

Enteric Glia in Intestinal Inflammation

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Background

The etiology of the human inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis, remains unknown. There is a huge body of evidence establishing a central role of the mucosal immune system in the etiopathogenesis of IBD, but little is known about the contribution of the enteric nervous system (ENS). However, it has been repeatedly demonstrated that the ENS is affected in IBD, and in turn the ENS has been implicated in the pathogenesis of inflammatory diseases of the gut.

Similar to the organization of the central nervous system, the ENS is made up from two major cell populations: enteric neurons and glial cells. Recently, it has been demonstrated that ablation of enteric glial cells results in mucosal disintegration and, consecutively, severe intestinal inflammation. From these findings, it was concluded that enteric glia may be essential to maintain the integrity of the intestinal mucosa. In our own work, we have previously demonstrated that short-term immune-stimulation of EGC induces synthesis and secretion of the key inflammatory cytokine IL-6.

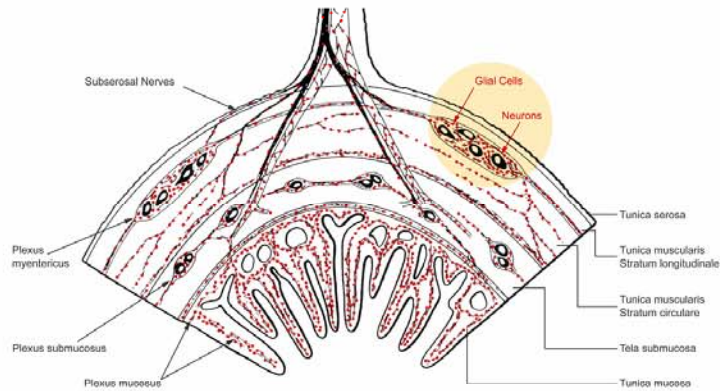


Fig.1: Enteric glia accompany nerve strands throughout all layers of the intestinal wall, from mucosa to serosa. Schematic of a cross-section through the intestinal wall; enteric glia are symbolized by red dots

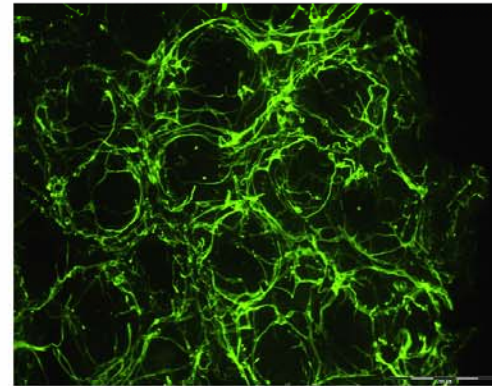


Fig.2: Epithelial crypt bases are surrounded by a network of glial cells and their processes. Whole mount preparation from the human plexus mucosus (sigmoid colon). After dissecting off the colonic mucosa with fine forceps, the tissue was stained with a polyclonal anti-S100 antibody visualized with a Cy2-coupled secondary antibody (green). All glial cell bodies and processes are labeled.

Aims

To investigate glial epithelial interactions in the human intestinal mucosa, employing *in vitro* and *in situ* techniques, and to assess the contribution of these interactions to the etiopathogenesis of IBD.

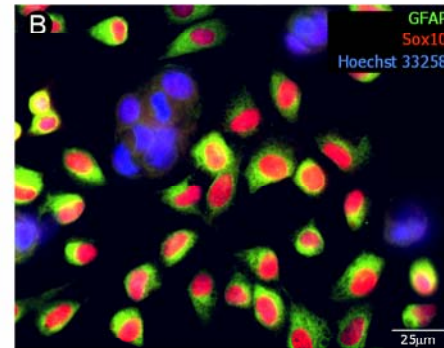
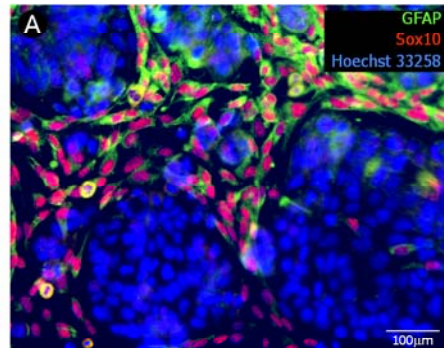
Research Plan/Methodology

Specifically, we will assess the following hypothesis:

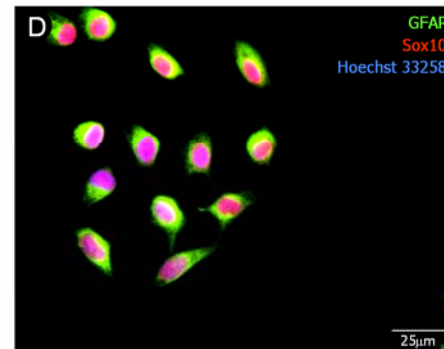
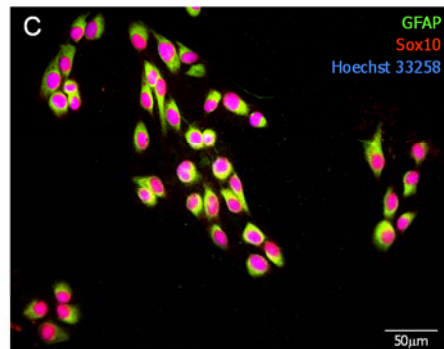
1. Enteric glia are involved in the regulation of mucosal defense. *In vitro*, co-culturing with enteric glia alters transepithelial resistance and macromolecular permeability of epithelial cell layers.
2. Enteric glia support epithelial cells. This is mediated by glial expression of neurotrophins, neurotrophic factors and/or epithelial growth factor and can be regulated by proinflammatory cytokines.
3. Enteric glia is affected in human IBD.
4. Glial expression of neurotrophins, neurotrophic factors and/or epithelial growth factor is altered in human IBD.

Results I

We have established and characterized primary enteric glia from human ileum myenteric and submucous plexuses (Fig. 3a-d).



Figs.3a,b: Mixed cultures of enteric glial cells and CaCo2. Note the selective labeling of glia with Sox10 and GFAP.



Figs.3c,d: Purified cultures of enteric glial cells. Note that all cells are homogeneously labeled with Sox10 and GFAP.

Results II

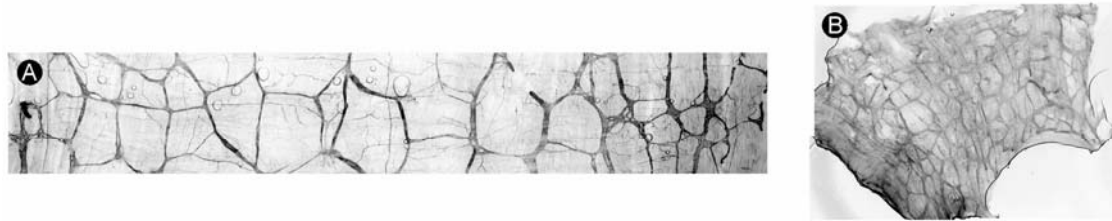


Fig.4: Wholemount preparation of human colonic myenteric plexus – overview. (A) normal, (B) CD.

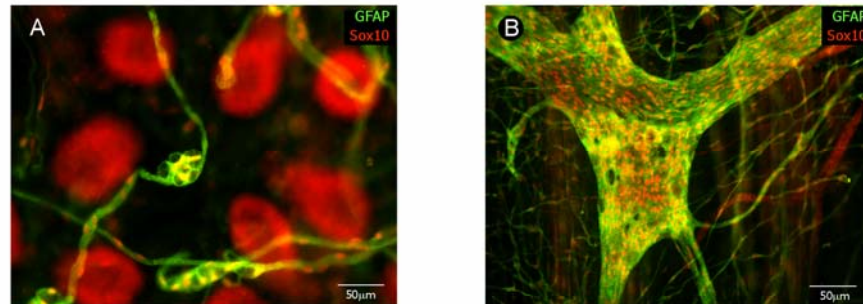


Fig.5a,b: Wholemount preparation of human colon submucosa (A) and myenteric plexus (B).

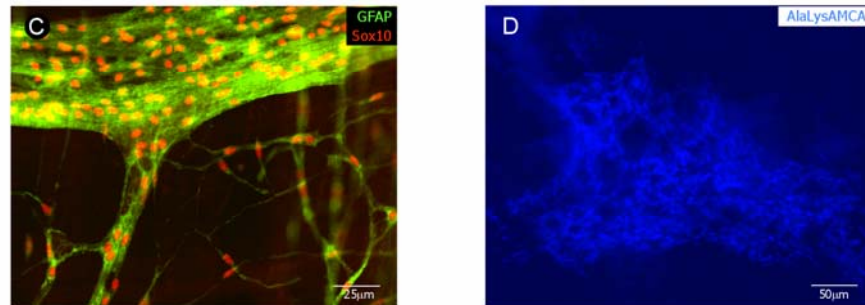


Fig.5c,d: Wholemount preparation of human colon myenteric plexus.

Primary antibody/markers	Description	Staining pattern
Sox 10	Transcription factor	nuclear
S100	Calcium binding protein	cytoplasmic
p75NGFR	Low-affinity nerve growth factor receptor	cytoplasmic
GFAP	Glial fibrillary acidic protein	cytoplasmic
B-FABP	Brain-derived fatty acid binding protein	not detectable in the human ENS
AlaLysAMCA (intravitaly!)	PEPT2-mediated dipeptide uptake	cytoplasmic and nuclear

Results III

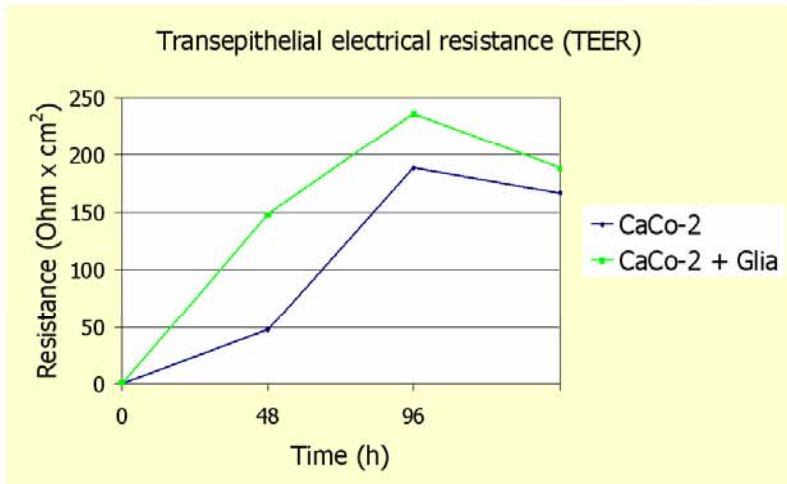


Fig.6: Coculturing human colon cancer cells (Caco-2) with enteric glial cells in a non-contact transwell-system increases transepithelial resistance (TEER).

These preliminary results were obtained using purified glial cell cultures (Figs.3c,d).

Summary

At this point, we have established

1. pure primary culture of human enteric glia and co-cultures of human enteric glia with intestinal epithelial cells in a transwell system which will allow us
 - to thoroughly characterize the effects of enteric glial cells on transepithelial resistance and macromolecular permeability of epithelial cell layers;
 - to assess if these effects are mediated by glial expression of neurotrophins, neurotrophic factors and/or epithelial growth factor;
 - to assess if glial expression of these factors can be regulated by proinflammatory cytokines;
2. the immunohistochemical marker profile of glial cells in the human ENS which allow us to
 - reliably detect and quantify all enteric glial cells in the non-inflamed as well as the inflamed human gut wall;
 - assess glial expression of neurotrophins, neurotrophic factors and/or epithelial growth factor in tissue specimens from human IBD.

Next Steps

1. We will proceed with our successful *in vitro* tissue culture work.
2. We will start to work on glial expression of neurotrophic factors and neurotrophins *in vitro* and *in vivo*.

Perspectives and Relevance of the Project to IBD

The project aims to elucidate the interactions between enteric glial cells and the gut mucosa and to analyze factors that mediate glial-epithelial interactions in the human intestine. It will thus help to better define the putative mechanisms underlying the physiological and pathophysiological roles of enteric glia for mucosal protection and regeneration in human intestinal inflammation.

In addition, we aim to elucidate if the postulated novel glia-epithelial crosstalk is correlated with the clinical course of disease in human IBD.

We expect that understanding the postulated glial-derived network of neurotrophins and neurotrophic factors and how it is being regulated during inflammatory conditions in the human intestine will significantly further our understanding of the neuro-glial element in the pathogenesis of human IBD, and we expect that such an understanding will ultimately identify a novel avenue for therapeutic intervention.