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Enteric glial reactivity to systemic LPS administration: Changes in GFAP and S100B protein

Raphaela da Cunha Franceschi[a,b], Patrícia Nardin[c], Clivia Valle Machado[a,b], Lucas Silva Tortorelli[c], Malcon Andrei Martinez-Pereira[d], Caroline Zanotto[c], Carlos-Alberto Gonçalves[b,c,*], Denise Maria Zancan[a,b]

[a] Laboratory of Comparative Neurobiology, Department of Physiology, Institute of Basic Health Sciences (ICBS), Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Brazil
[b] Graduate Program in Neuroscience, ICBS, UFRGS, Brazil
[c] Laboratory of Calcium-Binding Proteins, Department of Biochemistry, ICBS, UFRGS, Brazil
[d] Health Science Center, University of Cruz Alta, Brazil

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ABSTRACT

Lipopolysaccharide (LPS) is used to induce inflammation and promotes nervous system activation. Different regions of the brain present heterogeneous glial responses; thus, in order to verify whether systemic LPS-induced inflammation affects the enteric glia differently across the intestinal segments, we evaluated the expressions of two glial activity markers, GFAP and S100B protein, in different intestine segments, at 1 h, 24 h and 7 days after acute systemic LPS administration (0.25 or 2.5 mg kg^{-1}) in rats. Histological inflammatory analysis indicated that the cecum was most affected when compared to the duodenum and proximal colon at the highest doses of LPS. LPS induced an increased S100B content after 24 h in all three regions, which decreased at 7 days after the highest dose in all regions. Moreover, at 24 h, this dose of LPS increased *ex-vivo* S100B secretion only in the cecum. The highest dose of LPS also increased GFAP in all regions at 24 h, but earlier in the cecum, where LPS-induced enteric S100B and GFAP alterations were dependent on dose, time and intestine region. No associated changes in serum S100B were observed. Our results indicate heterogeneous enteric glial responses to inflammatory insult, as observed in distinct brain areas.

1. Introduction

For over forty years, astrocytic characteristics have been recognized in the enteric glia cell (EGC) (Gabella, 1971, 1981). The similarities between astrocytes and the enteric glia have been demonstrated in both morphological (Gabella and Trigg, 1984; Hanani and Reichenbach, 1994; Komuro et al., 1982) and functional (Aube et al., 2006; Ferri et al., 1982; Hoff et al., 2008; Jessen and Mirsky, 1980, 1983; Kimball and Mulholland, 1996; Nasser et al., 2006a) aspects. Even in response to different insults, such as inflammation and infection, similarities are observed between the responses of the astrocytes and the enteric glia (Cabarrocas et al., 2003; Coelho-Aguiar et al., 2015). Despite these similarities, there is growing evidence of important diversity among enteric glia (Boesmans et al., 2015; Rao et al., 2015).

The characteristic response of the astrocytes to these insults is called reactive astrogliosis, in which increased glial fibrillary acidic protein (GFAP) expression and extensively-modified enzymatic metabolism are observed (Anderson et al., 2014; Eddleston and Mucke, 1993; Kálmán, 2004; Ridet et al., 1997). Brain reactive gliosis is a complex process that has been intensively investigated in many conditions of injury and diseases. The response to injuries in the enteric glia has been less well characterized. The S100B protein, another astrocyte marker of glial activation in the brain tissue is also expressed by the glial elements of the enteric nervous system (Cirillo et al., 2011a) and its expression is affected by gastroin-

Abbreviations: BSA, bovine serum albumin; EGC, enteric glia cell; ENS, enteric nervous system; EGTA, ethylene-bis-(oxyethylenenitrilo)-tetracetic acid; GFAP, glial fibrillary acidic protein; HEPES, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; NR, Neutral Red; OPD, *o*-phenylenediamine; PMSF, phenylmethylsulfonyl fluoride; PBS, phosphate-buffered saline; RAGE, receptor for advanced glycation end-products; TLR, Toll-like receptor; UC, ulcerative colitis.

* Corresponding author at: Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Ramiro Barcelos 2600, Porto Alegre, RS 90035-003, Brazil.
E-mail address: casg@ufrgs.br (C.-A. Gonçalves).

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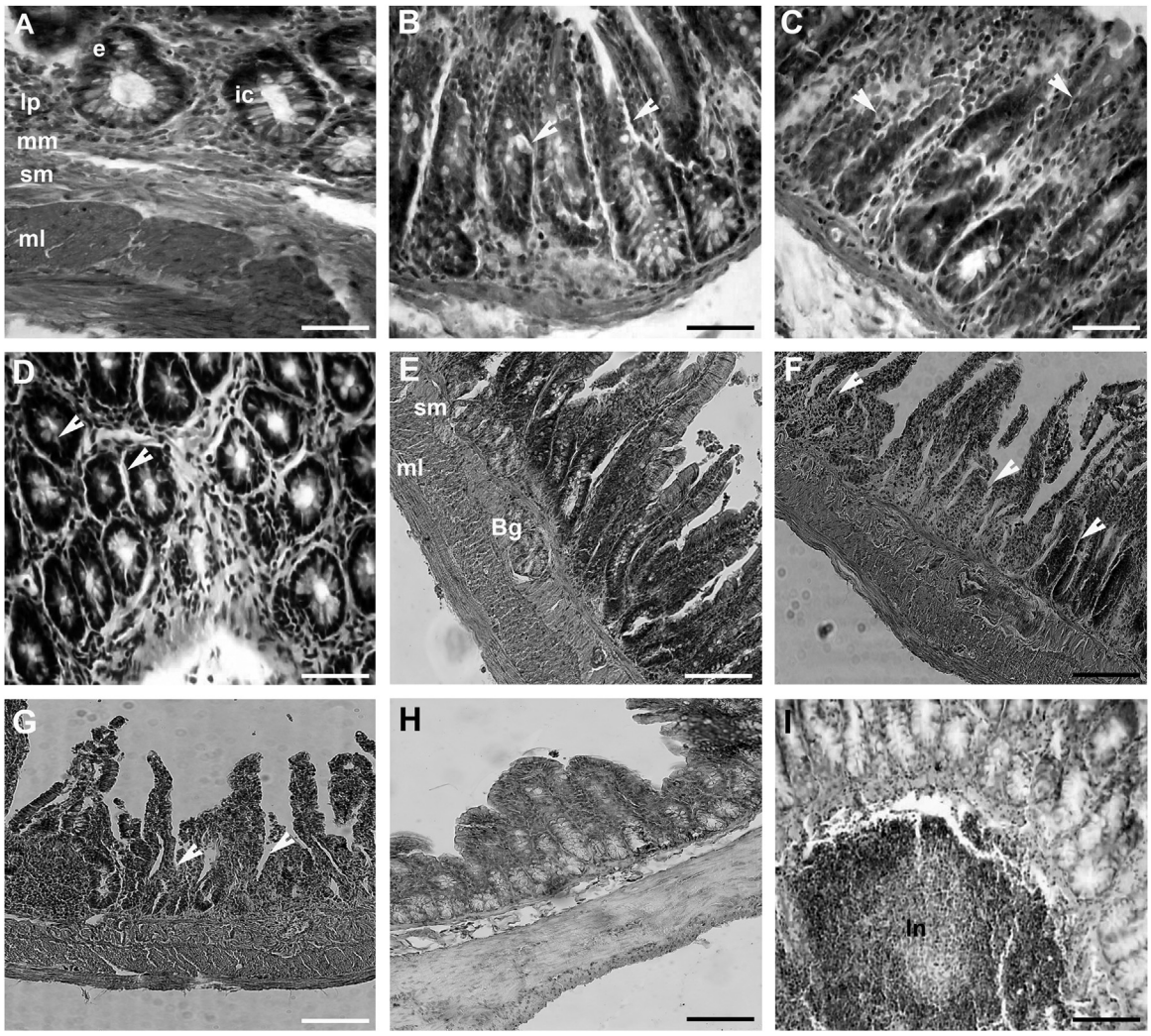

Fig. 1. Representative horizontal sections of the small and large intestines of rats treated with i.p. LPS (hematoxylin-eosin staining). (A) cecum, control: intestinal crypts (ic), epithelium (e), lamina propria (lp), muscularis mucosa (mm), submucosa (sm) and muscular (ml) layers. (B) cecum, LPS 0.25/24 h. (C) cecum, LPS 0.25/7 days. (D) cecum, LPS 2.5/24 h. (E) duodenum, control: Brunner's glands (Bg). (F) duodenum, LPS 0.25/24 h. (G) duodenum, LPS 2.5/24 h. (H) proximal colon, control. (I) proximal colon LPS 2.5/7 days. Dense infiltrate of inflammatory cells in the epithelium and lamina propria of the mucosa was observed (B–D; F, G). The arrowheads indicate the focal loss of intestinal crypt organization (B–D; F, G). After 7 days of LPS injection there was recovery of the organization and morphology of the crypts (I), as well as the lymph nodes without increased size and evidence of low lymphocyte activity for the clear identification of the mantle and germinal center, indicating a decrease in the inflammatory process. Scale bar = 100 μm (A–D); 75 μm (E–H); 50 μm (I).

testinal inflammatory disorders. In patients with celiac disease and ulcerative colitis (UC), S100B overexpression and release by the EGC network have been reported (Cirillo et al., 2009; Esposito et al., 2007). The increase in S100B protein observed in rectal biopsies of UC patients suggests that extracellular S100B works as a ligand for cell surface receptors via a RAGE-dependent mechanism (receptor for advanced glycation end-products) (Cirillo et al., 2011b).

Lipopolysaccharide (LPS) is commonly used to induce inflammation and activation of the innate immune system via Toll-like receptor (TLR) signaling (Hoshino et al., 1999), which is also found in EGC (Brahmachari et al., 2006; Guerra et al., 2011; von Boyen et al., 2004). The gastrointestinal tract is a large reservoir of both gram-positive and gram-negative bacteria (Ley et al., 2006), where the gram-negative bacteria works as a source of endotoxin LPS. Thus, the intestines represent both a major barrier and a major source of endotoxin (Mani et al., 2012). Intestinal diseases, systemic inflammatory response syndrome, or environmental and metabolic stresses result in a decrease in the barrier integrity of the intestinal epithelial cells (Mittal and Coopersmith, 2014; Shimizu et al., 2011). Considering the close proximity of the EGCs to intestinal epithelial cells and their production of mediators acting in mucosal barrier function, the EGC has been proposed to be an essential component for the integrity of the gut wall (Coquenlorge et al., 2014; von Boyen et al., 2004; Xiao et al., 2011).

LPS administration (by intraperitoneal or intracerebroventricular injection, in astrocyte cultures, or even in hippocampal slices) promotes an increase in the astrocyte expression of GFAP and alters extracellular levels of S100B (Brahmachari et al., 2006; Guerra et al., 2011). Enteroglial-derived GFAP and S100B protein expressions increase with LPS administration in dissected colon and cultured enteric glia cells (Cirillo et al., 2011b; Coquenlorge et al., 2014; von Boyen et al., 2004; Xiao et al., 2011). At present, there is one evidence of the existence of *in vivo* data to confirm the impact of EGC activity on the response to single high-dose of LPS (Rosenbaum et al., 2016) as we have observed in the brain tissue (Borges et al., 2012; Cardoso et al., 2015; Guerra et al., 2011; Ifuku et al., 2012). In this study, we evaluate the effects of *in vivo* systemic LPS administration, at different concentrations and times, on the enteroglial GFAP and S100B protein expressions on different segments of the