

# Enteric glia: A new player in inflammatory bowel diseases

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## Abstract

In addition to the well-known involvement of macrophages and neutrophils, other cell types have been recently reported to substantially contribute to the onset and progression of inflammatory bowel diseases (IBD). Enteric glial cells (EGC) are the equivalent cell type of astrocyte in the central nervous system (CNS) and share with them many neurotrophic and neuro-immunomodulatory properties. This short review highlights the role of EGC in IBD, describing the role played by these cells in the maintenance of gut homeostasis, and their modulation of enteric neuronal activities. In pathological conditions, EGC have been reported to trigger and support bowel inflammation through the specific over-secretion of S100B protein, a pivotal neurotrophic factor able to induce chronic inflammatory changes in gut mucosa. New pharmacological tools that may improve the current therapeutic strategies for inflammatory bowel diseases (IBD), lowering side effects (i.e. corticosteroids) and costs (i.e. anti-TNF $\alpha$  monoclonal antibodies) represent a very important challenge for gastroenterologists and pharmacologists. Novel drugs capable to modulate enteric glia reactivity, limiting the pro-inflammatory release of S100B, may thus represent a significant innovation in the field of pharmacological interventions for inflammatory bowel diseases.

## Keywords

enteric glia, S100B, enteric nervous system, inflammatory bowel diseases, nitric oxide

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## Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) represent the two major clinically defined forms of inflammatory bowel disease (IBD) that may affect the whole gastrointestinal tract and the colonic mucosa, respectively, and are associated with an increased risk of developing colon cancer.<sup>1,2</sup> Even though widespread, IBD is more common in developed countries, with the highest incidence rates and prevalence registered in North America and Europe. However, a substantial variation in the epidemiology of IBD has been lately observed with an alarming rise in prevalence in previously reported low-incidence areas, such as Asia, further pointing out the urgent need of new pharmacological approaches in the management of these diseases.

Usually, therapies for IBD include chronic administration of glucocorticosteroids and sulfasalazine derivatives. However, these drugs are

not always effective and cannot be used for long-term maintenance.<sup>3</sup> In fact, steroids are useful in the short-term treatment of acute flares, but they may induce a number of systemic adverse reactions during prolonged therapy.<sup>4,5</sup> Sulfasalazine and its derivative 5-aminosalicylic acid (5-ASA) are effective only in mild-to-moderate phases of the disease and in preventing relapses.<sup>4-7</sup> The introduction of monoclonal anti-tumor necrosis

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factor-alpha (TNF $\alpha$ ) antibodies (Infliximab and Adalimumab) in the therapy of IBD has radically changed their management, since these drugs are effective both in controlling moderate-to-severe forms of UC and CD and providing an efficient prevention of their relapses.<sup>8</sup> However, the long-term safety concerns of these drugs (i.e. the possibility to allow the developing of aggressive form of cancer, particularly leukemia and lymphoma<sup>9</sup>), together with the high costs, limit the ordinary use of these therapeutics. Moreover, a poor response to anti-TNF $\alpha$  therapy has been observed in some forms of UC and CD.<sup>10</sup> For these reasons, there is an urgent need for novel effective drugs, with manageable side toxicity and low costs for patients. In this perspective, the identification and characterization of new therapeutic targets for the development of innovative anti-IBD drugs appear to be crucial. The etiology of IBD has been extensively studied and several efforts have been made aiming at a better understanding of the pathophysiological mechanisms underlying the disease. Different studies have demonstrated the involvement of some risk factors including infectious agents, viruses and bacteria, autoimmune response, food allergies, hereditary factors, and co-morbid stressing conditions.<sup>11,12</sup> Generally, CD and UC have been univocally identified as autoimmune pathologies and the mucosal macrophages and lymphocytes infiltration has been considered as the main responsible for the chronic inflammation, occurring in the gut mucosa. Severe dysfunction of the mucosal immune system has been thus described to play an important role in the pathogenesis of IBD.<sup>13,14</sup> In general, a wide range of inflammatory cells in the gut, such as mucosal CD4+ T cells, are thought to play a central role in both the induction and the persistence of chronic inflammation by producing pro-inflammatory cytokines.<sup>15</sup> Several studies have demonstrated that the levels of T helper 1 (Th1)-related cytokines (e.g. TNF $\alpha$ , interferon gamma (IFN)- $\gamma$ , interleukin (IL)-12), as well as the concentration of other cytokines (e.g. IL-17A, IL-21, IL-23), are increased in the inflamed mucosa of these patients when compared to normal subjects.<sup>16–20</sup> Pro-inflammatory cytokines may profoundly affect intestinal mucosal homeostasis by inducing chronic inflammatory changes, including T cell and macrophage proliferation, expression of adhesion molecules and chemokines, and secretion of other pro-inflammatory cytokines

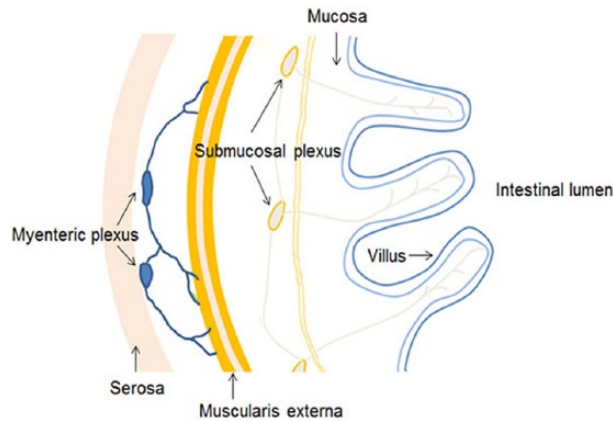
that perpetuate, in turn, the chronic inflammation in the gut.<sup>19,20</sup>

### *From mucosal inflammation concept to the enteric-driven neuroinflammation concept*

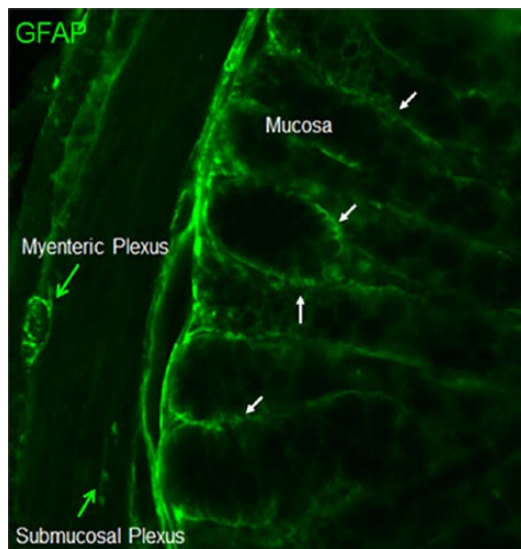
In recent years, it has become clear that the mucosal immune system alone may not account for all the aspects of IBD pathogenesis and pursuit of new players in CD and UC pathophysiology led to investigate the involvement of the enteric nervous system (ENS) in intestinal inflammation, enlarging the concept of inflammation to neuro-inflammation in IBD.

Although in patients with IBD morphological abnormalities of the ENS have been consistently described, only in the last decade have recent studies highlighted the changes occurring in both enteric neurons and enteric glial cells during intestinal inflammation.<sup>21–24</sup>

The ENS takes part to the peripheral nervous system and it is located within the wall of the gastrointestinal tract. It has been considered the “brain of the gut” since, independently from the central nervous system (CNS), it coordinates many aspects of digestive functions such as motility, blood flow, and immune/inflammatory processes.<sup>25</sup> Many features of digestive function are guided by the ENS, a complex network of neurons and glia that works independently from the central nervous system. The ENS originates from the neural crest, which invades, proliferates, and migrates within the intestinal wall until the whole bowel is colonized with enteric neural crest-derived cells (ENCDCs). Due to different factors and morphogens, the ENCDCs develop further, differentiating into glia and neuronal sub-types, interplaying to form a functional nervous system.<sup>26</sup> Histologically, the ENS is organized in two major ganglionated plexuses, the myenteric (Auerbach’s) and the submucosal (Meissner’s and Henle’s) plexus (Figure 1a). These ganglia contain neuron cell bodies and are interconnected by bundles of nerve processes. The myenteric plexus is located between the longitudinal and circular muscle throughout the gut, from the esophagus to the rectum. It mainly innervates the *muscularis externa* and controls intestinal motility. The sub-mucosal plexus, lying between the mucosa and the circular muscle, is involved in the regulation of bowel secretion, especially in the small intestine where it is most located. A number of



**Figure 1a.** Schematic representation of the gut wall. Myenteric plexus innervates the muscularis externa and controls the intestinal motility; the submucosal plexus innervates the submucosal blood vessels and is basically involved in the control of the intestinal secretory functions.



**Figure 1b.** GFAP immunofluorescence staining in the enteric nervous system. The figure shows the localization of myenteric plexus and submucosal plexus networks in mice intestine indicated by green arrows. White arrows indicate the close proximity of enteric glial cell processes with epithelial cells in the mucosa. Magnification 100 $\times$ .

morphological and functional distinct neurons are located in both plexuses, including primary afferent neurons, sensitive to chemical and mechanical stimuli, interneurons, and motor neurons. Most of the enteric neurons involved in motor functions are located in the myenteric plexus with some primary afferent neurons located in submucosal plexus.<sup>27</sup>

Though not fully understood, the neuroinflammatory process occurring during IBD refers to

several structural and functional abnormalities occurring in the ENS. In these conditions both macroscopic changes (hypertrophy and hyperplasia of nerve bundles and ganglia) and neurotransmitter release adaptations in the ENS are commonly observed.<sup>22</sup> In patients with IBD, morphological changes occurring in the ENS, ranging from the alteration of submucosal plexus structure to the retraction of neuronal fibers and the appearance of neuromatous lesions, are observed.<sup>23</sup>

Moreover, although the exact mechanism(s) at the basis of such alteration has/have not been fully understood, it is commonly accepted that the increased apoptosis of enteric neurons and EGC is closely correlated to several functional disturbances observed in IBD patients, such as gut dysmotility and increased sensory perception.<sup>21–23</sup>

#### *Enteric glia: The beautiful and the bad weather in the gut*

Enteric glial cells (EGC), the phenotypical equivalent of astrocytes into the CNS, are small cells with a star-like shape and are believed to represent the most abundant cells in the ENS;<sup>28</sup> EGCs are for these reasons considered as active partners in ENS function.<sup>29</sup> They display dynamic responses to neuronal inputs and may take part into the release of neuro-active factors. At present, EGCs of human gut are usually identified by the expression of the S100B and GFAP protein, as well as by the expression of more recently recognized markers such as Sox 10.<sup>30–33</sup> EGCs surround enteric neuron bodies and axons,<sup>34</sup> as well as intestinal blood vessels<sup>35</sup> while their processes extend into the mucosa.<sup>36</sup> Despite the previous assumption that EGCs may serve as mechanical support for enteric neurons, nowadays the knowledge on these cells is consistently expanded. Functionally, EGCs are believed to be responsible for many of peripheral neurons functions through the release of a variety of soluble factors.<sup>37</sup> Under physiological conditions, major histocompatibility complex class I (MHC I) molecules are constitutively expressed by EGCs, while MHC class II molecules are almost undetectable.<sup>38,39</sup> It has become increasingly clear that EGCs play a pivotal role in the regulation of intestinal homeostasis, leading to a more multifaceted and comprehensive knowledge of these cells.<sup>40</sup> Beside their trophic and cytoprotective functions toward enteric neurons, enteric glial cells play a key role in

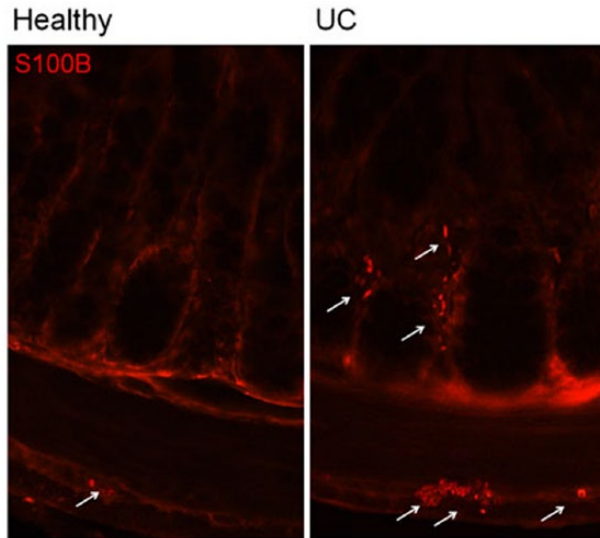
the intestinal epithelial barrier homeostasis and integrity.<sup>41</sup> The intestinal barrier regulates the passage of the intestinal contents, preventing the diffusion of microbes and other pathogens (including viruses) through the mucosa. EGCs are in close proximity of gut epithelial cells and similarly to their counterparts in the CNS, they may affect intestinal permeability through the release of several mediators, directly controlling epithelial barrier functions.<sup>42</sup> Among the different EGC-related mediators, glial-derived neurotrophic factor (GDNF) plays a fundamental role in the preservation of mucosal integrity under the enteric glia surveillance. GDNF indeed exerts anti-inflammatory effects via a dual mechanism; on one hand, it inhibits EGCs apoptosis in an autocrine manner; on the other, via a paracrine mechanism, it lowers the level of pro-inflammatory cytokines, such as IL-1 $\beta$  and TNF $\alpha$ , that significantly increases during IBD and gut infections.<sup>43</sup> Recently it has been shown that EGCs can assure the integrity of the intestinal barrier through the release of S-nitrosoglutathione (GSNO).<sup>44</sup> The effect of this metabolite is associated with the overexpression of tight junction associated-proteins, for example zonula occludens-1 (ZO-1) and occludin, that in turn prevents the intestinal barrier breakdown during an inflammatory insult, linking with actin cytoskeleton ring and myosin light chain (MLC). Thus, EGCs play important functions in the maintenance of ENS homeostasis, but they may also proliferate and be activated in response to injury and inflammation, undergoing reactive gliosis (entero-gliosis).<sup>45,46,47</sup> In these conditions, EGCs activity is profoundly altered and, following injury and inflammation, these activated cells undergo a dynamic process associated with an increased proliferation and a pro-inflammatory phenotype.<sup>45,47,48</sup> Enteroglia activation is characterized by the over-release of neurotrophins, growth factors, and cytokines that, in turn, recruit infiltrating immune cells such as macrophages, neutrophils, and mast cells in the colonic mucosa.<sup>47-49</sup> During the onset and perpetuation of the inflammatory state of the mucosa, EGCs control of mucosal integrity is markedly altered.<sup>50</sup> These results are in line with several studies<sup>50-53</sup> showing that EGCs can regulate the expression of genes responsible for adhesion, differentiation, and proliferation of epithelial cells,<sup>54</sup> further confirming the importance of enteric glia in the epithelial barrier homeostasis. In the context of

signaling molecules released by EGCs, the production of transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) and vascular endothelial growth factor (VEGF) might be involved in the onset and metastasis of colon cancer during IBD, thanks to their effects on the epithelial cells proliferation and formation of new blood vessels.<sup>55</sup> Very interestingly, different evidences let hypothesize that EGCs act as *primum movens* in triggering and amplifying the inflammatory cascade during chronic inflammatory insult of the gut.<sup>45,46,56</sup> An intriguing correlation between the degree of enteric gliosis and severity of gut inflammation has been also reported and an intimate interaction between EGCs and the mucosal immune system has been observed.<sup>50,57</sup>

#### *EGC drive neuroinflammation in IBD: The role of S100B protein and its partnership with nitric oxide*

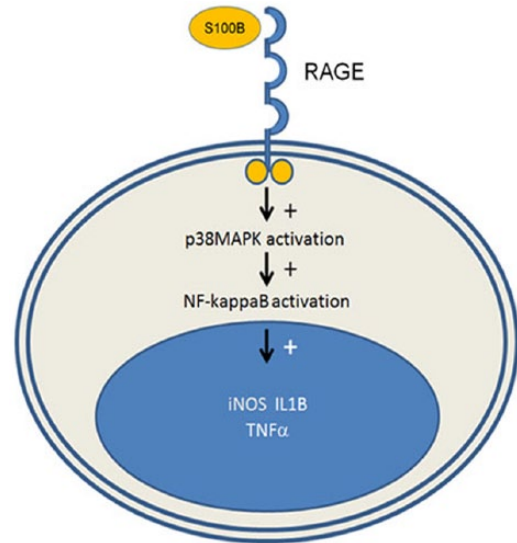
In recent years, it has been assumed that EGCs are involved in the chronic mucosal inflammation in UC; and many EGC-related signaling molecules thought to orchestrate such neuroinflammatory cross-talk are under extensive investigation.<sup>58,59</sup> The S100B protein, one of the typical markers of EGC, seems to play a crucial role in IBD.<sup>45</sup> S100B is the homodimer of subunit and belongs to a Ca<sup>2+</sup>-Zn<sup>2+</sup> binding proteins super-family that comprises more than 20 proteins.<sup>35</sup> In the gut, S100B protein is constitutively expressed by EGCs<sup>45</sup> while other members of S100 family, such as S100A8, S100A9, and S100A12 are expressed only under inflammatory conditions by phagocytes and intestinal epithelial cells.<sup>45,60</sup> The role exerted by S100B in gut inflammation has been only recently highlighted.<sup>45,61</sup> S100B is a pivotal signaling molecule that participates at the onset and progression of the inflammatory status, as it coordinates a wide range of signal activation pathways, directly correlated with the severity of tissue damage.<sup>61</sup> This is highlighted by the observation that rectal specimens from early diagnosed UC patients show an increased S100B protein expression (Figure 2a).<sup>45</sup>

The upregulation of S100B runs in parallel with an increased production of NO via the stimulation of iNOS protein expression. This is a very important point since a large group of studies pointed out that in UC patients an abnormal NO secretion by pro-inflammatory cytokines has been observed due to the progressive activation of iNOS protein.<sup>62,63</sup> In more detail, researches performed by comparing



**Figure 2a.** S100B protein in ulcerative colitis (UC). Immunofluorescence analysis showing the upregulation of S100B protein in UC versus healthy specimen. Arrows indicate S100B protein expression spots in both in myenteric plexus and in mucosa. Magnification 100 $\times$ .

patients with UC and healthy subjects demonstrated that rectal specimens from UC patients show an increased immunoreactivity for S100B protein and a significantly enhanced protein secretion and expression.<sup>56</sup> This upregulation cannot be considered a mere epiphenomenon because it is related to the specific activation of inducible NO synthase (iNOS) protein leading to an increase of NO level. Thus, S100B upregulation has a prominent role during ulcerative colitis. The correlation between S100B upregulation and NO production is very interesting since, as previously described, UC is characterized by abnormal mucosal NO production.<sup>63</sup> EGCs ability of modulating NO levels via S100B release has been confirmed also in absence of a chronic inflammatory scenario. In fact, the administration of micromolar concentration of exogenous S100B is able to induce a concentration-dependent activation of iNOS expression and a subsequent enhanced NO production, in rectal mucosa of healthy subjects.<sup>56</sup> In line with this it has been suggested that EGCs modulate the NO-dependent inflammatory response through the release of S100B within the intestinal milieu and let hypothesize that S100B release might be a first step in the onset of inflammation.<sup>64</sup> Once released, S100B may accumulate at the RAGE (Receptor for



**Figure 2b.** Schematic representation of the S100B pro-inflammatory signaling. The linkage between S-100B and RAGE is able to increase the production of NO and other pro-inflammatory cytokines via the activation of NF $\kappa$ B.

Advanced Glycation End products) site in micromolar concentrations.<sup>65,66</sup> Such interaction leads then to mitogen-activated protein kinase (MAPK) phosphorylation and consequent nuclear factor-kappaB (NF- $\kappa$ B) activation which, in turn, induces the transcription of different cytokines, such as iNOS protein, IL-1B, TNF $\alpha$  (Figure 2b).<sup>67,68</sup> Although the mechanisms by which EGCs and their signaling molecule S100B may coordinate such a complex inflammatory scenario is just initially conceived; more recently, a close relationship with toll-like receptors (TLRs) activation has been proposed.<sup>61</sup> In fact, the direct interaction between S100B and RAGE receptors during colitis has to be considered an initial event triggering the activation of a specific downstream pathway involved in the maintenance of a persistent enteroglia-sustained inflammation in the human gut. RAGE is also involved in the enteroglia TLRs signaling network, and it has become clear that, since EGCs express different TLR subtypes depending upon their pathophysiological functions;<sup>69</sup> these cells may be involved in a wider network of neuro-immunological pathways. Supporting this hypothesis, a specific S100B/RAGE/TLR-4 axis during gut inflammation has thus been observed as a pivotal molecular mechanism sustaining EGC activation during UC.<sup>61</sup>

## Conclusions and perspectives: Could we consider EGC targeting as a novel approach to develop anti-IBD drugs?

The urgent need of new pharmacological approaches that may enlarge the tools against IBD, as well as a better knowledge on new molecular players involved in the triggering and perpetuation of inflammation in the gut is a very important challenge for pharmacologists and gastroenterologists. This review highlighted the importance of EGCs as fundamental cell type within the ENS that participate to the modulation of inflammatory responses in the human gut. ENS alterations, featured by apoptotic bodies of neurons and glia,<sup>70</sup> especially in submucosal plexi, is commonly observed in human IBD, and it has been postulated to play a fundamental role in the occurrence of disorders of intestinal motility and or secretion.<sup>21,23</sup>

EGCs trigger and promote chronic inflammation in the intestinal mucosa since these cells over-release S100B that in turn determinates NO production. Such detrimental loop is responsible for a substantial recruitment of other target cells, including immune cells. In fact, EGC-derived S100B is able to affect peripheral macrophages and intestinal mucosal immune cell. A better understanding of the molecular mechanisms underlying EGC dysfunction, might constitute a new approach to increase the efficacy of new enteric-glia oriented drugs that may overcome the lack of long-term effectiveness of immunosuppressant agents used for IBD.

In the next future, molecules capable to selectively target EGC-mediated neuroinflammation, might represent a novel approach to develop new therapeutic strategies for IBD. In this context, the possibility to interfere with the S100B/NO axis may pave the way to a significant improvement of the actual therapies against UC or CD. To this aim, in preclinical studies, we demonstrated that the specific inhibition of S100B protein activity with pentamidine, an antiprotozoal drug, resulted in a marked reduction of EGC-mediated neuroinflammation severity in mice.<sup>71</sup> Similar results were obtained *in vivo* in mice and in human UC-deriving cultured biopsies with palmitoylethanolamide (PEA), an endogenous autacoid local inflammation antagonist (ALIA)-mide, able to downregulate S100B protein expression and to inhibit S100B-dependent activation of TLR-4 on enteric

glia.<sup>61</sup> Both pharmacological approaches resulted in a significant downregulation of inflammatory parameters, and improved significantly the disease course through a selective enteroglia-specific targeting, although in a preclinical evidence.

In conclusion, most of the studies on the role of enteric glia have been carried out in preclinical animal models of IBD. Although this might appear as a limitative factor due to the unpreventable differences emerging by the IBD process *in vivo* and the human disease, EGCs powerfully emerge as a very intriguing target on which develop selective drugs to treat CD and UC.

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