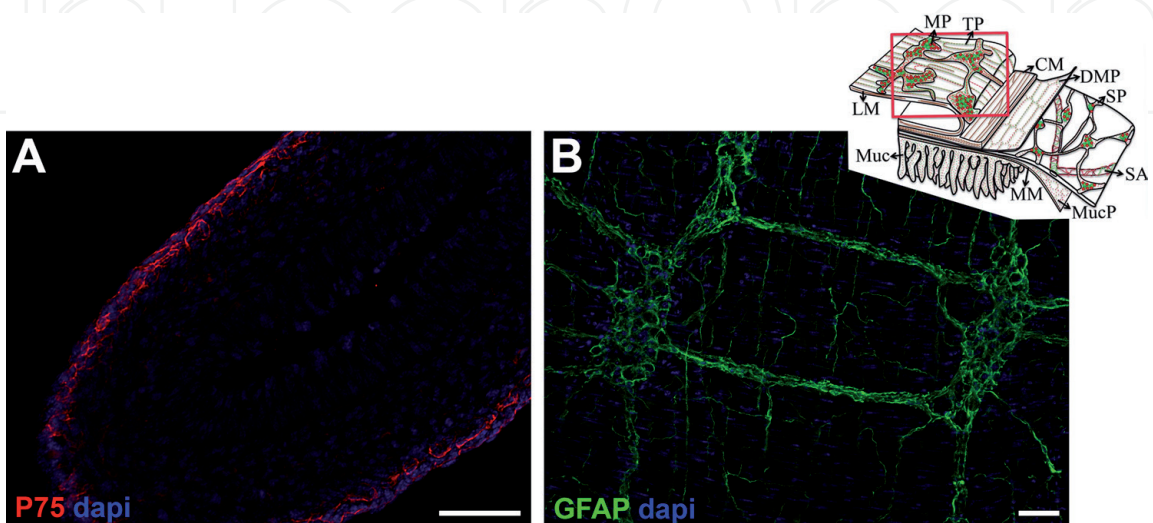


Figure 1. (A) Transverse section of mouse embryo gut at embryonic day (E)14.5 stained for the glial marker P75. The cells are not yet organized in ganglia. (B) Longitudinal muscle with the adherent myenteric plexus (LMMP) of adult mouse colon. The enteric glial network is evidenced by GFAP staining.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

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 S100 β -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-glia 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 GFR α 1–GFR α 3 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 Ca²⁺ 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 Ca²⁺ transients probably trigger different modes of gliotransmission, such as Ca²⁺–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 Ca²⁺ 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- α 2 [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-HT₄) 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, S100 β , 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)-1 β , 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-1 β , interferon- γ (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-1 β 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, gastroesophageal 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-contraction 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, TNF α , and NO) during 5-FU-induced intestinal mucositis via S100B/RAGE/NF κ B [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

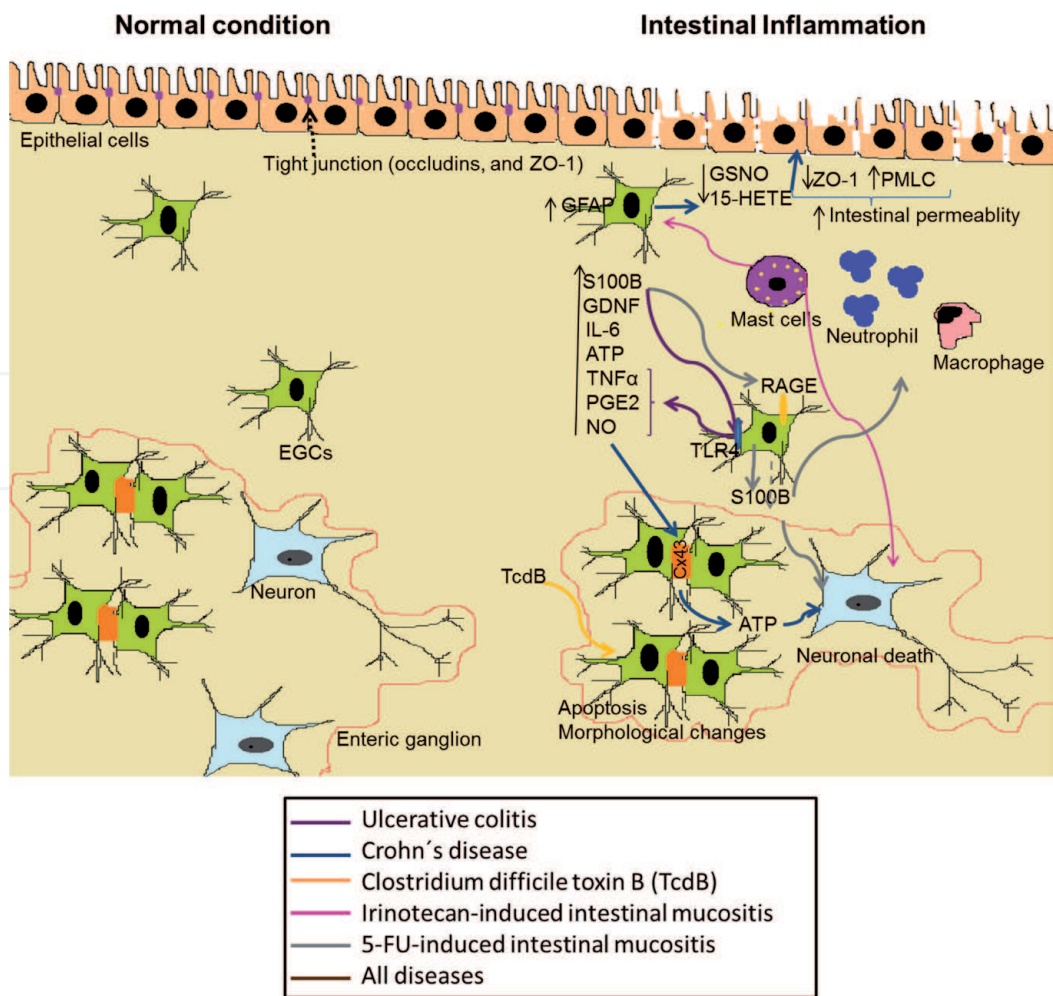


Figure 2. Mediator release by EGCs during intestinal inflammation and their role in the pathogenesis of intestinal inflammatory diseases. During intestinal inflammation promoted by ulcerative colitis, Crohn's disease, colitis induced by *C. difficile*, irinotecan- and 5-FU-induced intestinal mucositis, 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 TNF α , 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-1 β , 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 resolution 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 phagocytose 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-1 β and TNF α [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-1 β , 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.

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References

- [1] Coelho-Aguiar J, Bon-Frauches AC, Gomes ALT, Verissimo CP, Aguiar DP, Matias D, et al. The enteric glia: Identity and functions. *Glia*. 2015;**63**(6):921-935
- [2] Faure C, Chalazonitis A, Rheume C, Bouchard G, Sampathkumar S-G, Yarema KJ, et al. Gangliogenesis in the enteric nervous system: Roles of the polysialylation of the neural cell adhesion molecule and its regulation by bone morphogenetic protein-4. *Developmental Dynamics: An Official Publication of the American Association of the Anatomists*. 2007 Jan;**236**(1):44-59
- [3] Rollo BN, Zhang D, Simkin JE, Menheniott TR, Newgreen DF. Why are enteric ganglia so small? Role of differential adhesion of enteric neurons and enteric neural crest cells. *F1000Research*. 2015;**4**:113
- [4] Bondurand N, Natarajan D, Barlow A, Thapar N, Pachnis V. Maintenance of mammalian enteric nervous system progenitors by SOX10 and endothelin 3 signalling. *Development*. 2006;**133**:2075-2086
- [5] Woodward MN, Sidebotham EL, Connell MG, Kenny SE, Vaillant CR, Lloyd DA, et al. Analysis of the effects of endothelin-3 on the development of neural crest cells in the embryonic mouse gut. *Journal of Pediatric Surgery*. 2003;**38**:1322-1328
- [6] Mohr R, Neckel P, Zhang Y, Stachon S, Nothelfer K, Schaeferhoff K, et al. Molecular and cell biological effects of 3,5,3'-triiodothyronine on progenitor cells of the enteric nervous system in vitro. *Stem Cell Research*. 2013;**11**:1191-1205
- [7] Gulbransen BD, Sharkey KA. Novel functional roles for enteric glia in the gastrointestinal tract. *Nature Reviews. Gastroenterology & Hepatology* [Internet]. 2012;**9**(11):625-632. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22890111>
- [8] Boesmans W, Lasrado R, Vanden Berghe P, Pachnis V. Heterogeneity and phenotypic plasticity of glial cells in the mammalian enteric nervous system. *Glia* [Internet]. 2015;**63**(2):229-241. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25161129>
- [9] Gabella G. Glial cells in the myenteric plexus. *Zeitschrift fur Naturforschung Teil B, Chemie, Biochemie, Biophysik, Biologie und verwandte Gebiete*. 1971;**26**(3):244-245
- [10] Jessen KR, Duance VC, Mirsky R, Timpl R, Bannerman PGC. Light microscopic immunolocalization of laminin, type IV collagen, nidogen, heparan sulphate proteoglycan and fibronectin in the enteric nervous system of rat and Guinea pig. *Journal of Neurocytology*. 2005;**15**(6):733-743
- [11] Gershon MD, Rothman TP. Enteric glia. *Glia*. 1991;**4**(2):195-204
- [12] Jessen KR, Mirsky R. The origin and development of glial cells in peripheral nerves. *Nature Reviews. Neuroscience*. 2005;**6**(9):671-682
- [13] Hanani M, Francke M, Hartig W, Grosche J, Reichenbach A, Pannicke T. Patch-clamp study of neurons and glial cells in isolated myenteric ganglia. *American Journal of Physiology. Gastrointestinal and Liver Physiology*. 2000;**278**(4):G644-G651
- [14] Jessen KR, Mirsky R. Glial cells in the enteric nervous system contain glial fibrillary acidic protein. *Nature* [Internet]. 1980;**286**(5774):736-737. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/6997753>

- [15] Ferri GL, Probert L, Cocchia D, Michetti F, Marangos PJ, Polak JM. Evidence for the presence of S-100 protein in the glial component of the human enteric nervous system. *Nature* [Internet]. 1982;**297**(5865):409-410. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/7043279>
- [16] Riethmacher D, Sonnenberg-Riethmacher E, Brinkmann V, Yamaai T, Lewin GR, Birchmeier C. Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. *Nature*. 1997;**389**(6652):725-730
- [17] Young HM, Jones BR, McKeown SJ. The projections of early enteric neurons are influenced by the direction of neural crest cell migration. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 2002;**22**(14):6005-6018
- [18] Rao M, Nelms BD, Dong L, Salinas-Rios V, Rutlin M, Gershon MD, et al. Enteric glia express proteolipid protein 1 and are a transcriptionally unique population of glia in the mammalian nervous system. *Glia* [Internet]. 2015;**63**(11):2040-2057. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26119414>
- [19] Ruhl A. Glial cells in the gut. *Neurogastroenterology and Motility* [Internet]. 2005;**17**(6):777-790. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16336493>
- [20] Gabella G. Ultrastructure of the nerve plexuses of the mammalian intestine: The enteric glial cells. *Neuroscience* [Internet]. 1981;**6**(3):425-436. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/7219723>
- [21] McClain JL, Grubisic V, Fried D, Gomez-Suarez RA, Leininger GM, Seigny J, et al. Ca²⁺ responses in enteric glia are mediated by connexin-43 hemichannels and modulate colonic transit in mice. *Gastroenterology* [Internet]. 2014;**146**(2):497-507. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24211490>
- [22] Gulbransen BD. Enteric glia. *Colloquium Series on Neuroglia in Biology and Medicine: From Physiology to Disease* [Internet]. 2014;**1**(2):1-70. Available from: <http://www.morganclaypool.com/doi/10.4199/C00113ED1V01Y201407NGL002>
- [23] Gulbransen BD, Bashashati M, Hirota SA, Gui X, Roberts JA, MacDonald JA, et al. Activation of neuronal P2X7 receptor-pannexin-1 mediates death of enteric neurons during colitis. *Nature Medicine*. 2012;**18**(4):600-604
- [24] Brown IA, McClain JL, Watson RE, Patel BA, Gulbransen BD. Enteric glia mediate neuron death in colitis through purinergic pathways that require connexin-43 and nitric oxide. *Cellular and Molecular Gastroenterology and Hepatology* [Internet]. 2016;**2**(1):77-91. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26771001>
- [25] Van Landeghem L, Mahe MM, Teusan R, Leger J, Guisle I, Houlgatte R, et al. Regulation of intestinal epithelial cells transcriptome by enteric glial cells: Impact on intestinal epithelial barrier functions. *BMC Genomics* [Internet]. 2009;**10**:507. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19883504>
- [26] Cherian PP, Siller-Jackson AJ, Gu S, Wang X, Bonewald LF, Sprague E, et al. Mechanical strain opens connexin 43 hemichannels in osteocytes: A novel mechanism for the release of prostaglandin. *Molecular Biology of the Cell*. 2005;**16**(7):3100-3106
- [27] Leapheart CL, Qureshi F, Cetin S, Li J, Dubowski T, Baty C, et al. Interferon-gamma inhibits intestinal

restitution by preventing gap junction communication between enterocytes. *Gastroenterology*. 2007;**132**(7):2395-2411

[28] Cremaschi D, James PS, Meyer G, Smith MW. Positional dependence of enterocyte membrane potential in hamster and rabbit enterocytes. *Comparative Biochemistry and Physiology. A, Comparative Physiology*. 1984;**78**(4):661-666

[29] Liu YA, Chung YC, Pan ST, Shen MY, Hou YC, Peng SJ, et al. 3-D imaging, illustration, and quantitation of enteric glial network in transparent human colon mucosa. *Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society*. 2013;**25**(5):e324-e338

[30] Grubisic V, Parpura V. The second brain in autism spectrum disorder: Could connexin 43 expressed in enteric glial cells play a role? *Frontiers in Cellular Neuroscience*. 2015;**9**:242

[31] Bush TG, Savidge TC, Freeman TC, Cox HJ, Campbell EA, Mucke L, et al. Fulminant jejuno-ileitis following ablation of enteric glia in adult transgenic mice. *Cell* [Internet]. 1998;**93**(2):189-201. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9568712>

[32] Cornet A, Savidge TC, Cabarrocas J, Deng WL, Colombel JF, Lassmann H, et al. Enterocolitis induced by autoimmune targeting of enteric glial cells: A possible mechanism in Crohn's disease? *Proceedings of the National Academy of Sciences of the United States of America* [Internet]. 2001;**98**(23):13306-13311. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11687633>

[33] Wang N, Li K, Song S, Chen J. Gastric electrical stimulation improves enteric neuronal survival. *International*

Journal of Molecular Medicine [Internet]. 2017;**40**(2):438-446. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28627584>

[34] Steinkamp M, Gundel H, Schulte N, Spaniol U, Pflueger C, Zizer E, et al. GDNF protects enteric glia from apoptosis: Evidence for an autocrine loop. *BMC Gastroenterology* [Internet]. 2012;**12**:6. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/22251670>

[35] Savidge TC, Newman P, Pothoulakis C, Ruhl A, Neunlist M, Bourreille A, et al. Enteric glia regulate intestinal barrier function and inflammation via release of S-nitrosoglutathione. *Gastroenterology* [Internet]. 2007;**132**(4):1344-1358. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17408650>

[36] Schäfer KH, Mestres P. The GDNF-induced neurite outgrowth and neuronal survival in dissociated myenteric plexus cultures of the rat small intestine decreases postnatally. *Experimental Brain Research* [Internet]. 1999;**125**(4):447-452. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/10323291>

[37] Steinkamp M, Geerling I, Seufferlein T, von Boyen G, Egger B, Grossmann J, et al. Glial-derived neurotrophic factor regulates apoptosis in colonic epithelial cells. *Gastroenterology* [Internet]. 2003;**124**(7):1748-1757. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12806607>

[38] Zhang DK, He FQ, Li TK, Pang XH, Cui DJ, Xie Q, et al. Glial-derived neurotrophic factor regulates intestinal epithelial barrier function and inflammation and is therapeutic for murine colitis. *The Journal of Pathology* [Internet]. 2010;**222**(2):213-222. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20632386>

- [39] Bauman BD, Meng J, Zhang L, Louiselle A, Zheng E, Banerjee S, et al. Enteric glial-mediated enhancement of intestinal barrier integrity is compromised by morphine. *The Journal of Surgical Research* [Internet]. 2017;**219**:214-221. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/29078884>
- [40] Zhang W, Segura BJ, Lin TR, Hu Y, Mulholland MW. Intercellular calcium waves in cultured enteric glia from neonatal Guinea pig. *Glia*. 2003;**42**(3):252-262
- [41] MacEachern SJ, Patel BA, McKay DM, Sharkey KA. Nitric oxide regulation of colonic epithelial ion transport: A novel role for enteric glia in the myenteric plexus. *The Journal of Physiology*. 2011;**589**(13):3333-3348
- [42] Esposito G, Capoccia E, Turco F, Palumbo I, Lu J, Steardo A, et al. Palmitoylethanolamide improves colon inflammation through an enteric glia/toll like receptor 4-dependent PPAR-alpha activation. *Gut* [Internet]. 2014;**63**(8):1300-1312. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24082036>
- [43] Aoki E, Semba R, Kashiwamata S. Evidence for the presence of L-arginine in the glial components of the peripheral nervous system. *Brain Research*. 1991;**559**(1):159-162
- [44] Nagahama M, Semba R, Tsuzuki M, Aoki E. L-arginine immunoreactive enteric glial cells in the enteric nervous system of rat ileum. *Biological Signals and Receptors* [Internet]. 2001;**10**(5):336-340. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11490099>
- [45] Jessen KR, Mirsky R. Astrocyte-like glia in the peripheral nervous system: An immunohistochemical study of enteric glia. *The Journal of Neuroscience* [Internet]. 1983;**3**(11):2206-2218. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/6138397>
- [46] Abdo H, Mahe MM, Derkinderen P, Bach-Ngohou K, Neunlist M, Lardeux B. The omega-6 fatty acid derivative 15-deoxy-delta(1)(2), (1)(4)-prostaglandin J2 is involved in neuroprotection by enteric glial cells against oxidative stress. *The Journal of Physiology* [Internet]. 2012;**590**(11):2739-2750. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22473776>
- [47] Abdo H, Derkinderen P, Gomes P, Chevalier J, Aubert P, Masson D, et al. Enteric glial cells protect neurons from oxidative stress in part via reduced glutathione. *The FASEB Journal* [Internet]. 2010;**24**(4):1082-1094. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19906678>
- [48] von Boyen GB, Steinkamp M, Reinshagen M, Schafer KH, Adler G, Kirsch J. Nerve growth factor secretion in cultured enteric glia cells is modulated by proinflammatory cytokines. *Journal of Neuroendocrinology* [Internet]. 2006;**18**(11):820-825. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17026531>
- [49] Costagliola A, Van Nassauw L, Snyders D, Adriaensen D, Timmermans J-P. Voltage-gated delayed rectifier K v 1-subunits may serve as distinctive markers for enteroglial cells with different phenotypes in the murine ileum. *Neuroscience Letters*. 2009;**461**(2):80-84
- [50] Grubisic V, Parpura V. Two modes of enteric gliotransmission differentially affect gut physiology. *Glia*. 2017;**65**(5):699-711
- [51] Boesmans W, Ameloot K, van den Abbeel V, Tack J, Vanden Berghe P. Cannabinoid receptor 1 signalling dampens activity

and mitochondrial transport in networks of enteric neurones. *Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society*. 2009;**21**(9):958-e77

[52] Kimball BC, Mulholland MW. Enteric glia exhibit P2U receptors that increase cytosolic calcium by a phospholipase C-dependent mechanism. *Journal of Neurochemistry* [Internet]. 1996;**66**(2):604-612. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/8592130>

[53] Gomes P, Chevalier J, Boesmans W, Roosen L, van den Abbeel V, Neunlist M, et al. ATP-dependent paracrine communication between enteric neurons and glia in a primary cell culture derived from embryonic mice. *Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society*. 2009;**21**(8):870-e62

[54] Gulbransen BD, Sharkey KA. Purinergic neuron-to-glia signaling in the enteric nervous system. *Gastroenterology* [Internet]. 2009;**136**(4):1349-1358. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19250649>

[55] Gulbransen BD, Bains JS, Sharkey KA. Enteric glia are targets of the sympathetic innervation of the myenteric plexus in the Guinea pig distal colon. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 2010;**30**(19):6801-6809

[56] Broadhead MJ, Bayguinov PO, Okamoto T, Heredia DJ, Smith TK. Ca^{2+} transients in myenteric glial cells during the colonic migrating motor complex in the isolated murine large intestine. *The Journal of Physiology* [Internet]. 2012;**590**(2):335-350. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22063626>

[57] Schafer KH, Ginneken C, Copray S. Plasticity and neural stem cells in the enteric nervous system. *Anatomical Record* [Internet]. 2009;**292**(12):1940-1952. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19943347

[58] Dupin E, Coelho-Aguiar JM. Isolation and differentiation properties of neural crest stem cells. *Cytometry Part A: The Journal of the International Society for Analytical Cytology*. 2013;**83**(1):38-47

[59] Kruger GM, Mosher JT, Bixby S, Joseph N, Iwashita T, Morrison SJ. Neural crest stem cells persist in the adult gut but undergo changes in self-renewal, neuronal subtype potential, and factor responsiveness. *Neuron*. 2002;**35**(4):657-669

[60] Joseph NM, He S, Quintana E, Kim Y-G, Nunez G, Morrison SJ. Enteric glia are multipotent in culture but primarily form glia in the adult rodent gut. *The Journal of Clinical Investigation*. 2011 Sep;**121**(9):3398-3411

[61] Almond S, Lindley RM, Kenny SE, Connell MG, Edgar DH. Characterisation and transplantation of enteric nervous system progenitor cells. *Gut*. 2007;**56**(4):489-496

[62] Bondurand N, Natarajan D, Thapar N, Atkins C, Pachnis V. Neuron and glia generating progenitors of the mammalian enteric nervous system isolated from foetal and postnatal gut cultures. *Development (Cambridge, England)*. 2003 Dec;**130**(25):6387-6400

[63] Rauch U, Hansgen A, Hagl C, Holland-Cunz S, Schafer K-H. Isolation and cultivation of neuronal precursor cells from the developing human enteric nervous system as a tool for cell therapy in dysganglionosis. *International Journal of Colorectal Disease*. 2006;**21**(6):554-559

- [64] Laranjeira C, Sandgren K, Kessaris N, Richardson W, Potocnik A, Vanden Berghe P, et al. Glial cells in the mouse enteric nervous system can undergo neurogenesis in response to injury. *The Journal of Clinical Investigation*. 2011 Sep;**121**(9):3412-3424
- [65] Belkind-Gerson J, Graham HK, Reynolds J, Hotta R, Nagy N, Cheng L, et al. Colitis promotes neuronal differentiation of Sox2+ and PLP1+ enteric cells. *Scientific Reports* [Internet]. 2017;7(1):2525. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28566702>
- [66] Liu M-T, Kuan Y-H, Wang J, Hen R, Gershon MD. 5-HT4 receptor-mediated neuroprotection and neurogenesis in the enteric nervous system of adult mice. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 2009 Aug;**29**(31):9683-9699
- [67] Gershon MD. Serotonin is a sword and a shield of the bowel: Serotonin plays offense and defense. *Transactions of the American Clinical and Climatological Association*. 2012;**123**:268-280
- [68] Matsuyoshi H, Kuniyasu H, Okumura M, Misawa H, Katsui R, Zhang G-X, et al. A 5-HT(4)-receptor activation-induced neural plasticity enhances in vivo reconstructs of enteric nerve circuit insult. *Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society*. 2010;**22**(7):806-813
- [69] Belkind-Gerson J, Hotta R, Nagy N, Thomas AR, Graham H, Cheng L, et al. Colitis induces enteric neurogenesis through a 5-HT4-dependent mechanism. *Inflammatory Bowel Diseases*. 2015;**21**(4):870-878
- [70] Kulkarni S, Micci M-A, Leser J, Shin C, Tang S-C, Fu Y-Y, et al. Adult enteric nervous system in health is maintained by a dynamic balance between neuronal apoptosis and neurogenesis. *Proceedings of the National Academy of Sciences of the United States of America*. 2017;**114**(18):E3709-E3718
- [71] Pham TD, Gershon MD, Rothman TP. Time of origin of neurons in the murine enteric nervous system: Sequence in relation to phenotype. *The Journal of Comparative Neurology*. 1991;**314**(4):789-798
- [72] Sasselli V, Pachnis V, Burns AJ. The enteric nervous system. *Developmental Biology*. 2012 Jun;**366**(1):64-73
- [73] Young HM, Bergner AJ, Muller T. Acquisition of neuronal and glial markers by neural crest-derived cells in the mouse intestine. *The Journal of Comparative Neurology*. 2003 Jan;**456**(1):1-11
- [74] Belkind-Gerson J, Carreon-Rodriguez A, Benedict LA, Steiger C, Pieretti A, Nagy N, et al. Nestin-expressing cells in the gut give rise to enteric neurons and glial cells. *Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society*. 2013;**25**(1):61-69
- [75] Bannerman PG, Mirsky R, Jessen KR, Timpl R, Duan VC. Light microscopic immunolocalization of laminin, type IV collagen, nidogen, heparan sulphate proteoglycan and fibronectin in the enteric nervous system of rat and Guinea pig. *Journal of Neurocytology*. 1986;**15**(6):733-743
- [76] Neunlist M, Aubert P, Bonnaud S, Van Landeghem L, Coron E, Wedel T, et al. Enteric glia inhibit intestinal epithelial cell proliferation partly through a TGF-beta1-dependent pathway. *American Journal of Physiology. Gastrointestinal and Liver Physiology*. 2007;**292**(1):G231-G241

- [77] Bernardini N, Segnani C, Ippolito C, De Giorgio R, Colucci R, Faussone-Pellegrini MS, et al. Immunohistochemical analysis of myenteric ganglia and interstitial cells of Cajal in ulcerative colitis. *Journal of Cellular and Molecular Medicine* [Internet]. 2012;**16**(2):318-327. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21426484>
- [78] von Boyen GB, Schulte N, Pfluger C, Spaniol U, Hartmann C, Steinkamp M. Distribution of enteric glia and GDNF during gut inflammation. *BMC Gastroenterology* [Internet]. 2011;**11**:3. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21235736>
- [79] Bassotti G, Villanacci V, Rostami Nejad M. Chronic constipation: no more idiopathic, but a true neuropathological entity. *Gastroenterology and Hepatology from Bed to Bench* [Internet]. 2011;**4**(3):109-115. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24834167>
- [80] Schreiber S, Nikolaus S, Hampe J, Hämling J, Koop I, Groessner B, et al. Tumour necrosis factor alpha and interleukin 1beta in relapse of Crohn's disease. *Lancet* [Internet]. 1999;**353**(9151):459-461. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/9989717>
- [81] Celikbilek A, Celikbilek M, Sabah S, Tanik N, Borekci E, Dogan S, et al. The serum S100B level as a biomarker of enteroglia activation in patients with ulcerative colitis. *International Journal of Inflammation* [Internet]. 2014;**2014**:986525. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/24790767>
- [82] Cirillo C, Sarnelli G, Esposito G, Grosso M, Petruzzelli R, Izzo P, et al. Increased mucosal nitric oxide production in ulcerative colitis is mediated in part by the enteroglia-derived S100B protein. *Neurogastroenterology and Motility* [Internet]. 2009;**21**(11):1209-e112. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/19558426>
- [83] Esposito G, Cirillo C, Sarnelli G, De Filippis D, D'Armiento FP, Rocco A, et al. Enteric glial-derived S100B protein stimulates nitric oxide production in celiac disease. *Gastroenterology* [Internet]. 2007;**133**(3):918-925. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17678654>
- [84] Costa DVS, Bon-Frauches AC, Silva AMHP, Lima-Júnior RCP, Martins CS, Leitão RFC, et al. 5-fluorouracil induces enteric neuron death and glial activation during intestinal mucositis via a S100B-RAGE-NFκB-dependent pathway. *Scientific Reports* [Internet]. 2019;**9**(1):665. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/30679569>
- [85] Nogueira LT, Costa DV, Gomes AS, Martins CS, Silva AM, Coelho-Aguiar JM, et al. The involvement of mast cells in the irinotecan-induced enteric neurons loss and reactive gliosis. *Journal of Neuroinflammation* [Internet]. 2017;**14**(1):79. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28388962>
- [86] Burda JE, Sofroniew MV. Reactive gliosis and the multicellular response to CNS damage and disease. *Neuron* [Internet]. 2014;**81**(2):229-248. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24462092>
- [87] Cheadle GA, Costantini TW, Bansal V, Eliceiri BP, Coimbra R. Cholinergic signaling in the gut: A novel mechanism of barrier protection through activation of enteric glia cells. *Surgical Infections* [Internet]. 2014;**15**(4):387-393. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24828283>
- [88] Pochard C, Coquenlorge S, Jaulin J, Cenac N, Vergnolle N, Meurette G, et al.

Defects in 15-HETE production and control of epithelial permeability by human enteric glial cells from patients with Crohn's disease. *Gastroenterology* [Internet]. 2016;**150**(1):168-180. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/26433161>

[89] Hopkins RJ, Wilson RB. Treatment of recurrent. *Gastroenterology Report* [Internet]. 2018;**6**(1):21-28. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/29479439>

[90] Martin JS, Monaghan TM, Wilcox MH. *Clostridium difficile* infection: Epidemiology, diagnosis and understanding transmission. *Nature Reviews. Gastroenterology & Hepatology* [Internet]. 2016;**13**(4):206-216. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/26956066>

[91] Peery AF, Crockett SD, Murphy CC, Lund JL, Dellon ES, Williams JL, et al. Burden and cost of gastrointestinal, liver, and pancreatic diseases in the United States: Update 2018. *Gastroenterology* [Internet]. 2019;**156**(1):254-272. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/30315778>

[92] Davies KA, Longshaw CM, Davis GL, Bouza E, Barbut F, Barna Z, et al. Underdiagnosis of *Clostridium difficile* across Europe: The European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea (EUCLID). *The Lancet Infectious Diseases* [Internet]. 2014;**14**(12):1208-1219. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/25455988>

[93] Walker AS, Eyre DW, Wyllie DH, Dingle KE, Griffiths D, Shine B, et al. Relationship between bacterial strain type, host biomarkers, and mortality in *Clostridium difficile* infection. *Clinical Infectious Diseases* [Internet]. 2013;**56**(11):1589-1600. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/23463640>

[94] Jose S, Mukherjee A, Abhyankar MM, Leng L, Bucala R, Sharma D, et al. Neutralization of macrophage migration inhibitory factor improves host survival after *Clostridium difficile* infection. *Anaerobe* [Internet]. 2018;**53**:56-63. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/29944928>

[95] Yu H, Chen K, Sun Y, Carter M, Garey KW, Savidge TC, et al. Cytokines are markers of the *Clostridium difficile*-induced inflammatory response and predict disease severity. *Clinical and Vaccine Immunology* [Internet]. 2017;**24**(8):e00037-17. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28592627>

[96] Nakagawa T, Mori N, Kajiwarra C, Kimura S, Akasaka Y, Ishii Y, et al. Endogenous IL-17 as a factor determining the severity of *Clostridium difficile* infection in mice. *Journal of Medical Microbiology* [Internet]. 2016;**65**(8):821-827. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/27166143>

[97] Buonomo EL, Madan R, Pramoonjago P, Li L, Okusa MD, Petri WA. Role of interleukin 23 signaling in *Clostridium difficile* colitis. *The Journal of Infectious Diseases* [Internet]. 2013;**208**(6):917-920. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/23776194>

[98] Fettucciari K, Ponsini P, Gioè D, Macchioni L, Palumbo C, Antonelli E, et al. Enteric glial cells are susceptible to *Clostridium difficile* toxin B. *Cellular and Molecular Life Sciences* [Internet]. 2017;**74**(8):1527-1551. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/27891552>

[99] Neunlist M, Barouk J, Michel K, Just I, Oreshkova T, Schemann M, et al. Toxin B of *Clostridium difficile* activates human VIP submucosal neurons, in part via an IL-1beta-dependent pathway. *American Journal of Physiology*.

Gastrointestinal and Liver Physiology [Internet]. 2003;**285**(5):G1049-G1055. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/12801886>

[100] Xia Y, Hu HZ, Liu S, Pothoulakis C, Wood JD. Clostridium difficile toxin A excites enteric neurones and suppresses sympathetic neurotransmission in the Guinea pig. Gut [Internet]. 2000;**46**(4):481-486. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/10716676>

[101] Pothoulakis C, Castagliuolo I, LaMont JT, Jaffer A, O'Keane JC, Snider RM, et al. CP-96,345, a substance P antagonist, inhibits rat intestinal responses to *Clostridium difficile* toxin A but not cholera toxin. Proceedings of the National Academy of Sciences of the United States of America [Internet]. 1994;**91**(3):947-951. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/7508124>

[102] Gutiérrez RL, Riddle MS, Porter CK. Increased risk of functional gastrointestinal sequelae after *Clostridium difficile* infection among active duty United States military personnel (1998-2010). Gastroenterology [Internet]. 2015;**149**(6):1408-1414. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/26255560>

[103] Macchioni L, Davidescu M, Fettucciari K, Petricciuolo M, Gatticchi L, Gioè D, et al. Enteric glial cells counteract *Clostridium difficile* toxin B through a NADPH oxidase/ROS/JNK/caspase-3 axis, without involving mitochondrial pathways. Scientific Reports [Internet]. 2017;**7**:45569. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28349972>

[104] Fettucciari K, Macchioni L, Davidescu M, Scarpelli P, Palumbo C, Corazzi L, et al. *Clostridium difficile* toxin B induces senescence in enteric glial cells: A potential new mechanism

of *Clostridium difficile* pathogenesis. Biochimica et Biophysica Acta, Molecular Cell Research [Internet]. 2018;**1865**(12):1945-1958. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/30296499>

[105] Flamant M, Aubert P, Rolli-Derkinderen M, Bourreille A, Neunlist MR, Mahé MM, et al. Enteric glia protect against *Shigella flexneri* invasion in intestinal epithelial cells: A role for S-nitrosoglutathione. Gut [Internet]. 2011;**60**(4):473-484. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/21139062>

[106] El Basha NR, Zaki MM, Hassanin OM, Rehan MK, Omran D. Giardia assemblages a and B in diarrheic patients: A comparative study in Egyptian children and adults. The Journal of Parasitology [Internet]. 2016;**102**(1):69-74. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/26509291>

[107] Pavanelli MF, Colli CM, Bezagio RC, Góis MB, de Melo G, de Almeida Araújo EJ, et al. Assemblages A and B of *Giardia duodenalis* reduce enteric glial cells in the small intestine in mice. Parasitology Research [Internet]. 2018;**117**(7):2025-2033. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/29728828>

[108] Soveri LM, Hermunen K, de Gramont A, Poussa T, Quinaux E, Bono P, et al. Association of adverse events and survival in colorectal cancer patients treated with adjuvant 5-fluorouracil and leucovorin: Is efficacy an impact of toxicity? European Journal of Cancer [Internet]. 2014;**50**(17):2966-2974. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/25266443>

[109] Soares PM, Mota JM, Gomes AS, Oliveira RB, Assreuy AM, Brito GA, et al. Gastrointestinal dysmotility in 5-fluorouracil-induced intestinal mucositis outlasts inflammatory process

resolution. *Cancer Chemotherapy and Pharmacology* [Internet]. 2008;**63**(1):91-98. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/18324404>

[110] Robinson AM, Stojanovska V, Rahman AA, McQuade RM, Senior PV, Nurgali K. Effects of oxaliplatin treatment on the enteric glial cells and neurons in the mouse ileum. *The Journal of Histochemistry and Cytochemistry* [Internet]. 2016;**64**(9):530-545. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/27389702>

[111] Knowles CH, Lindberg G, Panza E, De Giorgio R. New perspectives in the diagnosis and management of enteric neuropathies. *Nature Reviews. Gastroenterology & Hepatology*. 2013;**10**(4):206-218

[112] Cogliandro RF, De Giorgio R, Barbara G, Cogliandro L, Concordia A, Corinaldesi R, et al. Chronic intestinal pseudo-obstruction. *Best Practice & Research. Clinical Gastroenterology*. 2007;**21**(4):657-669

[113] Goldstein AM, Thapar N, Karunaratne TB, De Giorgio R. Clinical aspects of neurointestinal disease: Pathophysiology, diagnosis, and treatment. *Developmental Biology*. 2016 Sep;**417**(2):217-228

[114] Selgrad M, De Giorgio R, Fini L, Cogliandro RF, Williams S, Stanghellini V, et al. JC virus infects the enteric glia of patients with chronic idiopathic intestinal pseudo-obstruction. *Gut*. 2009 Jan;**58**(1):25-32

[115] Tabbers MM, Di Lorenzo C, Berger MY, Faure C, Langendam MW, Nurko S, et al. Evaluation and treatment of functional constipation in infants and children: Evidence-based recommendations from ESPGHAN and NASPGHAN. *Journal of Pediatric Gastroenterology and Nutrition*. 2014 Feb;**58**(2):258-274

[116] Bassotti G, Villanacci V, Maurer CA, Fisogni S, Di Fabio F, Cadei M, et al. The role of glial cells and apoptosis of enteric neurones in the neuropathology of intractable slow transit constipation. *Gut* [Internet]. 2006;**55**(1):41-46. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16041063>

[117] Bassotti G, Villanacci V, Antonelli E, Morelli A, Salerni B. Enteric glial cells: New players in gastrointestinal motility? *Laboratory Investigation* [Internet]. 2007;**87**(7):628-632. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17483847>

[118] Burnstock G, Lavin S. Interstitial cells of Cajal and purinergic signalling. *Autonomic Neuroscience : Basic & Clinical*. 2002 Apr;**97**(1):68-72

[119] Braak H, Del Tredici K. Invited article: Nervous system pathology in sporadic Parkinson disease. *Neurology* [Internet]. 2008;**70**(20):1916-1925. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18474848>

[120] Chalazonitis A, Rao M. Enteric nervous system manifestations of neurodegenerative disease. *Brain Research* [Internet]. 2018;**1693**(Pt B):207-213. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=29360466

[121] Lebouvier T, Chaumette T, Paillusson S, Duyckaerts C, Bruley d, Varannes S, et al. The second brain and Parkinson's disease. *The European Journal of Neuroscience* [Internet]. 2009;**30**(5):735-741. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19712093>

[122] Natale G, Pasquali L, Paparelli A, Fornai F. Parallel manifestations of neuropathologies in the enteric and central nervous systems.

Neurogastroenterology and Motility [Internet]. 2011;23(12):1056-1065. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=21951862

[123] Winkler J, Ehret R, Buttner T, Dillmann U, Fogel W, Sabolek M, et al. Parkinson's disease risk score: Moving to a premotor diagnosis. *Journal of Neurology* [Internet]. 2011;258(Suppl 2):S311-S315. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21560061>

[124] Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging* [Internet]. 2003;24(2):197-211. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12498954>

[125] Braak H, de Vos RA, Bohl J, Del Tredici K. Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. *Neuroscience Letters* [Internet]. 2006;396(1):67-72. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16330147>

[126] Lebouvier T, Neunlist M, Bruley des Varannes S, Coron E, Drouard A, N'Guyen JM, et al. Colonic biopsies to assess the neuropathology of Parkinson's disease and its relationship with symptoms. *PLoS One* [Internet]. 2010;5(9):e12728. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20856865>

[127] Shannon KM, Keshavarzian A, Mutlu E, Dodiya HB, Daian D, Jaglin JA, et al. Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. *Movement Disorders* [Internet]. 2012;27(6):709-715. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21766334>

[128] Clairembault T, Leclair-Visonneau L, Coron E, Bourreille A, Le Dily S, Vavasseur F, et al. Structural alterations of the intestinal epithelial barrier in Parkinson's disease. *Acta Neuropathologica Communications* [Internet]. 2015;3:12. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25775153>

[129] Dickson DW, Fujishiro H, Orr C, DelleDonne A, Josephs KA, Frigerio R, et al. Neuropathology of non-motor features of Parkinson disease. *Parkinsonism & Related Disorders* [Internet]. 2009;15(Suppl 3):S1-S5. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20082965

[130] Pfeiffer RF. Gastrointestinal dysfunction in Parkinson's disease. *Parkinsonism & Related Disorders* [Internet]. 2011;17(1):10-15. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20829091>

[131] Clairembault T, Kamphuis W, Leclair-Visonneau L, Rolli-Derkinderen M, Coron E, Neunlist M, et al. Enteric GFAP expression and phosphorylation in Parkinson's disease. *Journal of Neurochemistry* [Internet]. 2014;130(6):805-815. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24749759>

[132] Devos D, Lebouvier T, Lardeux B, Biraud M, Rouaud T, Pouclet H, et al. Colonic inflammation in Parkinson's disease. *Neurobiology of Disease* [Internet]. 2013;50:42-48. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23017648>

[133] Fang X, Xu RS. Protective effect of simvastatin on impaired intestine tight junction protein ZO-1 in a mouse model of Parkinson's disease. *Journal of Huazhong University of Science and Technology. Medical Sciences*

[Internet]. 2015;**35**(6):880-884. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=26670440

[134] Schwiertz A, Spiegel J, Dillmann U, Grundmann D, Burmann J, Fassbender K, et al. Fecal markers of intestinal inflammation and intestinal permeability are elevated in Parkinson's disease. *Parkinsonism & Related Disorders* [Internet]. 2018;**50**:104-107. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=29454662

[135] Adams-Carr KL, Bestwick JP, Shribman S, Lees A, Schrag A, Noyce AJ. Constipation preceding Parkinson's disease: A systematic review and meta-analysis. *Journal of Neurology, Neurosurgery, and Psychiatry* [Internet]. 2016;**87**(7):710-716. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=26345189

[136] Chaudhuri KR, Odin P. The challenge of non-motor symptoms in Parkinson's disease. *Progress in Brain Research* [Internet]. 2010;**184**:325-341. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20887883

[137] Fasano A, Visanji NP, Liu LW, Lang AE, Pfeiffer RF. Gastrointestinal dysfunction in Parkinson's disease. *Lancet Neurology* [Internet]. 2015;**14**(6):625-639. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=25987282

[138] Berg D, Postuma RB, Adler CH, Bloem BR, Chan P, Dubois B, et al. MDS research criteria for prodromal Parkinson's disease. *Movement*

Disorders [Internet]. 2015;**30**(12):1600-1611. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=26474317

[139] Seguela L, Capuano R, Sarnelli G, Esposito G. Play in advance against neurodegeneration: Exploring enteric glial cells in gut-brain axis during neurodegenerative diseases. *Expert Review of Clinical Pharmacology* [Internet]. 2019;**12**(6):555-564. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=31025582

[140] Klingelhoefer L, Reichmann H. Pathogenesis of Parkinson disease—The gut-brain axis and environmental factors. *Nature Reviews. Neurology* [Internet]. 2015;**11**(11):625-636. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=26503923

[141] Lane CA, Hardy J, Schott JM. Alzheimer's disease. *European Journal of Neurology* [Internet]. 2018;**25**(1):59-70. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=28872215

[142] Leblhuber F, Geisler S, Steiner K, Fuchs D, Schutz B. Elevated fecal calprotectin in patients with Alzheimer's dementia indicates leaky gut. *Journal of Neural Transmission (Vienna)* [Internet]. 2015;**122**(9):1319-1322. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=25680441

[143] Joachim CL, Mori H, Selkoe DJ. Amyloid beta-protein deposition in tissues other than brain in Alzheimer's disease. *Nature* [Internet]. 1989;**341**(6239):226-230. Available from:

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2528696

[144] Arai H, Lee VM, Messinger ML, Greenberg BD, Lowery DE, Trojanowski JQ. Expression patterns of beta-amyloid precursor protein (beta-APP) in neural and nonneural human tissues from Alzheimer's disease and control subjects. *Annals of Neurology* [Internet]. 1991;**30**(5):686-693. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1763893

[145] Holmes C, Cunningham C, Zotova E, Woolford J, Dean C, Kerr S, et al. Systemic inflammation and disease progression in Alzheimer disease. *Neurology* [Internet]. 2009;**73**(10):768-774. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19738171

[146] Solleiro-Villavicencio H, Rivas-Arancibia S. Effect of chronic oxidative stress on Neuroinflammatory response mediated by CD4(+)T cells in neurodegenerative diseases. *Frontiers in Cellular Neuroscience* [Internet]. 2018;**12**:114. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=29755324

[147] da Silveira AB, de Oliveira EC, Neto SG, Luquetti AO, Fujiwara RT, Oliveira RC, et al. Enteroglial cells act as antigen-presenting cells in chagasic megacolon. *Human Pathology* [Internet]. 2011;**42**(4):522-532. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=21208643

[148] Geboes K, Rutgeerts P, Ectors N, Mebis J, Penninckx F, Vantrappen G, et al. Major histocompatibility class

II expression on the small intestinal nervous system in Crohn's disease. *Gastroenterology* [Internet]. 1992;**103**(2):439-447. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/1378803>

[149] Turco F, Sarnelli G, Cirillo C, Palumbo I, De Giorgi F, D'Alessandro A, et al. Enteroglial-derived S100B protein integrates bacteria-induced toll-like receptor signalling in human enteric glial cells. *Gut* [Internet]. 2014;**63**(1):105-115. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23292665>

[150] Gabanyi I, Muller PA, Feighery L, Oliveira TY, Costa-Pinto FA, Mucida D. Neuro-immune interactions drive tissue programming in intestinal macrophages. *Cell* [Internet]. 2016;**164**(3):378-391. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26777404>

[151] De Schepper S, Verheijden S, Aguilera-Lizarraga J, Viola MF, Boesmans W, Stakenborg N, et al. Self-maintaining gut macrophages are essential for intestinal homeostasis. *Cell*. 2018;**175**(2):400-415

[152] Cardoso V, Chesne J, Ribeiro H, Garcia-Cassani B, Carvalho T, Bouchery T, et al. Neuronal regulation of type 2 innate lymphoid cells via neuromedin U. *Nature*. 2017 Sep;**549**(7671):277-281

[153] Klose CSN, Mahlakoiv T, Moeller JB, Rankin LC, Flamar A-L, Kabata H, et al. The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation. *Nature*. 2017 Sep;**549**(7671):282-286

[154] Ibiza S, Garcia-Cassani B, Ribeiro H, Carvalho T, Almeida L, Marques R, et al. Glial-cell-derived neuroregulators control type 3 innate lymphoid cells and gut defence. *Nature* [Internet]. 2016;**535**(7612):440-443.

Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27409807>

[155] Veiga-Fernandes H, Coles MC, Foster KE, Patel A, Williams A, Natarajan D, et al. Tyrosine kinase receptor RET is a key regulator of Peyer's patch organogenesis. *Nature*. 2007;**446**(7135):547-551

[156] Capoccia E, Cirillo C, Gigli S, Pesce M, D'Alessandro A, Cuomo R, et al. Enteric glia: A new player in inflammatory bowel diseases. *International Journal of Immunopathology and Pharmacology* [Internet]. 2015;**28**(4):443-451. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26526203>

[157] Kermarrec L, Durand T, Gonzales J, Pabois J, Hulin P, Neunlist M, et al. Rat enteric glial cells express novel isoforms of Interleukine-7 regulated during inflammation. *Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society*. 2019;**31**(1):e13467

[158] Kermarrec L, Durand T, Neunlist M, Naveilhan P, Neveu I. Enteric glial cells have specific immunosuppressive properties. *Journal of Neuroimmunology* [Internet]. 2016;**295-296**:79-83. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27235353>

[159] Koretz K, Momburg F, Otto HF, Moller P. Sequential induction of MHC antigens on autochthonous cells of ileum affected by Crohn's disease. *The American Journal of Pathology* [Internet]. 1987;**129**(3):493-502. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/3425689>

[160] Ruhl A, Franzke S, Collins SM, Stremmel W. Interleukin-6 expression and regulation in rat enteric glial cells. *American Journal of Physiology. Gastrointestinal and Liver Physiology* [Internet]. 2001;**280**(6):G1163-G1171.

Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11352809>

[161] Ruhl A, Franzke S, Stremmel W. IL-1beta and IL-10 have dual effects on enteric glial cell proliferation. *Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society*. [Internet]. 2001 Feb;**13**(1):89-94

[162] Rao M, Rastelli D, Dong L, Chiu S, Setlik W, Gershon MD, et al. Enteric glia regulate gastrointestinal motility but are not required for maintenance of the epithelium in mice. *Gastroenterology* [Internet]. 2017;**153**(4):1068-1081. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28711628>

[163] Aube AC, Cabarrocas J, Bauer J, Philippe D, Aubert P, Doulay F, et al. Changes in enteric neurone phenotype and intestinal functions in a transgenic mouse model of enteric glia disruption. *Gut* [Internet]. 2006;**55**(5):630-637. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16236773>

[164] Nasser Y, Fernandez E, Keenan CM, Ho W, Oland LD, Tibbles LA, et al. Role of enteric glia in intestinal physiology: Effects of the gliotoxin fluorocitrate on motor and secretory function. *American Journal of Physiology. Gastrointestinal and Liver Physiology* [Internet]. 2006;**291**(5):G912-G927. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16798727>