Volume 57(1):83-94, 2013
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Aligned alteration of enteric neurons, smooth muscle cells and inflammatory markers involved in stricture formation in a rat model of Crohn's disease

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Crohn's disease (CD) is a multifactorial, relapsing disorder with chronic inflammation involving all layers of the gut wall. The development of obstructive strictures associated with CD causes major complications in patients. Because no effective therapies are available to prevent stricturing we wanted to gain a better understanding of its pathogenesis by developing a rat model suitable to investigate the involvement of the enteric neurons, the intestinal smooth muscle cells (SMCs) and different inflammatory markers in the intestinal stricturing.

Colitis was induced by an enema of 2,4,6-trinitrobenzenesulfonic acid (TNBS, 10 mg) in 25% ethanol. Tissue samples were taken from control, as well as once, twice and three times treated rats from the inflamed segment, and also proximal and distal to the inflamed segment of the colon in different time points between 2 and 120 days. Quantitative features of myenteric neurons were investigated after HuC/D immunohistochemistry. The expression of multiple inflammatory markers was determined by RT-PCR. The strictures were studied by transmission electron microscopy.

The number of myenteric neurons decreased significantly in all three colonic segments in the acute phase of inflammation. However, 8 days after the TNBS treatments no further changes in the neuronal number was detected until the end of the investigation. Strictures developed at 60th day after the first TNBS treatment and the frequency of strictures increased until day 120th. Thickened muscle layers, expanded intercellular spaces and matrix deposition characterized the strictures. Loosed SMCs with the morphological sign of apoptosis was frequently seen, while enteric ganglia were morphologically intact. HO-1 mRNA was upregulated in all samples from the TNBS-treated rats in the acute phase of the inflammation, and the HO-1 level remained high until day 120th. The increased activity of MMP9 after repeated treatments referred to severe local tissue injury. TGF-β2, but not TGF-β3 was expressed in each tissue samples from the rats with colitis. This expression profile of TGF-β isoforms is characteristic to CD.

The repeated induction of TNBS colitis enhanced intestinal stricturing making this rat model suitable to investigate its pathogenesis. Our preliminary findings indicate that aligned alteration of enteric neurons, smooth muscle cells (SMCs) and different inflammatory markers have a critical role in the development of intestinal strictures. After repeating TNBS treatments, decreased extension of mucosal inflammation was observed when compared to rats treated with TNBS only once. Therefore, a preconditioning effect of repeated TNBS treatment was suggested. Based on the evaluation of quantitative properties of the enteric neurons seemed that this preconditioning did not evolve in the enteric neurons. Ultrastructural morphometry revealed an increased amount of extracellular matrix deposition and increased number of SMCs with proapoptotic markers. Consequently, the distance between SMCs and myenteric ganglia increased, which might be responsible for the default innervation of SCMs and the formation of intestinal strictures.

This research was supported by the European Union co-funded by the European Social Fund (Project number: TÁMOP 4.2.4.A/2-11-1-2012-0001, National Excellence Program in the convergence region).

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Characterization of the innate and adaptive immune responses induced by the opportunistic human pathogen *Candida parapsilosis*

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The genus *Candida* comprises more than one hundred species, of which less than 20 have been associated with human infections. Depending on the age group and geographical region, *C. parapsilosis* is the second or third most common species after *C. albicans* and *C. glabrata* causing invasive candidiasis. Although in recent years there has been a great progress in the understanding of immune responses induced by *C. albicans*, little is known about the immunity against *C. parapsilosis*.

During our study, we examined the innate and adaptive immune responses induced by *C. albicans* and *C. parapsilosis*. Firstly, we compared the cytokine responses evoked by *C. albicans* and *C. parapsilosis* using an *in vitro* model of human peripheral blood mononuclear cells (PBMCs). PBMCs were stimulated with heat killed *C. albicans* or *C. parapsilosis*, and the cytokine production was measured by enzyme-linked immunosorbent assay (ELISA). *C. parapsilosis* induced similar quantities of TNFα and IL-6, and slightly lower amounts of IL-1β in human PBMCs compared to *C. albicans*. However, stimulation of PBMCs with *C. parapsilosis* resulted in higher IL-10 and lower IFNγ production compared to *C. albicans*, indicating a skewed T helper cell response. Furthermore, *C. parapsilosis* induced much lower IL-17 and IL-22 production compared to *C. albicans*. Following intracellular cytokine staining, flow cytometric analysis confirmed that the decreased production of IL-17 and IL-22 was in line with a lower number of IL-17 producing cells. Blocking of the three classical