

The Enteric Glia

Identity and Functions

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Enteric glial cells were first described at the end of the 19th century, but they attracted more interest from researchers only in the last decades of the 20th. Although, they have a different embryological origin, the enteric GLIA share many characteristics with astrocytes, the main glial cell type of the central nervous system (CNS), such as in their expression of the same markers and in their functions. Here we review the construction of the enteric nervous system (ENS), with a focus on enteric glia, and also the main studies that have revealed the action of enteric glia in different aspects of gastrointestinal tract homeostasis, such as in the intestinal barrier, in communications with neurons, and in their action as progenitor cells. We also discuss recent discoveries about the roles of enteric glia in different disorders that affect the ENS, such as degenerative pathologies including Parkinson's and prion diseases, and in cases of intestinal diseases and injury.

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Initial Studies on Enteric Nervous System

The enteric nervous system (ENS) was described in the late 19th century, by Starling and Bayliss, who studied isolated segments of dog bowel. They observed a muscle movement that they named the “law of the intestine,” which today is known as the peristaltic reflex (Bayliss and Starling, 1899). Preceding Starling and Bayliss, Meissner and Auerbach described the existence of neurons throughout the bowel wall (Auerbach, 1864). Later, Ramon y Cajal in 1892, using silver staining, identified different neuron types in the gut, and in 1899 Dogiel classified the enteric neurons according to their microarchitecture (Dogiel, 1899; reviewed by Furness, 2006). The first observation of enteric glial cells was reported by Dogiel in 1899. For the next 70 years, the enteric glial cells

were thought to be Schwann cells. In the early 1970s, Gabella established that enteric glial cells are a distinct type of glia, differing from those elsewhere in the peripheral nervous system (PNS), with an independent and characteristic identity (Gabella, 1972).

Establishment of the ENS

Embryological Origin of the ENS

The ENS is derived from the neural crest (NC). This cell population arises from the ectoderm and is composed of multipotent cells located in the dorsal edge of the neural tube, which dissociate and follow different migration paths (Coelho-Aguiar et al., 2013; Dupin and Coelho-Aguiar, 2013; Le Douarin and Kalchauer, 1999). This structure gives rise to a diverse range of

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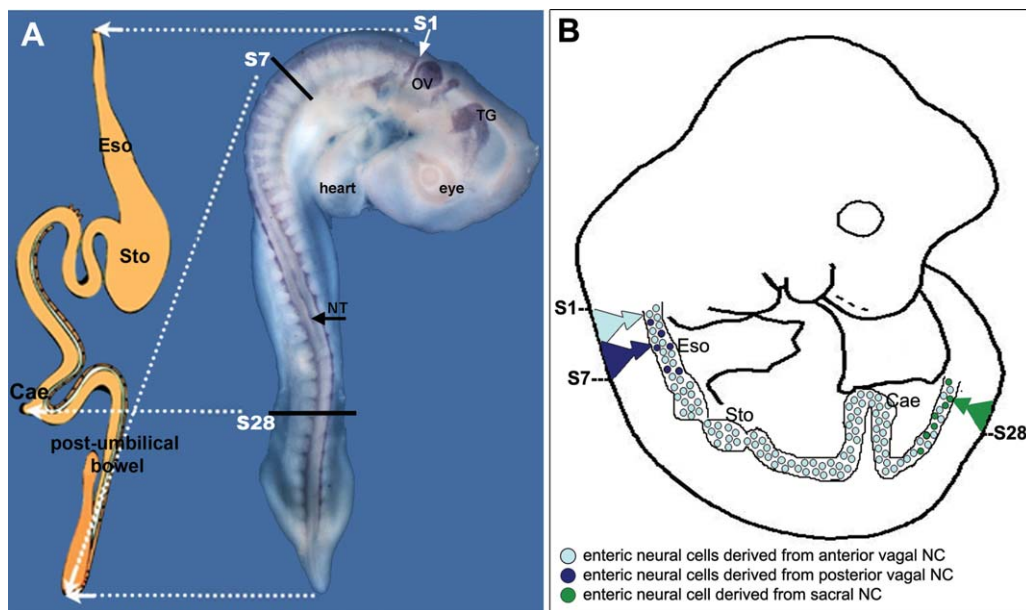


FIGURE 1: Origin of ENS. (A) Three-day chicken embryo with *in situ* hybridization for the detection of the transcription factor Sox10. The location of the NC cells subpopulations that give rise to ENS is shown. (B) Schematic presentation of the origin of enteric neural cells in an E14 mouse embryo. The vagal NC (somites 1–7) originates enteric ganglia throughout the length of the digestive system, with those corresponding to somites 6 and 7 contributing only to the foregut. The sacral NC (posterior to somite 28) contributes to the postumbilical ENS. Cae, caecum; Eso, esophagus; NT, neural tube; OV, otic vesicle; S, somite; Sto, stomach; TG, trigeminal ganglia. Figure adapted from (A) Le Douarin and Kalcheim, 1999; and (B) Durbec et al., 1996. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

phenotypes, including mesenchymal phenotypes of the head and neck, endocrine cells, melanocytes, and the entire PNS, including the ENS (Le Douarin and Kalcheim, 1999; Le Douarin et al., 2004).

In 1954, Yntema and Hammond showed that in chicken embryos, removal of the NC from the postoptical zone to the anterior spinal cord leads to a lack of ganglia in the digestive tract (Yntema and Hammond, 1954). Later, in 1973, Le Douarin and Teillet carried out grafts of the quail neural tube into chick embryos at the same embryonic stage (Le Douarin and Teillet, 1973), and showed that the vagal NC (originating at the level of somites 1–7) is the main source of enteric ganglion cells. These cells migrate in the rostrocaudal direction to colonize the digestive tract from the esophagus, and extend through the stomach and pancreas to the large intestine and rectum. Le Douarin and Teillet also showed using avian embryos that part of the NC of the post-umbilical gut originates from sacral NC cells (posterior to somite 28), which migrate to the digestive tract through the Remak nerve and pelvic ganglia, and mix with cells derived from the vagal NC (Burns, 2005; Le Douarin and Kalcheim, 1999; Le Douarin and Teillet, 1973; Le Douarin et al., 2004). Figure 1 shows the details of the description by Le Douarin and colleagues. Later, Durbec and coworkers discovered, using mouse embryos, that different subsets of the vagal NC colonize different gut regions, with the posteriormost

region of the vagal NC, corresponding to the anteriormost trunk NC cells (somites 6–7), contributing only to the colonization of the foregut (Durbec et al., 1996) (Fig. 1B).

In mouse embryos, vagal NC cells delaminate at embryonic day (E) 8.5–9.0 and transiently colonize the postbranchial region ventral to the dorsal aorta. The vagal NC cells colonize the digestive tract antero-posteriorly over 4 days, completing this process at E13.5 (Fig. 2). In human embryos, NC cells invade the foregut prior to week 4 and colonize it over the next 2–3 weeks (Young and Newgreen, 2001; Wallace and Burns, 2005). Recently, Nishiyama and coworkers discovered in mouse embryos that at E11 and E12, some enteric NC cells cross the mesentery between the small bowel and the proximal colon. These transmesenteric NC cells then give rise to a large part of the colonic ENS (Nishiyama et al., 2012).

Impaired differentiation of the NC cells can result in ENS congenital disorders, like Hirschsprung's disease, in which the incomplete formation of enteric ganglia of the distal colon causes obstruction of the gastrointestinal transit (Heanue and Pachnis, 2007).

ENS Organization and Enteric Glial Cell Types

Similarly to the central nervous system (CNS), the ENS is composed of neurons and their axons, as well as glial cells that form a complex neural network located in the wall of

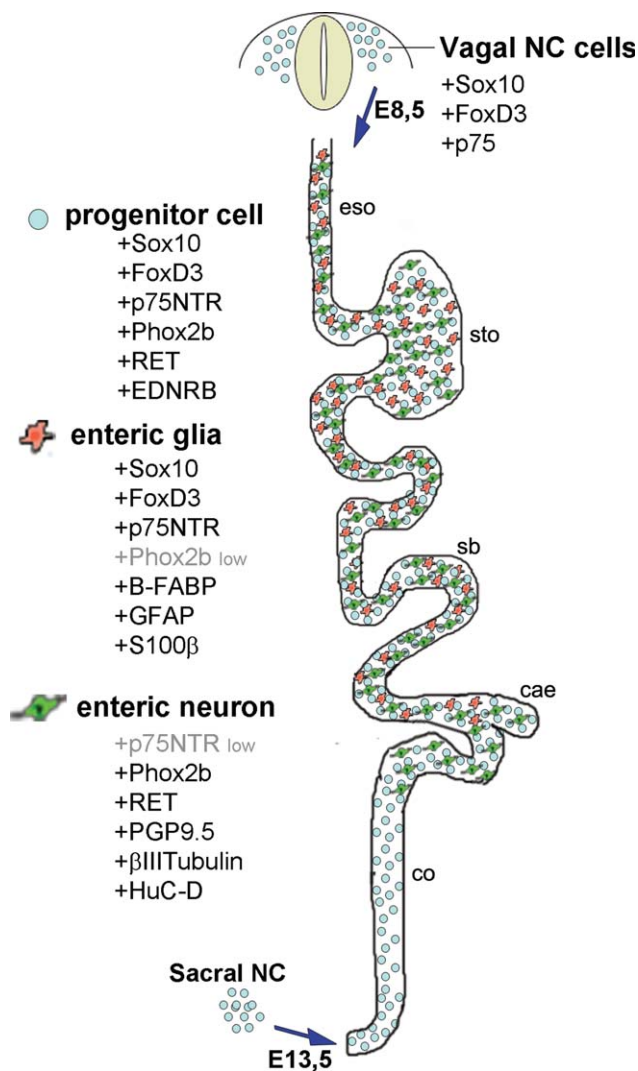


FIGURE 2: Colonization of mouse GIT by enteric NC cells. Vagal NC cells delaminate at E8.5–9.0 and express Sox10, FoxD3, Phox2b, and p75NTR. The enteric NC progenitor cells begin to express RET and Phox2b prior to entering the GIT. After they differentiate, enteric NC cells lose EDNRB expression. Enteric neurons retain expression of Phox2b and begin to express PGP9.5, βIII-Tubulin, and HuC-D. After they differentiate, enteric glia continue to express Sox10, FoxD3, and p75NTR; and they gain B-FABP, GFAP, and S100β expression. At E13.5, vagal NC cells complete their migration. At this time, progenitors can be found throughout the GIT, and differentiated enteric neurons and glial cells are present behind the wave front. Sacral NC begins the hindgut colonization at E13.5. cae, caecum; co, colon; eso, esophagus; sb, small bowel; sto, stomach. Figure adapted from Sasselli et al., 2012. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the GIT, including the pancreas and gall bladder. In the gut, there are nerve fiber bundles and connections between enteric neural cells, with innervation of muscles, blood vessels, and epithelial and endocrine cells. Enteric neurons and glial cells are grouped in the ganglia, and as in the CNS, connective cells, collagen fibrils, and blood vessels are lacking (Komuro et al., 1982). In mammals, enteric neurons and glial cells are

organized mainly in two ganglionated plexuses: the myenteric plexus, which is more prominent and is located between the longitudinal and circular muscle layers; and the inner and outer submucosal plexuses between the circular and submucosal layers (Furness, 2006; Timmermans et al., 2001) (Fig. 3A–D). In addition, a small number of enteric neural bodies are present in the lamina propria of the mucosa, the mucosal plexus, and in the subserosal plexus in the connective-tissue layer. Clusters of nerve cells in the muscle layers are known as the deep muscle plexus and the circular muscle plexus (Bernstein and Vidrich, 1994; Furness, 2006; Furness and Costa, 1980).

Enteric glial cells are smaller than neurons and have innumerable gliofilaments and processes of different shapes and sizes. They are located in direct contact with neurons membranes, both in the enteric ganglia and in the nerve strands, and lack myelin sheaths (Gabella, 1972). In the guinea-pig ileum myenteric ganglia, enteric glial cells are twice as abundant as neurons (Gabella, 1981). These glial cells of the gut are more similar in structure and morphology to the astrocytes than to other glial-cell types of the PNS, such as Schwann cells (Gabella, 1981). Similarly to the cells of the astrocytic lineage, glial fibrillary acidic protein (GFAP) (Jessen and Mirsky, 1980), S100 calcium-binding protein beta (S100β) (Ferri et al., 1982), glutamine synthetase (Jessen and Mirsky, 1983), and brain-type fatty acid-binding protein (BFABP) are markers of enteric glial progenitors and/or mature enteric glial cells (Fig. 3). Interestingly, enteric glial cells seem to express the same GFAP isoforms as astrocytes, while the GFAP expressed by nonmyelinating Schwann cells is a different polypeptide (Jessen and Mirsky, 1985). We have isolated and immunostained for GFAP and S100β glial cells from mice ENS, to compare their morphology and properties to those of astrocytes, and also to tumoral astro-glial cells (Fig. 3E–G). Their similarities are apparent in spite of their different embryonic origins, from NC for the enteric glia and from the “neural tube” for the astrocytes. Additional markers that have been used to identify enteric glial cells are the low-affinity nerve growth factor receptor (p75NTR) and SRY-related HMG-box (Sox) 8/9/10 (Hoff et al., 2008; Young et al., 2003), which are also expressed by enteric NC progenitors.

As the astrocytes, enteric glial cells show different morphological features in the intestinal microenvironment (Gulbransen and Sharkey, 2012; Hanani and Reichenbach, 1994; Oberheim et al., 2009). Hanani and Reichenbach described the intraganglionic type I or protoplasmic enteric glia, which are similar to protoplasmic astrocytes; and the type II or fibrous enteric glia in the fiber tracts, which resemble the fibrous astrocytes of white matter in the brain (Hanani and Reichenbach, 1994). Gulbransen and Sharkey (2012)

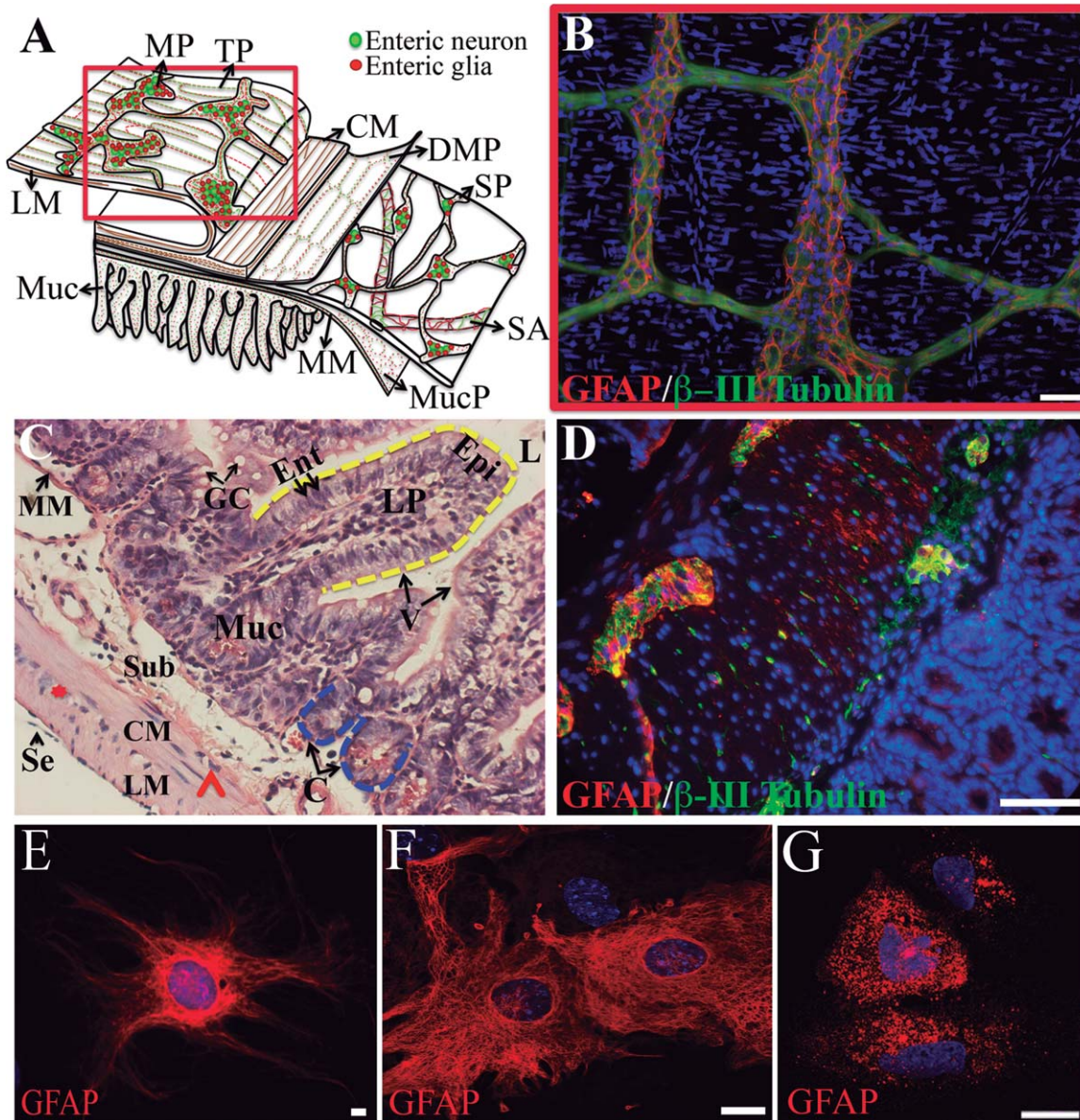


FIGURE 3: Enteric glial distribution in the gut; and GFAP immunostaining of different glial cell types. (A) Drawing of the intestine showing layers and the main plexuses (modified from Furness and Costa, 1980). (B) Delaminated longitudinal muscular layer with adhered myenteric plexus stained for GFAP and β III-Tubulin, identifying enteric glia (red) and neurons (green). (C) Hematoxylin-Eosin (HE) staining of ileum from adult mouse, showing most of the gut components. (D) Cross-section of ileum immunostained for GFAP (red) and β III-Tubulin (green). (E–G) Examples of different enteric and astro-glial types expressing GFAP *in vitro*. (E) Enteric glia of adult mouse large intestine. (F) Astrocytes from cerebral cortex of newborn mouse. (G) Tumoral glial cell from human glioblastoma. Nuclei were stained with DAPI (blue). C, crypts (blue dotted line); CM, circular muscle; DMP, deep muscular plexus; Ent, enterocytes; Epi, epithelium (yellow dotted line); GC, goblet cells; L, lumen; LM, longitudinal muscle; LP, lamina propria; MM, muscularis mucosae; MP, myenteric plexus (red asterisk in C); Muc, mucosa; MucP, mucosal plexus; SA, submucosal artery; Se, serosa; SP, submucous plexus (red arrow in C); Sub, submucosa; TP, tertiary plexus; V, villi. Scale bars: 50 μ m in A–D; 10 μ m in E–G.

demonstrated the type III or mucosal enteric glia in the sub-epithelial space, and the type IV or intramuscular enteric glia, the elongated glia located close to nerve fibers in the muscle layers. However, recent studies suggest that the most appropriate nomenclature to describe enteric glia subpopulations is based their locations (Gulbransen and Sharkey, 2012). Therefore, EG_{Mucosa} and EG_{IM} (intramuscular) are related to the glia

of mucosa and muscle layers respectively. Those in the sub-mucosal and myenteric ganglia are termed EG_{SMP} and EG_{MP} (Gulbransen and Sharkey, 2012). The EG_{Mucosa} are crucial to maintenance of the gut barrier supplying the epithelial cells (Bush et al., 1998; Van Landeghem et al., 2009). Intragan-glionic glia (EG_{MP} and EG_{SMP}) interact closely with neurons, supporting these cells and participating in gliogenesis and

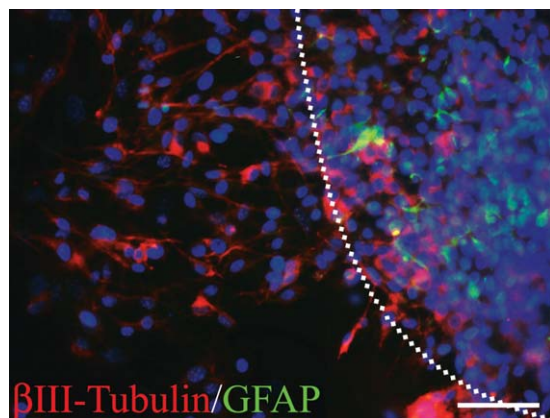


FIGURE 4: Neurosphere derived from ENS culture of newborn mouse, with β III-Tubulin-positive neurons migrating through the cell-culture plate. Enteric glial cells expressing GFAP can be seen in the center of the attached sphere (delimited by dotted line). Scale bar: 50 μ m.

neurogenesis (Boesmans et al., 2013; Gulbransen and Sharkey, 2012; Laranjeira et al., 2011). The role of EG_{IM} in the gut is not well established.

The gut is also colonized by cells from extrinsic nerves, which will become associated with nerve bundles innervating the muscles. These cells are nonmyelinating Schwann cells, and the EG_{IM} enteric glial group certainly includes them. (Rothman et al., 1986; Ruhl, 2005). Interestingly, Schwann cell myelin protein (SMP) expression can be induced in glial cells of the avian gut or inhibited in SMP-positive Schwann cells, when each is challenged with the microenvironment of the other (Dulac and Le Douarin, 1991).

Differentiation of Enteric Neural Cells

Enteric neuronal and glial differentiation is a coordinated developmental process that starts during the migration of NC cells to colonize the GIT. Neuronal progenitors start to differentiate first, and in mice can be detected as early as E10–E10.5 (Young et al., 1999). The first glial precursors appear at E11.5, located far behind the migratory front, and can be firstly detected by their BFABP expression (Young et al., 2003) (Fig. 2). We were able to demonstrate *in vitro* this sequence of migration and differentiation (Fig. 4).

Different growth factors are produced by the gut mesenchyme to promote neuronal and glial differentiation in the gastrointestinal tract (GIT); like glial cell line-derived neurotrophic factor (GDNF), bone morphogenetic proteins (BMPs), and neurotrophin-3 (NT3) (Chalazonitis et al., 2004; Heuckeroth et al., 1998). These factors have similar functions in the CNS (Grinspan et al., 2000; Oppenheim et al., 2000). Sonic hedgehog (Shh) represses the neuronal induction of GDNF in NC cells (Fu et al., 2004). At low concentrations, GDNF promotes the proliferation of neuron and glia progenitors. At high concentrations, GDNF pro-

motes neuronal differentiation (reviewed by Uesaka et al., 2013).

BMP4, in addition to inducing the expression of tyrosine kinase receptor C (TrkC), which favors the differentiation of dopaminergic TrkC-dependent neurons (Chalazonitis et al., 2004), also induces the differentiation of enteric glia. Overexpression of the BMP-antagonist Noggin suppress enteric glia differentiation. Activation of the BMP pathway enhances expression of Erb3, the receptor of glial growth factor 2 (GGF2). In turn, GGF2 acts in gliogenesis and also inhibits GDNF-induced neurogenesis (Chalazonitis et al., 2011).

Overexpression of Sox10, a member of the SoxB1 subgroup of transcription factors, inhibits enteric neuron and glia differentiation, maintaining both the neurogenic and gliogenic potential of the enteric NC progenitor cells (Bondurand et al., 2006). Sox2 is expressed in the gut from E10.5 (Heanue and Pachnis, 2011) and this expression overlaps with Sox10 in the enteric NC progenitors and then in differentiated glial cells (Bondurand and Sham, 2013) (Fig. 2). The forced expression of Sox2 has been shown to inhibit neuronal differentiation in the developing CNS (Lee et al., 2014).

During the differentiation of NC cells, Notch signaling inhibits neurogenesis and, on the other hand, promotes gliogenesis. Thus, Notch and Sox10 contribute to promoting gliogenesis and to the maintenance of ENS progenitors (Ngan et al., 2011; Okamura and Saga, 2008). The transcription factor Foxd3 is crucial for ENS development, including neural patterning and glial differentiation, self-renewal, multipotency, and establishment of NC-derived cells (Mundell and Labosky, 2011; Mundell et al., 2012). Foxd3 is expressed in enteric NC-derived progenitors and is gradually restricted to enteric glial cells (shown in Fig. 2).

Similar Functions Performed by Enteric Glia and Astrocytes

Enteric Glia Functions in Intestinal Epithelial Barrier

Mucosal epithelial tissue, which lines the GIT internally, forms an interface between the body and the external environment. The mucosal cells are tightly adhered and function as a barrier that controls the flow of nutrients and fluid and prevents the passage of microorganisms and toxic agents (reviewed by Neunlist et al., 2013).

Similarly to the astrocytes that control the adhesion of endothelial cells of blood vessels forming the BBB (Abbott et al., 2006), enteric glial cells play a decisive role in maintaining the intestinal barrier (Savidge et al., 2007b). The mucosal enteric glia is located very close to the epithelial edge (<1 μ m), and terminal end-feet processes occasionally appear in direct contact with the epithelial basal membrane and

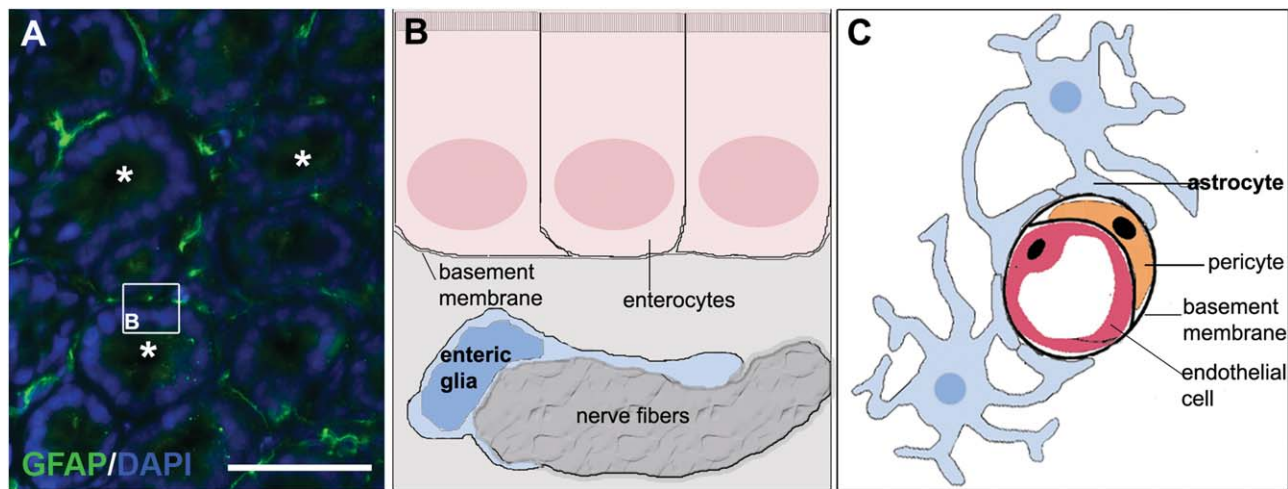


FIGURE 5: Arrangement of glial cells in intestinal epithelial barrier (A and B) and blood-brain barrier (BBB) (C). (A) Mucosal enteric glia immunostained for GFAP around colonic epithelial crypts (*). (B) Schematic representation of an enteric glial cell ensheathing a nerve tract composed of several fibers in close proximity (about 1 μ m) to the basement membrane of enterocytes, the intestinal epithelial cells. (C) BBB formed by endothelial cells and astrocytic end-feet touching the basement membrane. Scale bar: 50 μ m. See Neunlist et al. 2007; Bohórquez et al. 2014 and Abbott et al. 2006 for references.

blood capillaries in the lamina propria (Savidge et al., 2007a) (Fig. 5). Enteric glia control the proliferation of epithelial cells and also the adhesion between them. Transforming growth factor beta 1 (TGF β 1), secreted by enteric glia, negatively affects proliferation and increases the apical surface area of epithelial cells (Neunlist et al., 2007).

In transgenic mice with conditional ablation of GFAP-positive cells in the gut, the absence of enteric glia increases the permeability of the intestinal epithelia with a consequent inflammatory reaction, accompanied by enhanced gene expression of pro-inflammatory cytokines and alterations in the jejunum and ileum structures (Bush et al., 1998). The same sequence occurs when S-nitrosoglutathione (GSNO) production is inhibited in enteric glia. GSNO is a potent inducer of barrier properties in epithelia *in vivo* and *in vitro*, leading to increased expression of F-actin and the tight junction-associated proteins, and regulating epithelial permeability at the mucosal surface. GSNO also inhibits the increase in levels of tumor necrosis factor alpha (TNF α), and prevents the increase in permeability of the epithelial layer induced by the joint action of TNF α , interferon gamma (INF γ), and interleukin 1 beta (IL1 β) *in vitro* (Cheadle et al., 2013; Savidge et al., 2007a). GSNO released by the enteric glia protects intestinal epithelial cells from invasion by *Shigella flexneri*, an enteroinvasive pathogen that causes dysentery (Flamant et al., 2011).

Although their role in maintaining the intestinal epithelial barrier is well known, the functions of the enteric glia in healing of wounds to the intestinal mucosa are still poorly studied. Van Landeghem et al. (2011) showed that in mucosal damage, with dextran sulfate sodium (DSS) and diclofenac

treatments, conditional ablation of enteric glia worsened the wound and delayed the lesion healing, respectively. *In vitro*, enteric glial cells enhanced epithelial recovery through secretion of pro-epidermal growth factor (pro-EGF) and activation of the focal adhesion kinase (FAK) signaling pathway (Van Landeghem et al., 2011).

Enteric Glia as a Progenitor Cell

Some types of glial cells maintain a progenitor potential, and can replace and supplement damaged regions with new cells, even in adults. Examples are the astrocytes of the subventricular zone in the lateral ventricles and those in the subgranular zone of the dentate gyrus of the hippocampus, which undergo neurogenesis in the adult brain (reviewed by Ihrie and Alvarez-Buylla, 2008). Even in adult cerebral cortex, astrocytes can differentiate through direct conversion into functional neurons when induced by specific transcription factors (Heinrich et al., 2010). Müller glial cells of the retina can give rise to neurons in case of injury, for instance (Ooto et al., 2004; Stutz et al., 2014). In the PNS, in the adult carotid body, glial-like stem cells of this oxygen-sensing organ differentiate into new neurons under hypoxemia (Pardal, 2007).

Although neurogenesis is a well-known physiological function of some adult glial cell types, the presence of these properties in enteric glial cells remains a matter for discussion. As reviewed by Dupin and Coelho-Aguiar (2013), many NC-derived stem cells have been found at different body sites in adults. In the gut, these cells have been isolated from postnatal and adult rodents (Bondurand et al., 2003; Kruger et al., 2002) and humans (Almond et al., 2007; Rauch et al.,

2006). They can be isolated in two ways: by flow cytometry of gut neural cells expressing specific markers (Joseph et al., 2011; Kruger et al., 2002) or by growing dissociated neural cells of the gut in appropriate conditions for the formation of neurospheres (Almond et al., 2007; Bondurand et al., 2003; Schafer et al., 2003), as we could show in Fig. 4.

The first identification of these cells was reported by Kruger and coworkers, who isolated p75NTR-positive progenitor cells from postnatal and adult rat ENS, grew some in neurospheres, and found that they are able to generate colonies composed by neurons, glial cells and myofibroblasts; and are also able to give rise to glia and neurons after transplantation and migration through the NC pathway in the chick embryo (Kruger et al., 2002). Later, Joseph and coworkers found that CD49b (integrin $\alpha 2$) could be used to highly enrich enteric neural progenitors of adult mice, and these cells formed multipotent neurospheres in culture (Joseph et al., 2011). CD49b-positive cells have characteristics of enteric glia and express p75NTR, GFAP, S100 β , and Sox10.

Progenitor cells can also be found in the human gut. They have been isolated and grown as neurospheres from fetuses and 5-year-old children (Rauch et al., 2006) and from adults (Hetz et al., 2014; Metzger et al., 2009). When transplanted into murine gut explants, both embryonic and postnatal cells (Almond et al., 2007; Lindley et al., 2008, 2009; Metzger et al., 2009; Rauch et al., 2006) and cells from adult individuals (Hetz et al., 2014) were able to generate neurons and glia. In addition, some of these studies have reported that the contractility of the bowel was restored (Hetz et al., 2014; Lindley et al., 2008, 2009; Rauch et al., 2006).

To investigate the physiological function of these glial stem cells in the adult gut, Joseph and coworkers analyzed the fate of the CD49b-positive adult enteric glial cells under normal conditions and with different stimuli. They looked for neurogenesis and gliogenesis in the adult ENS, through the incorporation of the thymidine analog BrdU to detect dividing cells, and found that even in injury conditions, neurogenesis was very limited, while gliogenesis was substantial, even under physiological conditions (Joseph et al., 2011). At the same time, a companion study also demonstrated the glial identity of the adult neurogenic stem cells of the ENS. This study found that, in addition to giving rise to neurons in culture, cells expressing Sox10 and also the glial marker GFAP were able to undergo neurogenesis *in vivo* in response to chemical injury (Laranjeira et al., 2011).

While the study by Laranjeira et al. (2011) used fate-mapped Sox10-positive cells, Joseph et al. (2011) studied the GFAP-labeled cells of GFAP-Cre recombinase mice. It is possible that neurogenic enteric glia comprise a portion of cells expressing Sox10 and were not detected in GFAP-Cre animals. It is also possible that neurogenesis from enteric glia

occurs by direct conversion, without proliferation, as described in astrocytes of the cerebral cortex, and because of this, it was not possible to detect neurogenesis by BrdU incorporation.

Independently of these differences in results, when taken together these studies suggest that adult enteric glia give rise to new glial cells under injury conditions. However, they maintain limited neurogenic potential, which can be activated as “reactive potential” induced by tissue dissociation as well as triggered by injury. It is still unclear whether all glial cells of the intestine are able to perform neurogenesis, or whether only a subpopulation of these cells retains this potential.

Although we recognize the neurogenic capacity of enteric glia, little is known about the signals and signaling pathways, probably absent under physiological conditions, which are implicated in this process. One way to obtain enteric neurogenesis in adult animals *in vivo*, is to use agonists for the serotonin receptor 5-hydroxytryptamine 4 (5-HT₄), which leads to the activation of CREB (cAMP response element-binding protein) (Liu et al., 2009). A recent study showed that the switch from quiescence to proliferation and the neurogenesis that occur in the 5-HT₄ treated myenteric plexus of adult mice in an *ex vivo* organotypic culture corresponds to a change in the PI3K/AKT pathway through the phosphatase and tensin homolog (PTEN), a negative regulator of this pathway. Both a decrease in PTEN mRNA expression and an increase in the inactive form of PTEN were observed (Becker et al., 2013).

Although the event of neurogenesis in the adult gut remains a matter for discussion, it is well established that an appropriate environment, such as the fetal and postnatal colon, can elicit neurogenesis of these glial-like stem cells, making them a promising cell population for developing therapies for disorders of the ENS. The transplantation of progenitors from a healthy to a damaged part of the intestine, as performed for the mouse gut (Metzger et al., 2009), could be promising to restore the intestinal properties and functions.

Neuron-Glia Communication

In the CNS, neurons can activate the development of astrocytes, as we have described (Garcia-Abreu et al., 1996; Gomes et al., 1999a,b; Faria et al., 2006; Romão et al., 2013), while astrocytes modulate neuronal synaptic transmission and plasticity (Araque et al., 1999; Bezzi and Volterra, 2001; Diniz et al., 2012; Gomes et al., 1999a; Romão et al., 2008). Astrocytes also participate in heterocellular signaling through the release of gliotransmitters triggered by increased cytosolic fluid [Ca²⁺] (Araque et al., 2002; Malarkey et al., 2008; Romão et al., 2008), and participate in neuron differentiation (Gomes et al., 1999a,b). Astrocyte synaptic vesicles can



contain gliotransmitters such as excitatory and inhibitory amino acids (GABA, glutamate and D-serine) and nucleotides (ATP), the tissue plasminogen activator tPA, and other mediators such as neurotrophins [nerve growth factor (NGF), NT3, brain-derived neurotrophic factor (BDNF)] and cytokines (interleukins and tumor necrosis factors) (Bergami et al., 2008; Blum et al., 2008; Fujita et al., 2009; Kang et al., 2013; Lee et al., 2011, 2013; Perea and Araque, 2005; Zhuang et al., 2010).

Although enteric neuro-glial communication is not as well understood as in the CNS (Faria et al., 2006; Froes et al., 1999; Garcia-Abreu et al., 1996; Izmiryani et al., 2006; Romão et al., 2013), the idea that the output of enteric neurons is modulated by the enteric glia is addressed in a growing number of articles. This proposal is supported by the ideal position of the enteric glia in the enteric ganglion, interacting closely with neurons (Fig. 3A–D). Indeed, the enteric glial cells have mechanisms similar to those found in astrocytes: production of neurotransmitter precursors, expression of receptors for neurotransmitters, mechanisms for the uptake and degradation of neurotransmitters, and release of signaling molecules such as trophic factors that support the neuron–glia interactions (Cirillo et al., 2011; von Boyen and Steinkamp, 2006; von Boyen et al., 2006a).

The activation of the enteric glia by neurons elicits responses such as increased levels of intracellular Ca^{2+} or cyclic AMP (Broadhead et al., 2012; Christofi et al., 1993; Gulbransen et al., 2010; Segura et al., 2004). Stimulation of interganglionic neurons in the myenteric plexus of the guinea pig colon elicited increases in intracellular Ca^{2+} in enteric glial cells that was impaired by tetrodotoxin, a voltage-gated Na^+ channel blocker (Gulbransen and Sharkey, 2009). It has been demonstrated that in cases of gut inflammation, with elevated extracellular purine (ATP) concentrations, purine (ATP) is released from enteric neurons through pannexin channels directly to the enteric glia, which leads to Ca^{2+} responses in neurons and glia (Gulbransen et al., 2012). Indeed, activation of enteric glia seems to depend on the activation of purinergic receptors, suggesting that ATP released with neurotransmitters during enteric synaptic transmission acts as a signal to enteric glia. A recent study provided evidence that rat and human enteric glial cells respond to different excitatory neurotransmitters by changes in intracellular Ca^{2+} levels (Boesmans et al., 2013). This report showed that not only is there purinergic neuron–glia communication in the ENS, but also that the enteric glia are responsive to other neurotransmitter systems, since stimulation with acetylcholine and serotonin also induced an increase in the intracellular Ca^{2+} concentration in enteric glial cells.

In a mouse model of enteric glia disruption, changes were observed in the proportions of different types of enteric

neurons. An alteration in the proportions of cholinergic and nitrergic neurons in the myenteric plexus was followed by a delay in gastrointestinal motility and an increase in intestinal permeability in transgenic as compared with control mice (Aube et al., 2006). Furthermore, evidence suggests that a novel glial-derived mediator, 15-Deoxy- $\Delta^{12,14}$ -prostaglandin J2 (15d-PGJ2), an omega-6 derivative, has neuroprotective properties against oxidative stress in the ENS (Abdo et al., 2012; Bach-Ngohou et al., 2010). However, there is still no direct evidence that supports a hypothesis similar to that stated for the CNS homeostasis, i.e., that the enteric glia responds to neuronal activation with Ca^{2+} -dependent gliotransmitter release.

Role of Enteric Glia in Diseases

The Role of Enteric Glia in Neurodegenerative Diseases. In the last 20 years, the idea that the glia provides only structural support for neurons has proven incorrect with respect to the CNS. Likewise, this assumption does not hold true for the ENS. The cross-talk between the enteric glia and neurons contributes to neuronal maintenance, survival, and function (Ruhl, 2005; von Boyen and Steinkamp, 2006). In view of this role of enteric glia in protecting enteric neurons, any impairment to enteric glial cells may induce a neurodegenerative response.

Enteric glial cells are susceptible to a number of metabolic processes that are common to many neurodegenerative diseases. For example, mice with disrupted mitochondrial metabolism showed alterations in glial cell density in parallel to neuron loss. Although initially viable, these animals suffered premature death caused by severe gastrointestinal motility problems as a result of extensive and progressive neurodegeneration (Viader et al., 2011).

Glial dysfunction has been implicated in many neurodegenerative diseases and this seems to be true for both CNS and ENS. Parkinson's disease (PD), initially described as a CNS dysfunction, is presently considered a multicentric disease, and is an example of a neurodegenerative disorder capable of changing the behavior of the GIT. This pathology produces constipation as well as hyposmia as the main non-motor symptoms (Cersosimo and Benarroch, 2012), which can precede the development of motor symptoms by several years. Constipation is also observed in mice subjected to striatal injection of 6-hydroxydopamine (Blandini et al., 2009), considered an animal model of PD. The large-intestine tissue was compromised, showing extensive disorganization of the mucosa (Fig. 6A,B).

The pathological hallmark in PD is the formation of aggregates of α -synuclein in susceptible neurons (or glial cells) in the CNS, called Lewy bodies and Lewy neurites, which are

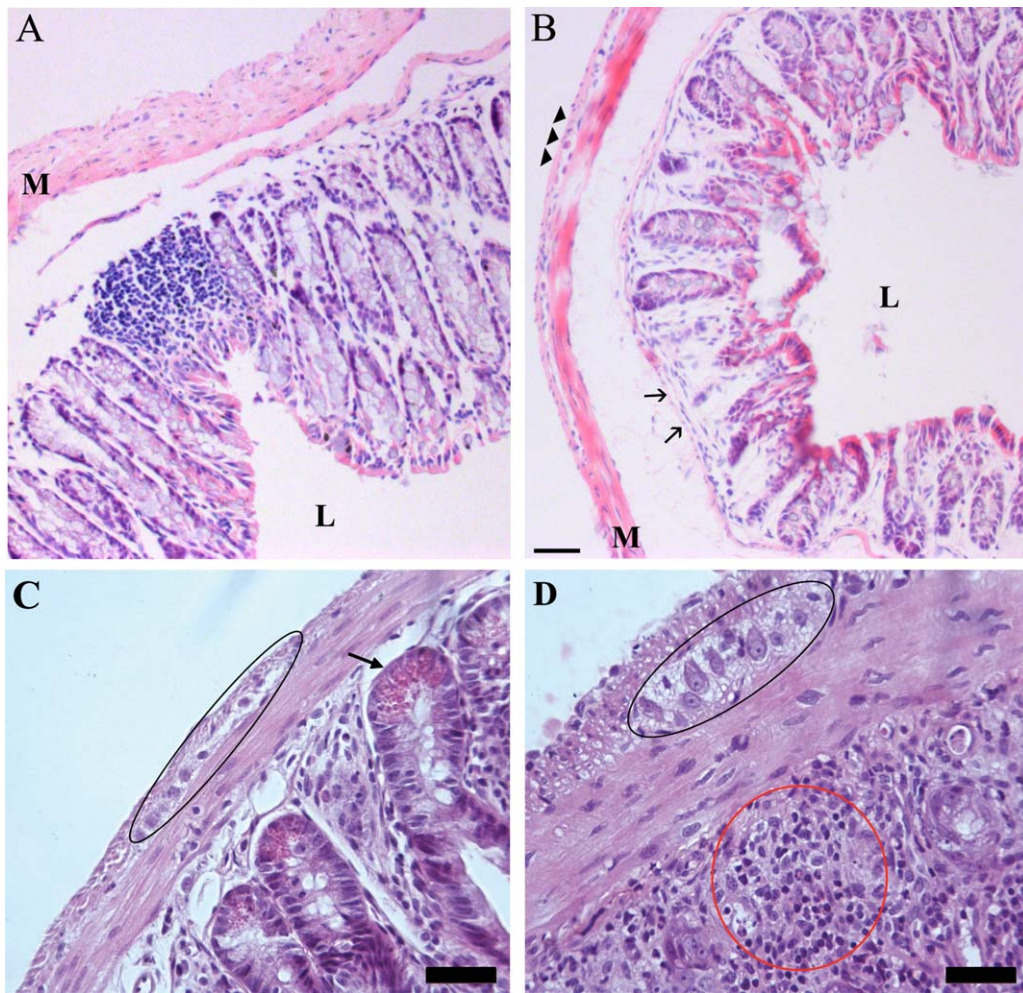


FIGURE 6: Gut morphology revealed by HE staining from wild-type and Parkinson's disease (PD) mice (A and B); and wild-type and Irinotecan induced-gut inflammation mice (C and D). (A) Colonic section from wild-type mouse showing the tissue integrity. (B) Experimental PD-mouse induced by intracerebral injection of 6-hydroxydopamine displays a disorganization of colonic tissue architecture (arrows). Observe the disruption of myenteric plexus (arrowhead). Original magnification, $\times 200$. (C) Ileum from wild-type mouse, showing intact crypts with Paneth cells (arrow) and myenteric plexus (ellipsis). (D) Mouse treated with the chemotherapeutic Irinotecan for 5 days. Observe the loss of crypt architecture and marked infiltration of inflammatory cells (red circle). There was clear hypertrophy of the longitudinal muscle layer and hypertrophic myenteric plexus (ellipsis). L: gut lumen; M: muscle. Scale bars: 40 μm in A, B; 20 μm in C–D. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

found in both the CNS and ENS (Braga et al., 2011). Braak suggested that the ENS is the initial site of α -synuclein aggregation, with subsequent spread to the CNS via the vagal pre-ganglionic fibers, reaching the dorsal motor nucleus of the vagus nerve (Braak et al., 2003). Lewy bodies/neurites are detectable in the presymptomatic stage of PD along the autonomic pathways and in the GIT (Lebouvier et al., 2010). Analysis of human colon biopsies obtained from 2 to 5 years before PD onset showed the presence of α -synuclein in neurodegeneration sites, suggesting that colonic α -synuclein staining can be considered a biomarker of premotor PD (Shannon et al., 2012). Other alterations found in the PD scenario, such as elevation in the levels of pro-inflammatory cytokine mRNAs—TNF α , INF γ , IL1 β , IL6-, and glial markers—GFAP, Sox10 and S100 β —in the large intestine of PD

patients, suggest an association between enteric inflammation and glial dysregulation (Devos et al., 2013). Recently, colonic biopsies from PD patients revealed the presence of enteric glial reactivity through elevation in GFAP expression and reduction in its phosphorylation, which have been associated with degenerative CNS diseases (Clairembault et al., 2014).

Prion diseases are other examples of neurodegenerative disorders correlated with alterations in the performance of enteric glial cells. The prion diseases are progressive and fatal neurodegenerative disorders caused by dissemination of pathological isoforms of the cellular prion protein (PrP^C) (Kovacs and Budka, 2008). These diseases have been reported to affect the CNS—astrocytes and astrocytomas (Erlich et al., 2007; Fonseca et al., 2012; Lima et al., 2007). Albanese et al. (2008) demonstrated that enteric glia constitutively express



the PrP^C. Moreover, the GIT is the major exogenous prion protein entry site, and studies have described prion uptake through the gut and have found replication sites early in the enteric plexus, prior to replication in the CNS (Kujala et al., 2011). These data suggest that enteric glial cells may play a critical function in prion neuroinvasion, i.e., the route of prion movement from the digestive system to the brain. Enteric glial cells of an animal model with a typical prion disease (chronic wasting disease) were able to accumulate the pathological prion protein, which was also found in the CNS as a result of centrifugal spread (Seelig et al., 2011). The cellular alterations induced by prions in astrocytes are well known (Erlich et al., 2007; Hartmann et al., 2013; Lima et al., 2007). These alterations are similar to those observed in enteric glia in the presence of a prion disease, functioning as a prion aggregation site and dissemination agent, and triggering toxic events (Cronier et al., 2012). Once again, this shows the intimate correlation between enteric glial cells and astrocytes.

The Role of Enteric Glia in Intestinal Inflammation and Injury. Enteric glial cells act in homeostasis and protection of the gut in different ways, including in the inflammation process (reviewed by von Boyen and Steinkamp, 2010). Consistent with this protective function, genetic ablation of enteric glial cells results in a fatal hemorrhagic jejuno-ileitis, the enteric plexus is disrupted and the epithelial layer loses its integrity, resulting in fatal inflammation (Bush et al., 1998). Similarly, the destruction of enteric glial cells by an autoimmune mechanism leads to enterocolitis (Cornet et al., 2001). Patients with Crohn's disease (CD) have a smaller glial network (Cornet et al., 2001; von Boyen et al., 2011), and plexitis has been related to the recurrence of CD after surgery (Sokol et al., 2009). In irritable bowel syndrome, inflammatory infiltrates are found in association with the enteric plexuses (Chadwick et al., 2002). Along the same line, lymphoplasmacellular infiltrate within the myenteric plexus is a common finding in intestinal dysmotility and megacolon (De Giorgio et al. 2012). Moreover, loss of neurons and glial cells in myenteric and submucosal plexus is characteristic of necrotizing enterocolitis (NEC), a disease occurring in premature infants (Sigge et al., 1998; Wedel et al., 1998). In a model of intestinal mucositis induced by the antineoplastic agent Irinotecan, we can also observe alterations in the myenteric plexus, as illustrated in Fig. 6C,D.

In inflammatory bowel diseases (IBD) such as CD and ulcerative colitis (UC), proliferation of enteric glial cells is altered, as is the expression of GFAP and S100 β . Increased GFAP expression is found in inflamed regions of the intestine in patients with CD and UC. However, in areas that are not inflamed, a higher level of GFAP is found in UC, but not in

CD (von Boyen et al., 2011). The reduced level of GFAP in non-inflamed intestine of Crohn's disease patients may be linked to injury to glia cells resulting from continuous inflammation. The glial-cell injury leads to reduction of glial-derived protective factors, such as GDNF, resulting in altered barrier function, which may be involved in the pathogenesis of Crohn's disease. Colon biopsies of patients with *Clostridium difficile* infections also showed increased GFAP levels, as well as an increased production of GDNF comparable to the colon of UC patients (von Boyen et al., 2011), suggesting that these events are not specific for IBD, but may be a reaction of the enteric glia network during gut inflammation.

High expression of GFAP is a well-known characteristic of reactive astrocytes in the CNS. These cells are important for healing in the brain and spinal cord (Hamby and Sofroniew, 2010; Neunlist et al., 2013). Whether this also occurs in the intestine is still not fully accepted.

The expression of the Ca⁺²/Zn⁺²-binding protein S100 β by enteric glial cells is increased early in the inflammatory process in response to the signal triggered by disruption of the intestinal barrier (Cirillo et al., 2009). It modulates signals of active inflammation, such as nitric-oxide (NO) production in intestinal mucosa by the enteric glial cells in patients with celiac disease and UC (Esposito et al., 2007; Cirillo et al., 2009). NO is a pro-inflammatory molecule involved in antibacterial response. Recent studies showed that, similarly to the astrocytes, human enteric glial cells express toll-like receptors (TLR), and pretreatment of these enteroglia cells with specific inhibitors of the TLR and S100 β pathways abolished NO release induced by infection with pathogenic bacteria (Bowman et al., 2003; Turco et al., 2014). In addition to inducing NO production, exogenous S100 β leads to an increase in lipid peroxidation, together with increased levels of phosphorylated-p38 mitogen-activated protein kinase (MAPK) and activation of nuclear factor-kappa B (NF- κ B) (Esposito et al., 2007). Recently, the same group showed that treatment with palmitoylethanolamide (PEA) prevents inflammation mediated by enteroglia expression of S100 β / TLR4, by activating PPAR α and causing a downstream inhibition of NF κ B-dependent inflammation (Esposito et al., 2013). Thus, S100 β can be considered a pro-inflammatory cytokine involved in inflammation caused by pathogenic bacteria and in gut diseases such as celiac disease and UC.

Similarly, the expression of S100 β by astrocytes in the CNS is associated with processes of reactive gliosis. In the CNS as well as in the ENS, S100 β protein has neurotrophic activity, modulating growth and differentiation at nanomolar concentrations (Hinkle, 1998; Huttunen et al., 2000); but at micromolar concentrations it is involved in neuroinflammatory and neurodegenerative responses (Rothermundt et al., 2003).

Activation of enteric glia during inflammation occurs by proinflammatory cytokines (von Boyen and Steinkamp,

2010). This could be clearly demonstrated *in vitro*, when the proinflammatory cytokine IL1 β increased the percentage of mouse enteric glial cells expressing GFAP (von Boyen et al., 2004). In animal models of intestinal inflammation, interleukin IL-1 β , via its IL-1 receptor, activates c-Fos in enteric glia (Miampamba and Sharkey, 1999), which stimulates the synthesis and release of IL-6, another proinflammatory cytokine (Rühl et al., 2001; Tjwa et al., 2003).

Although enteric glial cells and astrocytes show many similarities, these cells can respond in different ways to cytokines. IL-1 β inhibits proliferation, but IL-10, an anti-inflammatory cytokine, can suppress glial cell proliferation in low concentrations and enhance it in high concentrations (Rühl et al., 2001). In astrocytes cultured *in vitro*, both IL-10 and IL-1 β had concentration-related effects on proliferation rates (Mucke and Eddleston, 1993; Rühl et al., 2001). The dual effects of IL-1 β and IL-10 suggest that, during intestinal inflammation, there may exist an interplay between different cytokines regulating enteric glia proliferation, defining a particular identity for enteric glial cells.

Although proinflammatory cytokines, particularly TNF α and INF γ , activate enteric glial cells, they may also cause apoptosis when operating for a longer period. Reversal of this cell-death event was possible through treatment with anti-TNF α antibodies in patients with CD, allowing the glial structure to recover and the mucosa to heal (Colombel et al., 2010).

Enteric glial cells also function in gut inflammatory processes by increasing the expression of neurotrophic factors such as GDNF, BDNF, and NGF. In IBD, GDNF has an antiapoptotic effect on intestinal epithelial cells and also on enteric glial cells, functioning as a protective feedback loop (Steinkamp et al., 2012a). Another neurotrophic factor with antiapoptotic effects for the enteric glia is BDNF (Steinkamp et al., 2012b). In contrast, NGF seems to improve gut inflammation (von Boyen et al., 2006b).

Considering the role of glial cells in modulating the epithelial barrier and intestinal inflammation, further research in this field may lead to the development of new approaches focused on the neuro-glial axis, which may provide an alternative for the management of intestinal disorders.

Conclusion

Although they have different embryological origins and are also located in different regions, the astrocytic type of glia and the intestinal glia share many characteristics. The star-shaped appearance and intimate contact between glial cells and neurons are observed in both the CNS and the ENS. These cells also show two main specific glial markers, GFAP and S100 β . It seems that the differences between these two types of glia cells, one from the CNS and the other present

in the ENS, depend on the part of the neuro-system and also on their embryological histories, such as the continued expression of markers of premature cells, e.g., Sox2 by astrocytes and enteric glia, and Sox10 by only enteric glia. Notable functional similarities include their roles in protecting the barriers of their respective environments (the BBB in the CNS and the intestinal barrier in the ENS), direct action in neurotransmission, and the ability to act as progenitor cells. The enteric glia responds to injuries and produces growth factors to control inflammation.

Despite the great advances made recently in the understanding of enteric glia activity, we still know very little about these cells. Expanding knowledge of the biology of enteric glia would help to understand the functioning of the ENS, which is essential for the homeostasis of the GIT and consequently of the organism. Better knowledge would help to conceive feasible cell therapies to combat gastrointestinal ailments, including debilitating diseases such as Hirschsprung's disease, neurodegenerative disorders, and different types of gut inflammation.

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