activate IL-21R+ T cells, B cells, plasma cells and macrophages, suggesting a role of this cytokine-receptor interaction in the pathogenesis of CD. Thus, IL-21 may represent a promising target for treatment of CD.

**P064**

**IL-21 neutralization in experimental colitis: regulation of cytokines, chemokines and colonic transcripts**

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**Background:** Interleukin 21 (IL-21), a member of the common gamma-chain family of cytokines, is secreted by activated T cells and NK T cells, and has a diverse range of immunomodulatory effects dependent upon the immunological context. IL-21 is produced in the inflamed intestine of patients with IBD, mostly by activated CD4+ helper T cells.

**Methods:** Using a CD4+CD25+ SCID adoptive transfer colitis model, we evaluated the effect of a murine monoclonal anti-IL-21 antibody. Mice were treated with anti-IL-21 mAb 25 mg/kg (i.p.) 3 × in either a prophylactic (day 0–42) or a therapeutic (day 21–56) setup. Colon biopsies were analyzed using either luminex or deep sequencing technology. Mesenteric lymph node CD4+ T cells were isolated from mice with colitis and stimulated with different combinations of anti-CD3 and IL-21 or analyzed directly by intracellular FACS analysis.

**Results:** IL-21 production by the transferred CD4+ T cells was found increased over time and correlated with clinical disease. Moreover, a significant reduction of clinical signs of colitis was shown in both anti-IL-21 mAb prophylactic and therapeutically treated mice, when compared to control Ig-treated mice. In anti-IL-21 mAb prophylactic treated mice, several cytokines (including TNF-a and IL-1b) were significantly down-regulated, revealed by deep sequencing analysis of colon biopsies. Additionally, in anti-IL-21 mAb treated mice, luminex analysis on colon biopsies showed a significant down regulation of CCL5, IL-17 and TNF-a. This is in agreement with an up-regulation of the same molecules when mesenteric lymph node CD4+ T cells isolated from mice with colitis were stimulated in vitro with anti-CD3 and IL-21. The percentage of IFN-γ and IL-21 positive CD4+ T cells in mesenteric lymph nodes was also significantly down regulated in anti-IL-21 treated mice. Moreover, anti-IL-21 mAb treatment significantly down-regulated colon transcripts for metallo matrix proteases (MMPs), activation markers, adhesions molecules and chemokines (including MMP3, MMP9, CD44, Integrin a4b7, ICAM-1, CXCL13, CCL5, CCL20) and up-regulated transcripts involved in mucosal barrier function (including Muc2, TFF3 and Occludin).

**Conclusions:** These results suggest that IL-21 is a relevant target in experimental colitis regulating several pro-inflammatory cytokines and chemokines as well as key molecules in mucosal barrier function. Thus, blockade of this pathway may be of therapeutic benefit in patients with IBD.

**P065**

**Humoral immune response to intestinal microbiota in inflammatory bowel diseases**

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**Background:** Evidence suggests that inflammatory bowel disease (IBD) results from an inappropriate inflammatory response to intestinal microbes in a genetically susceptible host.

**Methods:** 22 pts with IBD exacerbation (15 ulcerative colitis [UC], 7 Crohn’s disease [CD]) and 15 healthy controls were included into the study. IgM and IgG to lipopolysaccharide (Lps) of E. coli O14, protein antigens of E. coli M 17, P. aeruginosa, P. mirabilis, C. albicans, K. pneumoniae, Strep. spp., S. aureus were evaluated by immunossay. Mean age in UC was 36.1±6.4 years, CD = 37.4±6.6, controls = 30.4±6.6.

**Results:** There was decreasing of IgM and IgG to majority of antigens in UC and CD compared to controls (Tables 1, 2). IgM to Strept. spp. was increased in UC (p < 0.05), IgG to Lps of E. coli O14 was decreased in CD (p < 0.05). Humoral immunity correlated with IBD courses: in UC IgM to Lps of E. coli O14 and distribution of the disease (r = 0.6; p < 0.05), in CD – IgM to Strept. spp. (r = 0.8; p < 0.05), IgG to P. mirabilis (r = 0.7; p < 0.05) and disease duration. Some associations with clinical parameters were showed: bloody diarrhea was associated in UC with IgM to Lps of E. coli O14 (r = −0.6; p < 0.05), IgG to P. mirabilis (r = 0.6; p < 0.05) and in CD with IgM to S. aureus (r = 0.8; p < 0.05), IgG to P. mirabilis (r = −0.9; p < 0.05). The abdominal distention correlated in CD with IgM to C. albicans (r = 0.8; p < 0.05) and with IgG to Lps of E. coli O14 (r = 0.7; p < 0.05).

**Table 1**

<table>
<thead>
<tr>
<th>IgM</th>
<th>UC, n (%)</th>
<th>CD, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norm</td>
<td>T</td>
<td>Norm</td>
</tr>
<tr>
<td>Lps of E. coli O14</td>
<td>7 (46.7)</td>
<td>3 (20)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>7 (46.7)</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>C. albicans</td>
<td>6 (40)</td>
<td>8 (53.3)</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>2 (13.3)</td>
<td>9 (60)</td>
</tr>
<tr>
<td>Strep. spp.</td>
<td>1 (6.7)</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>Total</td>
<td>5 (33.3)</td>
<td>6 (40)</td>
</tr>
</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>IgG</th>
<th>UC, n (%)</th>
<th>CD, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norm</td>
<td>T</td>
<td>Norm</td>
</tr>
<tr>
<td>Lps of E. coli O14</td>
<td>7 (46.7)</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>11 (73.3)</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>C. albicans</td>
<td>9 (60)</td>
<td>6 (40)</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>6 (40)</td>
<td>8 (53.3)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>2 (13.3)</td>
<td>6 (40)</td>
</tr>
<tr>
<td>Total</td>
<td>5 (33.3)</td>
<td>6 (40)</td>
</tr>
</tbody>
</table>

**Conclusions:** The majority of pts with IBD have decreased IgM and IgG to intestinal microbes compared to control. IM changes correlated with IBD course and clinical features.

**P066**

**Human intestinal V62+ T-cells promote mucosal inflammation in Crohn’s disease and are selectively ablated by aziathoprine therapy**

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**Background:** Tumor-derived and microbial phosphoantigens specifically activate Vγ9Vδ2 (Vδ2T) cells which are found only in humans and higher primates. We have demonstrated that Vδ2T-cells populate the human intestinal mucosa and enhance inflammatory responses by conventional colonic T-cells, so we hypothesized that Vδ2T-cells promote pathological gut inflammation in Crohn’s disease (CD).

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

**Results:** ViT2T-cells up-regulate the expression of gut-homing integrin α4β7 upon phosphoantigen stimulation in vitro, and circulating ViT2T-cells in CD patients contained an elevated proportion of α4β7+ cells (CD median 80.8%, controls 64.9%; p = 0.010), and CD69+ cells (p = 0.006) that correlated with reduced ViT2T-cell frequency (p = 0.0018), suggesting increased activation/trafficking to the gut. Intestinal biopsies from both CD patients and controls contained CD103- and CD103+ ViT2T-cells that produced IFN-γ and TNFα, but the influence of ViT2T-cell subset balance on IFN-γ production by mucosal γδ T-cells was disrupted in CD. Blood ViT2T-cells rarely expressed CD103, but this integrin was significantly induced upon HDMAPP activation in the presence of TGF-β1, suggesting that TGF may regulate ViT2T-cell mucosal phenotype. There was an extensive, selective loss of ViT2T-cells in blood from CD patients receiving azathioprine therapy (p < 0.001), and azathioprine impaired proliferation and cytokine production by intestinal ViT2T-cells in vitro.

**Conclusions:** These data demonstrate that human intestinal ViT2T-cells exert pro-inflammatory effects in CD that are ablated by azathioprine therapy, which may help to resolve intestinal inflammation, but could also confer increased risk of malignancy due to loss of tumor surveillance by ViT2T-cells in blood.

**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 100–400 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 5 ng/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 markers 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...