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

P024

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 (PCIS) from different regions of the bowel, we studied the early onset of fibrosis in mouse jejunum, ileum and colon PCIS, 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. PCIS (estimated 300–400 μm) was prepared and incubated up to 48 hr with or without the presence of TGF-beta1 and PDGF. ATP content of the PCIS 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 PCIS 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 PCIS 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 PCIS, COL1A1, HSP47, CTGF and FN2 were significantly up-regulated compared to 48 hr control. In ileum PCIS 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 PCIS, CTGF (1.4 fold) and SYN (1.9 fold) were significantly increased compared to 48 hr control. Interestingly, in PCIS 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 PCIS 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 PCIS. This is in contrast to the result in colon PCIS, where only PDGF was able to induce these fibrosis markers. Moreover, PDGF increased CTGF and SYN only in ileum PCIS. These results indicate differences in the effect of TGF-beta1 and PDGF on the early onset of fibrosis in different regions of the intestine.

P025

Reduced *Butyricicoccus 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. *Butyricicoccus 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 pharmabiotic. 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 (TJP1), 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^T) 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: *Butyricicoccus 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.

P026

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)-α *via* ELISA and qRT-PCR, and LILRB1 and LILRA5 *via* qRT-PCR.
Results: Cultured monocytes produced low levels of the pro-inflammatory cytokine, TNF-α. Following stimulation with lipopolysaccharide (LPS), classically differentiated macrophages produced high levels of TNF-α. 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)-γ and interleukin (IL)-10 stimulation did not significantly affect TNF-α expression. Changes in TNF-α mRNA levels in response to cell stimulation paralleled the cytokine