P024 Regional differences in effect of TGF–beta1 and PDGF on the early onset of intestinal fibrosis

ARTICLE in JOURNAL OF CROHN S AND COLITIS · FEBRUARY 2014
Impact Factor: 6.23 · DOI: 10.1016/S1873-9946(14)60147-1

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normal development of Treg function and the regulation of DSS-induced colitis in SAMP1/YitFc mice.

PO24 Regional differences in effect of TGF-beta1 and PDGF on the early onset of intestinal fibrosis
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Background: Intestinal fibrosis (IF) is one of the major complications in inflammatory bowel disease patients. IF can cause narrowing of the intestinal lumen, which may lead to stricture formation. The mechanism of IF is still unknown and adequate models are lacking. By using precision-cut intestinal slice (PCS) from different regions of the bowel, we studied the early onset of fibrosis in mouse jejunum, ileum and colon PCS, in the presence of transforming growth factor (TGF)-beta1 and platelet-derived growth factor (PDGF).

Methods: Mouse jejunum, ileum and colon were excised and prepared as a segment embedded in agarose. PCS (estimated 300–400 mm) was prepared and incubated up to 48 hr with or without the presence of TGF-beta1 and PDGF. ATP content of the PCS was used to assess the general viability. The gene expression of different fibrosis markers including Pro-Collagen 1 A1 (COL1A1), heat shock protein 47 (HSP47), alpha-smooth muscle actin (SMA), connective tissue growth factor (CTGF), synaptophysin (SYN) and fibronectin (FN2) were determined.

Results: Mouse PCS from different segments were viable up to 48 hr. After 48 hr of incubation, HSP47 and FN2 gene expression were significantly up-regulated, compared to PCS directly after slicing, in jejunum (3.6 and 4.9 fold, respectively) and in ileum (4.9 and 5.5 fold, respectively). When incubated with 5 ng/mL TGF-beta1, in jejunum PCS, COL1A1, HSP47, CTGF and FN2 were significantly up-regulated compared to 48 hr control. In ileum PCS the gene expression of HSP47 (1.9 fold) and FN2 (3.9 fold) were also significantly increased.

In the presence of 50 ng/mL PDGF, only in ileum PCS, CTGF (1.4 fold) and SYN (1.9 fold) were significantly increased compared to 48 hr control. Interestingly, in PCS from the colon, 5 ng/ml TGF-beta1 did not affect the gene expression of the fibrosis markers. However, HSP47 (1.4 fold) and FN2 (1.7 fold) were significantly increased when colon PCS were incubated with 50 ng/mL PDGF.

Conclusions: TGF-beta1, but not PDGF, was able to induce HSP47 and FN2 in mouse jejunum and ileum PCS. This is in contrast to the result in colon PCS, where only PDGF was able to induce these fibrosis markers. Moreover, PDGF increased CTGF and SYN only in ileum PCS. These results indicate differences in the effect of TGF-beta1 and PDGF on the early onset of fibrosis in different regions of the intestine.

PO25 Reduced Butyricoccus pullicaecorum levels in mucosa of UC patients correlate with aberrant CLDN1 expression
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Background: Butyrate maintains colonic homeostasis by modulating a wide variety of cellular functions including the control of intestinal epithelial integrity. Butyricoccus pullicaecorum is a butyrate-producing bacterial strain that is found in reduced amounts in stool samples of patients with ulcerative colitis (UC) and is currently being investigated as a probiotic. Conditioned growth medium of B. pullicaecorum reduces TNF-induced colonic epithelial permeability in vitro, however its in vivo relevance is unknown. The aim of our research was to investigate the relationship between the presence of B. pullicaecorum in the colonic mucosa and the expression of tight junction protein 1 (TJJP1), occludin (OCLN) and claudin 1 (CLDN1), essential components of the tight junction complex which are partially regulated by butyrate.

Methods: The expression of these genes was analyzed by quantitative real-time PCR (qPCR) in a collection of colonic biopsies from healthy controls (N = 21) and UC patients with active disease (N = 26). Next, the effect of the conditioned growth medium of B. pullicaecorum (strain 25–3\textsuperscript{1}) on the expression of these genes was investigated in HT-29 cells in the presence or absence of TNF. Finally, B. pullicaecorum bacteria were quantified in an extended cohort of colonic mucosa of UC patients (N = 36) and healthy controls (N = 31) using a genus-specific qPCR.

Results: TJP1 and OCLN were significantly downregulated in colonic biopsies of UC patients (both P < 0.005), whereas CLDN1 expression was increased (P < 0.003). The conditioned growth medium of B. pullicaecorum increased the baseline expression of TJP1 and OCLN but did not decrease CLDN1 levels in HT-29 cells. TNF did not affect expression of TJP1 or OCLN but increased CLDN1 expression which was counteracted by 21% after co-incubation with the conditioned growth medium. B. pullicaecorum could be detected in colonic biopsies of 71% of healthy controls and in only 42% of UC patients (Fisher exact P = 0.026). In addition, in samples where B. pullicaecorum was detected, the absolute amount was lower in UC samples (P = 0.081). Interestingly, the quantity of B. pullicaecorum correlated with the deregulated expression of CLDN1 (R = –0.528).

Conclusions: Butyricoccus pullicaecorum is a mucus-adherent bacterium and is underrepresented in colonic biopsies of UC patients. Their reduced prevalence correlates with aberrant CLDN1 expression which can be reversed in vitro by the conditioned growth medium of B. pullicaecorum. Together, these data support a role for B. pullicaecorum in the preservation of colonic barrier integrity.

PO26 Recreating the intestinal macrophage in vitro: a potential role for stromal factors
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Background: We have previously shown that one inhibitory (LILRB1) and one activating (LILRA5) leukocyte immunoglobulin-like receptor are expressed by macrophages in the colonic lamina propria. We hypothesise that factors in the colonic stroma allow newly-recruited blood monocytes to differentiate into immune-tolerant intestinal macrophages. The aim of this study was to develop a cell culture model of intestinal-like macrophages, in order to elucidate the roles of these LILRs in the colon.

Methods: Conditioned media was generated from stroma of the colonic lamina propria. Peripheral blood monocytes were differentiated in vitro into classical macrophages with GM-CSF or into intestinal-like macrophages with GM-CSF and stromal-derived conditioned media. These cells were assessed for expression of tumour necrosis factor (TNF)-\textalpha via ELISA and qRT-PCR, and LILRB1 and LILRA5 via qRT-PCR.

Results: Cultured monocytes produced low levels of the pro-inflammatory cytokine, TNF-\textalpha. Following stimulation with lipopolysaccharide (LPS), classically differentiated macrophages produced high levels of TNF-\textalpha. When cells were differentiated to intestinal-like macrophages with stromal-derived conditioned media, this cytokine response was down-regulated in a dose- and time-dependent manner. Interferon (IFN)-\gamma and interleukin (IL)-10 stimulation did not significantly affect TNF-\textalpha expression. Changes in TNF-\textalpha mRNA levels in response to cell stimulation paralleled the cytokine