Methods: Cell suspensions prepared from peripheral blood and intestinal biopsies were stimulated with HDMAPP phosphoantigen and analyzed by flow-cytometry.

Results: Vi2T-cells up-regulate the expression of gut-homing integrin α4β7 upon phosphoantigen stimulation in vitro, and circulating Vi2T-cells in CD patients contained an elevated proportion of α7+ cells (CD median 80.8%, controls 64.9%; p = 0.010), and CD69+ cells (p = 0.006) that correlated with reduced Vi2T-cell frequency (p = 0.0018), suggesting increased activation/taxis to the gut. Intestinal biopsies from both CD patients and controls contained CD103+ and CD103+ Vi2T-cells that produced IFNγ and TNFα, but the influence of Vi2T-cell subset balance on IFNγ production by mucosal α7+ T-cells was disrupted in CD. Blood Vi2T-cells rarely expressed Vδ300 prepared as a segment embedded in agarose. PCIS (estimated onset of IF).

PCIS all cell types are present in their original tissue-matrix. When incubated with 5 ng/mL TGF-β, similarly, in mouse PCIS, gene expression of HSP47 (3.9 fold) and FIB (4.3 fold) was significantly up-regulated after 48 hr. However, incubation of human PCIS with 5 ng/mL of TGF-β, none of the investigated fibrosis genes was affected.

Conclusions: In rat, mouse and human PCIS an increase in gene expression of early-onset of fibrosis makers was found. In addition, TGF-β1 was able to induce fibrosis markers in rat and mouse, but not in human PCIS. Rodent and human PCIS are promising ex vivo models to study the early onset of intestinal fibrosis.

P067

Human and rodent ex vivo model for intestinal fibrosis in inflammatory bowel disease (IBD)

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Background: One of the major complications in IBD is intestinal fibrosis (IF). It is the result of the chronic inflammation of intestinal tissue. IF causes narrowing of the intestinal lumen and potential stricture formation. For the study of the cellular and molecular mechanism of IF in IBD adequate animal models are lacking. Our aim is to develop an ex vivo model for IF by using human and rodent precision-cut intestinal slice (PCIS). In PCIS all cell types are present in their original tissue-matrix environment and can be used as a model to study the early onset of IF.

Methods: Rat, mouse and human jejunal were excised and prepared as a segment embedded in agarose. PCIS (estimated 300-400 μm) was prepared and incubated up to 24 hr (rat) or 48 hr (human and mouse). ATP content of the PCIS was used to assess the general viability. Moreover, morphology (rat and human) was evaluated. 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 (FIB) were determined.

Results: Mouse PCIS were viable up to 48 hr. However, for rat and human PCIS ATP content was decreased to 25% (24 hr) and 70% (48 hr), respectively. ATP content of rat and human PCIS correlated well with morphology of the PCIS. In rat PCIS, after 24 hr, HSP47 (3.2 fold) and FIB (2.1 fold) gene expressions were significantly increased. In the presence of 5 ng/mL TGF-β, COL1A1 (1.8 fold), SMA (1.5 fold) and CTGF (2.1 fold) were significantly up-regulated compared to 24 hr control. Meanwhile HSP47 gene expression was slightly decreased (0.8 fold).

Similarly, in mouse PCIS, gene expression of HSP47 (3.9 fold) and FIB (4.3 fold) was significantly increased after 48 hr. When incubated with 5ng/mL TGF-β, COL1A1 and FIB were significantly up-regulated (2.0 fold) compared to 48 hr control, HSP47 and CTGF gene expression were slightly, but significantly increased (1.2 fold).

In human PCIS, after 48 hr, HSP47 (3.5-fold) and SYN (2.5 fold) were significantly up-regulated. However, incubation of human PCIS with 5 ng/ml of TGF-β, none of the investigated fibrosis genes was affected.

Conclusions: In rat, mouse and human PCIS an increase in gene expression of early-onset of fibrosis makers was found. In addition, TGF-β1 was able to induce fibrosis markers in rat and mouse, but not in human PCIS. Rodent and human PCIS are promising ex vivo models to study the early onset of intestinal fibrosis.

P068

High expression level of the T cell potassium channel, KV1.3, is a novel diagnostic marker of inflammatory activity in patients with ulcerative colitis

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Background: T cell KV1.3 and KCa3.1 channels are involved in T cell activation, motility and proliferation. These channels are thought to be expressed in all subsets of T cells. Moreover, KV1.3 is highly expressed in effector memory T cells that are believed to play an important role in autoimmune diseases. Their role in ulcerative colitis (UC) is yet to be explored. Our hypothesis was that the expression level of KV1.3 was associated with disease activity in patients with ulcerative colitis.

Methods: Biopsies from healthy individuals (n = 8) and patients (n = 26) with first-time attack or relapse of UC were obtained during endoscopic examination. Multiple biopsies were obtained from the inflamed mucosa. Biopsies were analysed by quantitative RT-PCR (qPCR) and immunohistochemistry. The degree of inflammation was assessed by a gastrointestinal pathologist (scores: 0–3; none, mild, moderate, severe).

Results: mRNA-expression levels of KV1.3 were significantly up-regulated in UC patients with active inflammation compared to controls with no symptoms of inflammatory bowel disease showed (p < 0.01). Expression levels of the other T-cell K+ channel, KCa3.1, were not different between the two groups. KV1.3 protein expression was localized to T-cell infiltrates and macrophages while the colonic mucosa did not express KV1.3. KCa3.1 protein expression was found in the mucosa of both patient groups and also in T-cell and macrophage infiltrates of the UC patients.

Conclusions: The mRNA and protein expression of KV1.3 channels in active UC is strongly associated with the degree of mucosal inflammation. Disease-related increases in KV1.3 might serve therefore as additional diagnostic or prognostic marker for UC. Moreover, the increased expression levels of KV1.3 support the hypothesis that UC could be an autoimmune disease, considering a pathomechanistic involvement of KV1.3-high T cells. Finally, blockers of KV1.3 may be of potential usage as a novel pharmacological treatment option.

P069

Glycine and glutamine down-regulate IL-6-induced claudin-2 expression in human colonic epithelial cell monolayer

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Background: It is well-known that tight junction contributes epithelial barrier function of intestinal mucosa, and claudins...