The multiple faces of inflammatory enteric glial cells: is Crohn's disease a gliopathy?

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Abstract

Gone are the days when enteric glial cells (EGC) were considered merely as satellites of enteric neurons. Like their brain counterpart astrocytes, EGC express an impressive number of receptors for neurotransmitters and intercellular messengers, thereby contributing to neuroprotection and to the regulation of neuronal activity. EGC also produce different soluble factors that regulate neighboring cells among which are intestinal epithelial cells. A better understanding of EGC response to an inflammatory environment, often referred to as enteric glial reactivity, could help define the physiological role of EGC and the importance of this reactivity in maintaining gut functions. In chronic inflammatory disorders of the gut such as Crohn's disease (CD) and ulcerative colitis (UC), EGC exhibit abnormal phenotype and their neighboring cells are dysfunctional, but it remains unclear whether EGC are only passive bystanders or active players in the pathophysiology of both disorders. The aim of the current paper is to review the physiological roles and properties of EGC, their response to inflammation, their role in the regulation of the intestinal epithelial barrier and to discuss the emerging concept of CD as being an enteric gliopathy.

Introduction: IBD, ENS and intestinal epithelial barrier

The inflammatory bowel diseases (IBD), which comprise ulcerative colitis (UC) and Crohn's disease (CD), are complex chronic inflammatory disorders of largely unknown cause in a genetically predisposed host. The incidence of both diseases varies between different countries but overall has increased greatly in recent years and IBD is now a major public health problem that affects approximately 3.6 million people in the United States and Europe (1). IBD is characterized by chronic or relapsing immune activation and inflammation within the gastrointestinal (GI) tract that severely alters GI functions. CD can affect any region of the gut but terminal ileum and proximal colon are the most frequent localizations. The lesions are often discontinuous and may involve all the layers of the gut. By contrast, the inflammation and ulcers in UC are more limited mainly involving the mucosa of the large intestine. Despite these differences, the inflammation of the gut observed in both disorders is concomitant to a breakdown in intestinal barrier function, abnormal secretion, changes in motility and visceral perception, all together contributing to symptom generation. IBD are associated with an increased risk of developing colorectal cancer, with a cumulative probability of about 18% for UC and 8% for CD patients, after 30 years of disease (77).

The etiology of IBD is not fully elucidated yet. However, there are now compelling evidences suggesting that (i) T cell and T cell trafficking to the gut and its associated lymphoid tissues are important components in disease pathogenesis (ii) the intestinal bacteria are critical for the development of IBD and (iii) genetic factors play an important role in susceptibility to the disease. Indeed, Card15⁄Nod2, DLG5 or OCTN1 genes, which are associated with a dysregulation of the immune response, have been identified as susceptibility genes for IBD. These observations provide the rationale for the main actual therapeutics (anti-TNF-α, -β4 integrin antibodies) and also the ones in development (anti-IL12, -IL23, -IL17, -MADCAM, -CCR9) (76). Despite an optimized use of immunosuppressive drugs and new biologic agents, preventing disease relapses remains a challenge and surgery is still required in approximately 1/3 of patients with IBD at some point during their lifetime. Primary and secondary failure to respond to approved therapies, and in some cases inability to provide a surgical treatment to a particular patient due to extension and/or location of lesions, are still unmet needs in the management of IBD. In the absence of a definitive cure, the research in the pathophysiology of IBD is necessary to improve disease outcome and prevention, and discover new effective and lasting treatments.

Aside from the study of the intestinal immune system and microbiota, interest has been focused on an essential component at the interface between the organism and the environment, the intestinal epithelial barrier (IEB)(55, 56, 68). This single layer of epithelial cells acts as a selective permeable barrier, permitting the absorption of nutrients, electrolytes, and water while maintaining

an effective defense against intraluminal toxins, antigens, and enteric microbiota (52). Among different protein-protein complexes that set up and maintain IEB cohesion, the tight junctions seal the intercellular space. The tight junctions control the diffusion of water and solutes thanks to multi-protein complexe formation (transmembrane proteins, scafolding proteins and regulatory molecules that include kinases) (85, 98) and defects in their composition have been associated with intestinal diseases. Claudin-2 upregulation, occludin downregulation or myosin light-chain kinase activation have been observed in both CD and UC (98, 110). In addition, the continual renewal of the surface epithelium (balance between cell shedding at the top of the villi and the generation of new cells in the crypts) is necessary to IEB homeostasis (98). During chronic inflammation, this homeostasis is lost, reflected by an increase in the electrical conductance directly over sites of epithelial apoptosis (34). IEB hyperpermeability has been described as an early feature of IBD (3, 69), and its reduction protects against the development of inflammation (4). Moreover, increased intestinal permeability has been observed in healthy first-degree relatives of patients (60) as well as in non-inflamed portions of the gut (86). It also precedes the onset (44) and the relapses of CD (2, 108), suggesting that this defect can occur independently of inflammation. In addition a good healing is associated with clinical remission (7, 70), altogether suggesting that a failing regulation of the IEB might contribute to IBD pathogenesis.

The IEB is regulated by its environment, among which is the enteric nervous system (ENS). The ENS is an integrative autonomous nervous network which extends from the lower 2/3 of the esophagus to the rectum and is organized in two major ganglionic plexus composed of neurons and enteric glial cells (EGC): the myenteric plexus (or Auerbach plexus) that controls the digestive motility, and the submucosal plexus (or Meissner plexus) that controls the IEB functions and the local blood flow (36, 81). It has been shown that ENS activation through the stimulation of the vagus nerve can modulate the integrity of the IEB by blocking the disorganization of tight junctions observed during IEB breakdown (27, 49). Most of the initial studies on the ENS and IBD have focused on neurons and showed morphological and numbering changes, nerve fiber hypertrophy and hyperplasia as well as alteration of the enteric neuronal cell bodies (82, 84). There is, nevertheless, mounting evidence that EGC might be also critically involved in IBD. In this short review, we will discuss the main physiological of characteristics of EGC, their response to inflammation, their role in the regulation of IEB and eventually their possible role in the pathophysiology of IBD.

EGC features

First observed by Dogiel in 1899 as nucleated satellite cells close to enteric neurons, EGC were more precisely characterized in 1971 by Gabella who distinghished them from peripheral Schwann cells (33). EGC originate from neural crest cells that migrate along the gut during embryonic stages and keep on developing after birth (48) to be present along the entire ENS at adulthood. EGC within the gut wall are found not only in the myenteric and the submucosal plexus (intraganglionic), but also associated with the nerve fibers (interganglionic) between smooth muscles (intramuscular) and in the lamina propria (subepithelial). EGC outnumber neurons by 4- to 10-fold (67) and are closely associated with them in ganglia. EGC are often described as the gut counterpart of astrocytes of the central nervous system (CNS) due to morphological, molecular and functional similarities.

EGC heterogenous shape and locations gave rise to morphological classifications (12, 39, 42) with four main subtypes of EGC. The type I is intraganglionic glia with short dense process tree, the type II is peripheral or interganglionic glia with long parrallel processes, the type III is extraganglionic glia with four major processes and the type IV are intramuscular elongated bipolar glial cells running in circular or longitudinal muscles.

At a molecular level, like astrocytes, the EGC express the glutamine synthase, the intermediate filaments vimentin and glial fibrillary acidic protein (GFAP), the rat neural antigen-2 (Ran-2) (45, 46) and the calcium binding protein S100β (30). Nine splice variants of GFAP have been described in the CNS, but the more recently discovered GFAPκ isoform is the one mainly expressed by EGC in biopsies (22). EGC also maintain strong expression of the transcription factor Sox-10, which is a central factor required for peripheral glial fate acquisition and also for EGC differentiation from neural crest cells. Some of these markers (vimentin, Ran-2, S100β) are also markers of Schwann cells but EGC do not express the galactocerebroside like myelin-forming Schwann cells and do not ensheath neuronal fibers. Looking for membrane or more specific EGC markers, Boesmans *et al.* reported that in the myenteric plexus EGC do not express the astocytic protein Aldh1L1 (13), while Rao and collaborators identified the glial proteo-lipo-protein 1 (PLP1) as a new EGC marker (73). In a recent and elegant study, the group from Pieter Vanden Berghe used mosaic analysis with double markers to characterize the morphology and the marker expression of EGC in mouse gastrointestinal tract (12). While most EGC located in the myenteric ganglia coexpressed GFAP, S100β and Sox-10, glia outside the plexi was more heterogeneous and expressed either one, two or three markers with different ratio in type I, II or III glial sub-type. Because GFAP, S100β and Sox10 are still the most commonly used markers to study EGC, this might be a shortcoming in the accurate evaluation of enteric glial functions.

EGC have been involved in almost every gut function including motility, IEB properties and host defense. Due to their strategic location across the gut wall, EGC are in close contact with different other cellular compartments (32, 68) and can respond to a broad range of stimuli, ranging from microbiota (48, 97) and diet (8, 66, 94) to neurotransmitters through cytokines (38, 65) (Figure 1). They respond to stimulation by changes in proliferation, in the expression of glial markers or soluble mediators and in their ability to sense other signals (regulation of receptor expression) as discussed below for the responses to inflammation (Table 1). EGC are also characterized by Ca2+ transients induced by purinergic (35, 38, 39, 40) serotonin, adrenergic, cholinergic and protease-activated receptor agonists, endothelins, and lysophosphatidic acid (10, 37). Heterogeneity of ligand-induced calcium response has been observed in EGC as only 85 %, 75 % or 35 % of the glial type I, II and III, respond to ATP stimulation, respectively, (12). To regulate their surrounding cells and IEB, EGC produce and release several soluble mediators (Figure 1) in response or not to environmental stimuli (see Table 1 for response to inflammation).

EGC regulation of the intestinal epithelial barrier

From animal studies published 10 to 20 years ago, EGC were considered as necessary to IEB homeostasis as EGC ablation led to intestinal inflammation along with an alteration of the mucosal integrity. Regardless of the experimental model that was used (GFAP positive cell poisoning or immune-depletion), EGC ablation induced an increase in intestinal vascular permeability and an increase in IEB paracellular permeability prior to signs of inflammation (5, 16, 25, 50, 80). Since then, several *in vitro* studies using primary EGC culture showed that EGC have the capability to reinforce the IEB (31, 90), enhance epithelial healing (99), inhibit epithelial cell proliferation (6, 64, 90) and even have a broader effect on epithelium by modifying its transcriptome (100). All these effects showed that EGC regulate IEB properties through the production and release of soluble factors. The S-nitrosoglutathione (GSNO) reinforces the IEB by both decreasing its permeability (26, 80) and increasing its resistance against *Shigella Flexneri* infection (31). EGC accelerate wound healing through the production of the pro-epithelial growth factor (pro-EGF) (99) and strongly inhibit intestinal epithelial cell proliferation through the release of transforming growth factor-β1 (TGFβ1) (64) or through the main PPARγ ligand, the 15-deoxy-(12,14)-prostaglandin J_2 ($15dPGJ_2$), that could also regulate intestinal epithelial cell differentiation (6). Rat or human EGC have the ability to produce several others n-3 and n-6 polyunsaturated fatty acid derivatives, including the 15-hydroxyeicosatetraenoic acid (15-HETE) and the 11β-Prostaglandin $F_{2\alpha}$ ($11\beta\text{–}PGF_{2\alpha}$), which regulate

IEB permeability (72) and healing (24) respectively. The neutrophic factor “glial cell line-derived neurotrophic factor” (GDNF) has also IEB reinforcing properties, especially epithelial antiapoptotic effect, but whether it concerns a direct glio-epithelial effects or it involves neurons or immune cells is not clear (58). In the same way, it is unclear whether these protective effects are due to glial or epithelial GDNF production (57). Mice genetically modified to overexpress or knock down soluble factor specifically in enteric glia should be studied to specify the importance of the glio-epithelial communication. An interesting recent work by Rao and colleagues concluded that the glio-epithelial communication is physiologically irrelevant, and tempers if not discredits the role of EGC on IEB control *in vivo*. Using the recently describe PLP1 glial marker (73), they analyzed mice depleted for PLP1 positive cells and showed that these cells were not required for the maintenance of gastrointestinal epithelium (74). In their mice model in which 80% of S100β positive cells are depleted in both the myenteric and the submucosal plexus, they observed no change in epithelial renewal or permeability, and no change in the sensitivity to dextran sodium sulfate-induced colitis. This led them to postulate that the IEB failure observed in the existing studies that used depletion of GFAP-positive EGC was mostly due to non-glial toxicity of ganciclovir. Nevertheless, their arguments are not entirely convincing. The ganciclovir toxicity on neighboring cells (16, 89) or the GFAP expression in rare epithelial cells observed in Rao’s work has not been previously described while they were looked for. More importantly Rao et al., did not comment the role of the 20% remaining EGC: what are their functions? As the co-expression of PLP1 and GFAP by EGC is quite low (35–54%)(73) we could suppose that the remaining EGC are mainly GFAP positive EGC. Better than erasing all the previous findings, this work could propose that only a small EGC subpopulation contributes in maintaining IEB homeostasis. This work should especially focus our research on the GFAP positive EGC and more broadly on the study of different EGC sub-populations. All works describing EGC impact on IEB have studied GFAP positive cells, and as GFAP is a marker of glial reaction to inflammation, the study of EGC in an inflammatory environment and more especially during inflammatory bowel diseases is again more interesting.

EGC response to inflammation

It has been shown that in response to injury, stress or inflammation, astrocytes of the CNS acquire new properties referred to as "reactive glia" or “astrogliosis” (71). This astrogliosis, characterized in particular by an upregulation of GFAP along with an increase in proliferation and cytokine secretion, have either beneficial or deleterious effects on the healing of brain tissue (54, 87, 88). For instance, although reactive astrocytes are involved in repairing the blood-brain barrier, it has also been shown that a long maintenance of this "reactive" state could have detrimental effects on

234 the complete recovery of CNS functions (41, 54). Given the similarities between CNS astrocytes and
235 EGC, it is tempting to hypothesize that EGC may also become reactive when exposed to an
236 inflammatory environment and induce IEB dysfunction when the deleterious environment is
237 maintained (17, 65).

238 Changes in EGC phenotype and soluble mediator production have been observed following
239 exposure to proinflammatory cytokines or bacterial components (Table 1). C-Fos expression is
240 induced by LPS and IFN-γ cocktail (21) or enteroinvasive *Escherichia coli* (97) in cultured EGC and by
241 IL-1β in isolated preparations of guinea pig ileum and colon (96). Such an enteric glial reactivity has
242 also been observed *in vivo* in various colitis models, in which c-Fos expression in the EGC was induced
243 by IL-1β (59, 83). The EGC activity evaluated by Ca^{2+} transient in response to bradykinin is also
244 increased in presence of IL-1β (61) or LPS (62). Pro-inflammatory cytokines also have effects on EGC
245 turnover depending on the concentration used: relatively low doses of IL-1β, IL-10 or a cocktail of
246 both cytokines inhibit EGC proliferation (79) while high dosage of IL-10 (79) or LPS and INF-γ cocktail
247 have an opposite effect (21). *In vivo* administration of trinitrobenzene sulfonic acid induced EGC
248 mitosis in myenteric ganglia (15) and EGC apoptosis has been detected in the gut of patients with CD
249 (93). We could suppose that the level of inflammation could explain these differences, but EGC
250 turnover can even be more complexe if we take into account that EGC can undergo gliogenesis (47)
251 or neurogenesis after injury *in vivo* (51). Again the existence of EGC subpopulations is adressed by
252 these works as gliogenesis has been revealed using GFAP lineage tracing (47) whether neurogenesis
253 has been revealed using Sox10 lineage tracing (51).

254 The expression levels of enteric glial markers are regulated by proinflammatory cytokines
255 and by the bacterial component LPS. In cultured EGC, GFAP is induced following treatment with TNF-
256 α, IL-1β, LPS or LPS and IFN-γ cocktail (19, 103, 107) while S100β is upregulated by LPS and INF-γ
257 cocktail (21) but also by enteroinvasive *Escherichia coli* (97). In a rat model of intestinal inflammation
258 induced by systemic LPS injection, an increase in GFAP expression was observed in the myenteric
259 plexus (75). Several studies performed on human samples from UC as well as in CD patients showed
260 that mucosal inflamed areas exhibited an increase in the expression of both GFAP and S100β when
261 compared to non-inflamed areas (25, 102, 104) (Table 2). GFAP expression is also increased in CD
262 inflamed colonic biopsies when compared to control patient biopsies (92, 93) but decreased in
263 uninflamed area of CD when compared to controls (25, 104) (Table 2). Concerning S100β, its
264 myenteric expression is downregulated in CD non-involed area (102) or UC human colon (9) in
265 comparison to control patients, but its submucosal expression is increased in biopsies from UC
266 patients when compared to control patients (19, 20, 29) (Table 2). In addition, an increase in S100β
267 submucosal expression in the proximal margin of resection from ileocolonic samples was found in CD

patients with endoscopic and clinical recurrence when compared to subjects without disease recurrence (53).

EGC response to inflammation also encompasses changes in receptor expression. The NGF receptor TrkA (106), endothelin-1 receptor ET-B (103), Toll like receptor TLR4 (29) and bradykinin receptor BR1 (61) expressions are increased in response to IL-1β and TrkA expression is also upregulated in response to LPS (106). The major histocompatibility complex (MHC) class II expression is increased in EGC after exposure to pathogenic enteroinvasive *Escherichia coli* (97).

Concerning soluble factor production, IL-1β induced monocyte chemotactic protein 1 expression (95) and IL-6 production that in turn can inhibit IL-6 glial production (78). IL-1β itself can be produced by EGC stimulated by LPS (62). Cultured EGC can also produce more endothelin-1 in response to IL-1β, TNF-α or LPS (103), more nitric oxide in response to LPS and INF-γ cocktail (21) or more prostaglandin E_2 in response to bradykinin (63). Gliospheres stimulated with LPS expressed more INF-γ messenger (75). Inflammation also induced an increase in the gliosecretion of neurotrophic factors by EGC, like GDNF and NGF which expression and production were induced by IL-1β, TNF-α or LPS in cultured EGC (105, 106). In human samples, GDNF expression is also increased in EGC of inflamed area of CD and UC patients when compared to controls, but GDNF expression is downregulated in non-inflamed area of CD patients and increased in non-inflamed area of UC patients when compared to controls (104).

These changes operated by EGC in an inflammatory environment suggest an increased bacterial sensing, neuronal and immune regulation by EGC, but very little is known regarding the functional consequences of this reactivity, especially on IEB regulation. The few we know concerns GDNF which is critically involved in barrier-protective mechanism of EGC after ischemia/reperfusion (109) and has an autocrine role on EGC by protecting them from apoptosis (91, 92). In a mouse model of colitis, GDNF decreases intestinal permeability and reduces the inflammatory response (111). A role for GSNO in the maintenance of IEB integrity has recently been proposed (18).

Taken together, these findings suggest that in inflammatory conditions, reactives EGC may acquire new phenotypic and functional properties, like reactive astrocytes in the CNS. Inflammation could induce changes in the expression of glial markers as well as in cytokine and neurotrophic factor production that may have a beneficial role on the IEB. Currently, we have little information on the functional impact of this reactive enteric glia on the IEB. In addition, the global impact of EGC when the inflammation is maintained and when the lesions are not repaired, like in IBD, remains obscure.

EGC in IBD: differences between CD and UC

As mentioned above, the phenotypic characterization of EGC from IBD patients showed that they differently express glial markers or mediators *in situ* (Table 2). While an increase in GFAP, S100β and GDNF expressions is observed in inflamed area of biopsies from both CD or UC patients when compared to non-inflamed area (25, 102, 104), GFAP, S100β and GDNF expressions are decreased in uninflamed area of CD but not UC, when compared to controls (25, 102, 104). GDNF expression is even increased in non-inflammaed area of UC patients when compared to controls (104).

Because EGC undergo apoptosis when stimulated with TNF-α and INF-γ (92) and in CD biopsies (92, 93) the *in situ* decrease in GFAP expression observed in CD is often interpretated as a loss of glial cells although none of the existing studies have performed EGC quantification. The cytoplasmatic staining pattern of GFAP makes EGC immunohistochemical quantification difficult, and it is therefore unlikely that this approach will permit glial quantification in IBD, regardless of the antibody used. On the other hand, the nuclear localization of Sox8/9/10-IR allows to identify and count all EGC individually in human ENS and it is highly likely that it will be helpful to determine if EGC loss occurs or not in IBD (43). If physiological reactive EGC could be characterized by high GFAP expression and high GDNF production, the pathological EGC remodeling could be characterized by low GFAP and GDNF expression and correspond to a loss of glial reactivity.

To further study the functions of EGC in IBD, we have isolated EGC from myenteric plexus of control subjects and IBD patients and cultured them to evaluate their impact on intestinal epithelial cells (IEC). We showed that EGC from controls enhance IEC spreading, speed up IEB repair (24) and decrease IEB permeability (72) as already shown in cultured rat EGC. In addition we have identified human EGC as a source of 15-HETE and 11β–$PGF_{2\alpha}$ to regulate IEB permeability (72) and healing (24), respectively. All these properties were lost in EGC from CD patients (24, 72). Using the same functional approaches, we have shown that if CD EGC had lost their ability to accelerate cell spreading and to decrease permeability, the EGC from UC patients had not and induced similar spreading and permeability than control EGC (Figure 2 and 3A and B). These data are the first evidence for the functional differences between CD and UC EGC. These findings should be interpreted cautiously as they are based on *in vitro* analyses of EGC. Indeed if cultured EGC are very useful to dissect functional cell-cell interactions, they have limitations due to the dedifferentiation that could occur in culture. For exemple modifications of the Ran-1 and -2 antigen expression have been observed between EGC in culture and *in situ* (45). But these human EGC studies are nonetheless informative as EGC from patients with different pathologies precisely present functional (24, 72) and molecular (24) differences out of their pathophysiological environment. These data suggest that in CD rather than in UC, EGC are phenotypically altered and undergo functional changes

that may contribute to the pathophysiology of these diseases. In addition it raises again the question of the different EGC sub-populations. The EGC used in our experiments were from myenteric plexus ganglia and could thus only originate from type I or II glial sub-types, but in culture they all express the GFAP, Sox10 and S100β markers with no difference between control, CD or UC EGC (24). This could propose that the functional difference observed in IEC spreading control is not directly linked to changes in the expression of one of these markers, but also that the glial sub-population regulating the IEB is the GFAP positive one. Further work is needed to define the role of mediators produced by different EGC sub-types.

Conclusion/Perspectives

IBD are diseases with well-known symptoms but a poorly understood etiology. In this context, EGC represent new interesting actors but their role in maintaining intestinal integrity still necessitates investigations. In this review, we focused on the impact of EGC on intestinal epithelial cells, but we do not have to neglect that EGC may also regulate other neighboring cells from enteroendocrine cells (14) to smooth muscles, immune cells and neurons. The high-throughput transcriptomic analyze of gliospheres, in response to LPS or not, even suggest a broader impact of EGC in regulating their neighbouring cells (75).

A better characterization of EGC in the context of IBD and other inflammatory pathologies where EGC reactivity has been observed concomitantly with gut dysfunction such as Parkinson's disease (23, 28) should allow better understanding of their involvement in pathophysiological processes. If we could propose that EGC reactivity represents the physiological temporary EGC reactions to environmental stimuli and is associated with the resolution of inflammation, the glial remodeling occurring along chronic disease development remains to be clarified. Further progress are expected in the near future with the development of *in vivo* imaging techniques (11, 101) and cell specific gene editing to better characterize EGC role *in vivo*, and, in particular, whether functionally distinct subclasses of EGC exist.

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Figure Legend

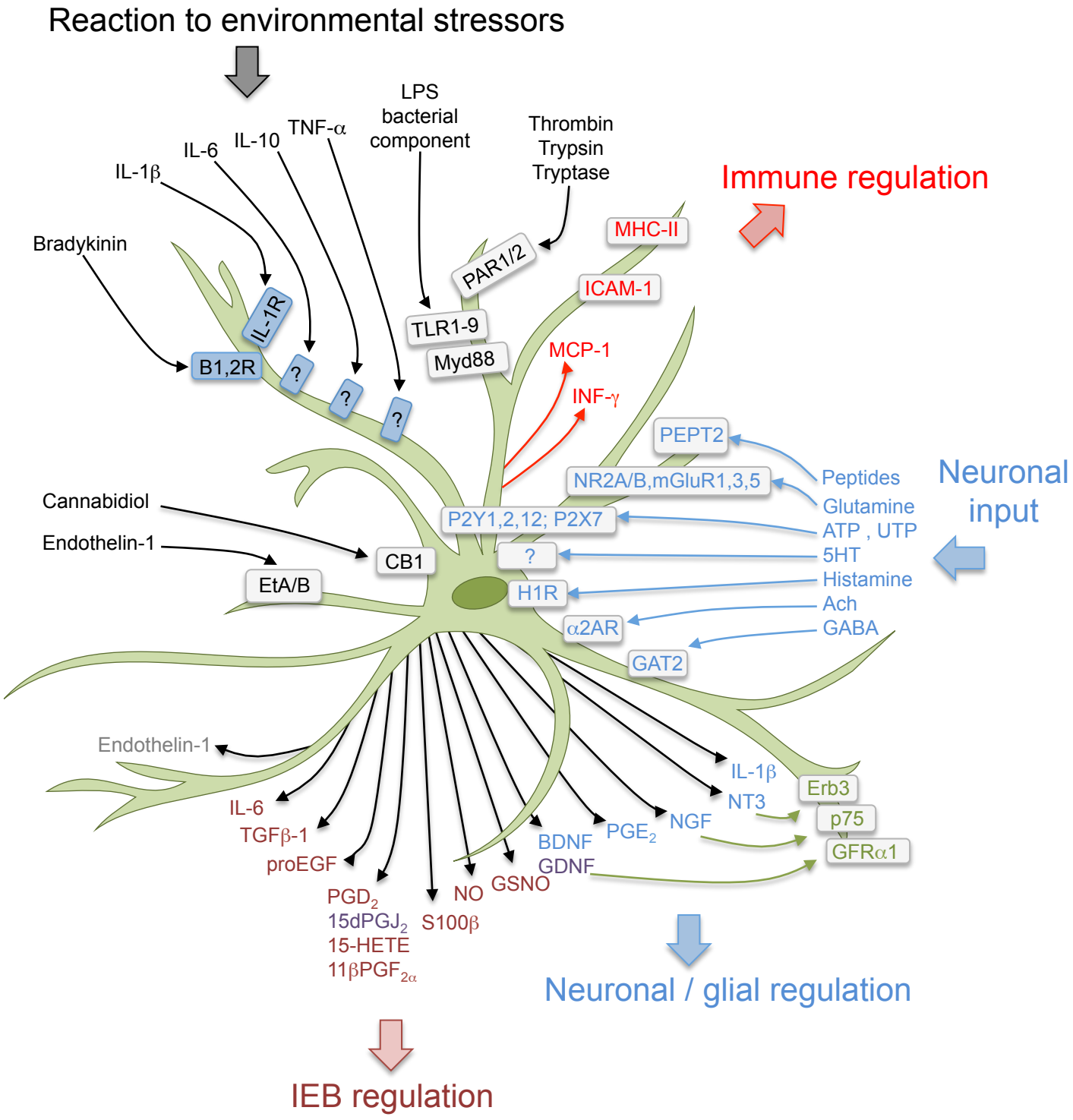
Fig. 1: Representative diagram of EGC at center stage in regulating communication in between gut cellular components. EGC can not only sense varied environmental stimuli (in black) but also produce and release numerous soluble mediators that especially regulate immune cells (in red), neuronal cells or glial cells themselves (in blue), intestinal epithelial cells (in burgundy) or even both epithelial and neuronal cells (in violet). Box with question mark represent receptor which identity has not been clearly described.

Table 1: Summary of EGC reactions to inflammation. EGC reactions to several inflammatory stimuli have been studied *in vitro* and *in vivo.* It encompasses changes in cell proliferation or apoptosis, changes in glial marker expression and soluble factor production.

Table 2: Summary of EGC features in IBD patients.
EGC from myenteric and/or submucosal plexus have been characterized in human specimens from CD, UC or control patients using Immunohistochemistry (IHC) Western blot analysis (WB) or enzyme-linked immunoabsorbent assay (ELISA).

Fig.2: **Epithelial permeability is increased by CD EGC but not by UC EGC.** Regulation of intestinal paracellular permeability by human EGC was assessed as described (72) excepted that cells were always cultivated in presence of 10% fetal calf serum. Shortly, sulfonic acid flux was measured through Caco-2 monolayer after 2 days of co-culture with EGC from control, CD or UC patients or without co-culture (WO EGC). n=6 to 17 patients per group, 6 independent experiments. Kruskal-Wallis test; *p<0,05

Fig. 3: **Epithelial spreading is increased by control or UC EGC but not by CD EGC. A**. Human EGC functional impact on IEB was assessed as described (24). Briefly spreading was measured (cell area after zonula occludens-1, ZO-1, immunostaining) on Caco-2 monolayer after 2 days of co-culture with EGC from control, CD or UC patients or without co-culture (WO EGC). n=7 to 17 patients per group, 6 independent experiments. Kruskal-Wallis test; *p<0,05 **B.** Representative pictures of ZO-1 immunostaining. Scale bar 100µm.



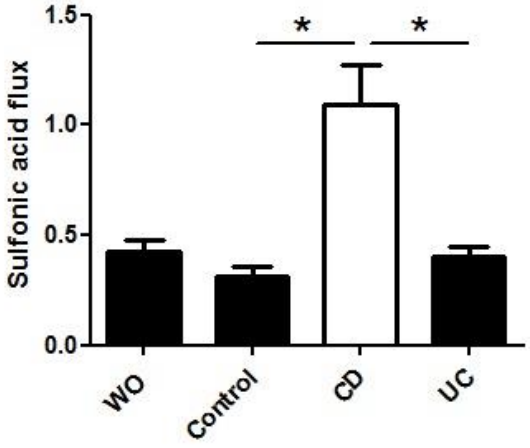


Fig.2

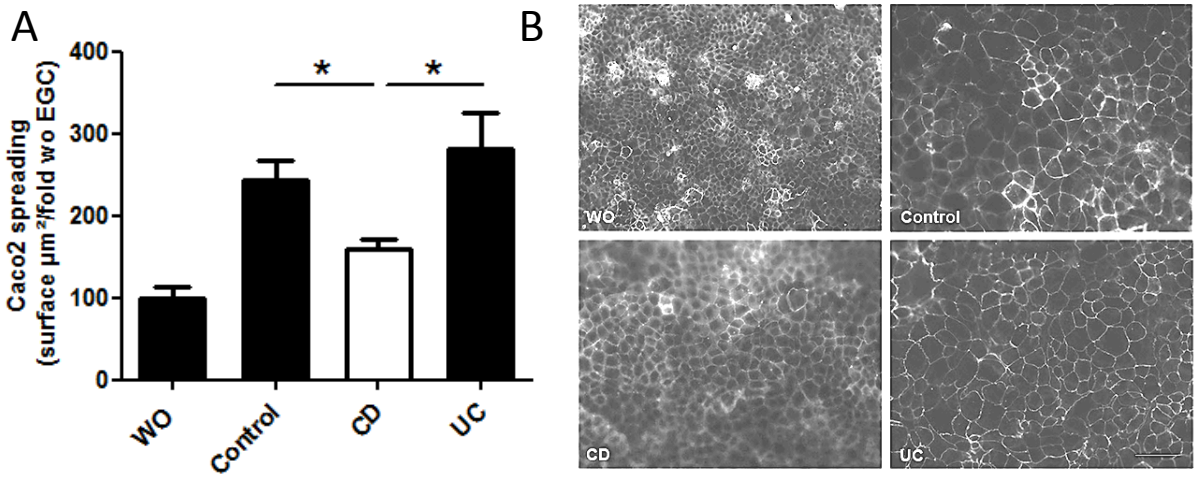


Fig. 3

EGC types	Stimuli / Receptors	Conditions	Effects of Stimulation	References
Primary or transformed culture of rat EGC from myenteric plexus	IL-1β	48h; 5 to 100ng.mL-1	Inhibition of cell proliferation	*79*
		12 to 24h ; 10 and 100ng.mL-1	Induction of IL-6 mRNA expression and production	*78*
		32h; 80ng.mL-1	Increased GDNF expression and production	*105*
		12 to 48 h; 10 to 100ng.mL-1	Increased NGF expression and production	*106*
		32h; 60ng.mL-1	Increased TrkA expression	
		24 to 48 h; 100 to 150ng.mL-1	Increased Et-1 production	*103*
		48 h; 100 ng.mL-1	Increased ET-B expression	
		32h; 10-80ng.mL-1	TLR4 upregulation	*29*
		32h; 80ng.mL-1	Increased percentage of GFAP+ cells, increased GFAP expression	*103 ; 107*
		24h; 10ng.mL-1	Increased IL-6 and MCP1 expression	*95*
		24h; 10ng.mL	Increased B1R expression and [Ca2+] response to bradykinin	*61*
	IL-10	48h ; 0.1ng.mL-1	Inhibition of cell proliferation	*79*
		48h ; 5 to 100ng.mL-1	Activation of cell proliferation	
	IL-1β+IL-10	48h ; 0.1 to 100ng.mL-1	Inhibition of cell proliferation	
	IL-6	24h ; 1 to 100ng.mL-1	Inhibition of IL-6 expression	*72*
	TNF-α	32h; 60pg.mL-1	Increased GDNF expression and production	*105*
		4 to 48 h; 40 to 100 pg.mL-1	Increased NGF expression and production	*106*
		8 to 48 h; 10 to 100 pg.mL-1	Increased Et-1 production	*103*
		32h; 60pg.mL-1	Increased percentage of GFAP+ cells, increased GFAP expression	
				105
	LPS	32h; 100µg.mL-1	Increased GDNF expression and production	*107*
		4 to 48h; 100µg.mL-1	Increased NGF expression and production	*108*
		32h; 20 to 200 µg.mL-1	Increased TrkA expression	
		4 to 48h; 100µg.mL-1	Increased Et-1 production	*103*
		32h; 100µg.mL-1	Increased percentage of GFAP+ cells, increased GFAP expression	
				107
		24h; 0,1µg.mL	Increased [Ca2+]i response to bradykinin	*62*
		6h; 0,1 or 10µg.mL	Increased IL-1β production	
	LPS+IFN-γ	72h; 1µg.mL-1+100U.mL-1	Activation of cell proliferation	*21*
		24h; 1µg.mL-1+100U.mL-1	Increased MHC expression	
			Increased c-Fos expression	
			Increased iNOS expression and NO production	
			Increased percentage of GFAP+ cells, increased GFAP expression	
			Increased S100β expression and production	
	TNF-α+IFN-γ	40h; 100ng.mL-1+100ng.mL-1	Increased apoptosis (activation of caspase-3/7)	*92; 93*
	EIEC		Increased c-FOS, S100β and MHCII expression, increased NO production and changes in TLR1,2,3,4,5,7,9 expression	*97*
	Et-1	32h; 1nM	Increased percentage of GFAP+ cells, increased GFAP expression	*103*
	Bradykinin	10 min; 10 or 100 nM	Increased PGE_2 production	*63*
Gliospheres	LPS	48h; 100 µg mL-1	Increased INF-γ expression	*75*
Isolated preparations of guinea pig ileum and colon	IL-1β	140min; 0.1-10ng.mL-1	Increased c-Fos expression in submucosal and myenteric plexus	*96*
Myenteric plexus of guinea pig ileum *in situ*		*in vivo* trinitrobenzene sulfonic acid induced inflammation	Activation of cell proliferation	*15*
Myenteric plexus of rat *in situ*	LPS	4h; *in vivo* LPS 2.5 mg/kg intravenously	Increased GFAP expression; Decreased S100β expression	*75*
Culture of human rectal biopsies	LPS+IFN-γ	24h; 10µg.mL-1+300U.mL-1	Increased S100β expression and release	*19*

Table 1

Sample types	Patients (number)	Methods	EGC responses	References
Non-inflamed and inflamed ileal and colonic biopsies	CD (43), UC (23), control (29)	IHC, ELISA, WB	Decreased GFAP expression in CD non inflamed area vs inflamed area or vs control patients	*25*
			Increased GFAP expression in inflamed vs non-inflamed area in CD as well as UC patients	
Non-inflamed and inflamed ileal and colonic full thickness samples	CD (16), UC (16), control (25)	IHC	Increased S100β expression in inflamed CD vs non-inflamed CD (submucosal and myenteric plexus)	*102*
			Increased S100β expression in inflamed UC vs non-inflamed UC or control (submucosal plexus only, no difference myenteric plexus)	
			Decreased S100β expression in non-inflamed CD vs control (myenteric plexus)	
Non-inflamed and inflamed colonic biopsies	CD (35), UC (30), control (26)	IHC, ELISA, WB	Increased GFAP expression and GDNF production in inflamed UC or CD vs control	*104*
			Decreased GFAP expression and no GDNF production in non-inflamed CD vs control or UC	
			Increased GDNF production in non-inflamed UC vs control	
Inflamed colonic biopsies	CD (10), control (26)	IHC	Increased apoptosis (cleaved Caspase-3 staining) in inflamed CD vs control	*92*
			Increased GFAP expression and GDNF production in inflamed CD vs control	
	CD (20), control (26)		Increased apoptosis (cleaved Caspase-3 staining) in inflamed CD vs control	*93*
			Increased GFAP expression and BDNF production in inflamed CD vs control	
Rectal biopsies	UC (35), control (30)	IHC, qPCR, WB, ELISA	Increased S100β (mRNA and protein) expression and release in UC vs control	*19, 20*
	UC (8), control (8)	IHC, WB	Increased GFAP and S100β expression in UC vs control	*29*
			Increased TLR4 expression in UC vs control	
Full thickness resected left colon	UC (10), control (10)	IHC	Reduced density of myenteric S100β in UC vs control	*9*
Ileocolonic samples	CD (48)		Increased S100β expression in proximal margin of resection in CD with disease recurrence	*53*

Table 2