studies. **Results:** TRPM8 was observed rarely in myenteric neurons and in lamina propria cells in healthy colon. In Crohn's disease (CD), there was a massive infiltration of strongly TRPM8 positive cells, specifically into the lamina propria. These cells were CD3 negative, so we looked for co-localization with other immune cell markers. The TRPM8 positive cells were also negative for neutrophil elastase, mast cell protease and CD68, and nerve markers protein gene product (PGP)9.5 and calcitonin gene-related peptide (CGRP). They were, however, clearly positive for a small proportion of CD45+ cells. We then went on to identify them as myeloid cells, most likely conventional DC, using CD11c as a marker. Therefore, in contrast to the mouse, TRPM8 in humans is located directly on DC, rather than on neighbouring nerves, and may therefore have a more direct immunomodulatory role. In preliminary studies we tested this by incubating biopsies from CD patients with the TRPM8 agonist icilin (1 μM), and measuring cytokine output into conditioned medium by multiplex bead assay. IL-1b, TNFa, IL-8, IL-6 and IL-10 were all reduced by icilin, by 53, 67, 45, 66 and 42% respectively (N=3). **Conclusions:** TRPM8 is positioned to regulate directly the activity of dendritic cells in Crohn's disease, and potentially other specific leucocyte populations, leading to potent suppression of inflammatory responses. Supported by Wellcome Trust 1. **de Jong PR, Takahashi N, Peiris M, *et al.*** TRPM8 on mucosal sensory nerves regulates colitogenic responses by innate immune cells via CGRP. *Mucosal Immunol*, 2014. 2. **Harrington AM, Hughes PA, Martin CM, *et al.*** A novel role for TRPM8 in visceral afferent function. *Pain* 152: 1459-1468, 2011. 3. **Ramachandran R, Hyun E, Zhao L, *et al.*** TRPM8 activation attenuates inflammatory responses in mouse models of colitis. *Proc Natl Acad Sci U S A* 110: 7476-7481, 2013.

Mo1719

Epithelial NF-κB Activation via TNF Signaling May Be Involved in the Development of Colitis-Associated Carcinogenesis

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Background & Aim: Prolonged inflammatory bowel diseases (IBD) such as ulcerative colitis and Crohn's disease may promote carcinogenesis in the epithelia. It has been reported that activation of NF-κB pathway in both intestinal epithelial cells and myeloid cells are significant for the development of colitis-associated cancer (CAC). We previously reported that specific up-regulation of the type 2 receptor for tumor necrosis factor (TNFR2) rather than TNFR1 expression was observed in the inflamed colonic epithelia and further in the CAC. It is known that TNFR2 signaling may induce NF-κB activation, but the role of its expression in the setting of CAC has not been elucidated. Here we analyzed TNFR2 signaling in the colonic epithelial cells. **Methods & Results:** As previously observed in animal models of colitis and CAC, the expression of TNFR2 was up-regulated in an epithelial cell line, MOC1, which was derived from murine colonic 'non-cancer' tissue, when stimulated with recombinant (r) IFN-γ. MOC1 cells incubated with both rIFN-γ and rTNF showed that NF-κB pathway was activated rather than apoptosis pathway. Furthermore, the activated NF-κB in MOC1 cells was associated with the expression of myosin light chain kinase (MLCK) as well as disrupted tight junction (TJ) in a rTNF dose-dependent manner. Such MLCK up-regulation and TJ disruption in MOC1 cells were abrogated by either anti-TNF mAb (MP6-XT22), TNFR2-specific siRNA or even MLCK inhibitor (ML-7). Using an animal model of CAC involving azoxymethane (AOM) and dextran sodium sulfate (DSS), the colonic lamina propria was found to have pro-tumorigenic cytokine production such as IL-1β, IL-6 and MIP-2 in association with epithelial NF-κB activation, TNFR2 and MLCK up-regulations and epithelial TJ disruption. AOM and DSS-administered mice with either MP6-XT22 or ML-7 treatment failed to show significant abrogation of colitis severity, however such treatments revealed the restored epithelial TJ and decreased pro-tumorigenic cytokine production in the colonic tissues in association with the reduced CAC development. **Conclusions:** Our studies showed that epithelial NF-κB activation via TNFR2 signaling in the context of IBD may be involved in the epithelial permeabilization and pro-tumorigenic cytokine production that result in the induction of CAC development.

Mo1720

IFN-γ Counteracts the Angiogenic Switch and Induces Vascular Permeability in DSS Colitis in Mice

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Background: Interferon (IFN)-γ is a central pathogenesis factor in inflammatory bowel disease (IBD) with pleiotropic effects on many different cell types. However, as yet, the immune modulatory functions of IFN-γ in IBD have been predominantly investigated. Based on previous studies showing that IFN-γ acts anti-angiogenic in colorectal carcinoma, we investigated the effects of IFN-γ on the vascular system in IBD. **Methods:** Colon tissues of IBD patients and dextran sulfate sodium (DSS)-induced colitis in C56/BL6 mice were subjected to immunohistochemistry, quantitative real time-PCRs and in situ hybridization in order to quantify cell activation, angiogenesis and immune responses. Vascular structure and permeability in mice were analyzed by ultramicroscopy and *in vivo* confocal laser endomicroscopy. For cell culture experiments, primary mouse intestinal endothelial cells or HUVEC were used. **Results:** We showed a significantly increased blood vessel density in IBD and DSS colitis. In mice, this was associated with a disorganized blood vessel structure and profound vascular leakage. As compared to genes associated with angiogenesis, genes associated with inflammatory cell activation including IFN-γ were more strongly up-regulated in colitis tissues. IFN-γ exerted direct effects on endothelial cells in IBD tissues in vivo, as demonstrated via the expression of IFN-γ-induced guanylate binding protein-1 (GBP-1). Stimulation of cultured primary murine intestinal endothelial cells with IFN-γ inhibited cell proliferation and induced massive remodeling of the actin cytoskeleton. In line with this, neutralization of IFN-γ in the acute DSS colitis model demonstrated that this cytokine exerts endogenous angiostatic activity in IBD and contributes to increased vascular permeability. **Conclusions:** The dissection of the pleiotropic activities of IFN-γ in IBD provides new insights to the pathological functions of this cytokine and may be of high relevance for the optimization of combination therapy approaches.

Mo1721

Methylation and Expression of the Genes That Have a Role in the Wnt Signaling Pathway in Ulcerative Colitis

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Introduction: Ulcerative colitis is a chronically inflammatory disease of colon. Wnt signaling regulates intestinal epithelial stem cell function. Secreted Frizzled-related protein (SFRP) and Dickkopf families inhibit Wnt signaling. A recent study has been contributed to the accumulating evidence that the Wnt pathway also plays a distinct role in inflammation and immunity. Our aim is to investigate the methylation and expression status of WNT signaling pathway genes in ulcerative colitis. **Methods:** Patient group composed of 20 people diagnosed with left sided ulcerative colitis and having surveillance colonoscopy, control group composed of 15 people having colon cancer screening, not having any complaints or endoscopic pathology. DNA and RNA are obtained from the biopsies inflamed and non-inflamed mucosa of the patient and control groups. SFRP2, SFRP4, SFRP5, APC1, APC2 and ACTB gene expressions are determined by Real-Time PCR and "Comparative CT (..Ct)" analysis. The methylation status of the genes are studied by using methylation specific PCR. **Results:** For APC2 gene methylation; in patient group 8 people are methylated (%40) and 12 are un-methylated (%60) whereas in the control group 1 is methylated (%6.7), 14 are un-methylated (93.3%) (p=0.018). In patient group, for SFRP5 gene, a significant relationship is observed between the methylation status and expressions (p=0.015). Regarding the other genes, no significant statistical relationship is observed among the methylation, expression and inflammation status between the patient and control groups. **Discussion / Conclusion:** In ulcerative colitis, it is suggested that the findings of the increase in the methylation of APC2, and the decrease in the expression due to the increase of SFRP5 methylation both have a relationship with inflammation. However, further studies are needed in order to suggest its relationship with ulcerative colitis.

Mo1722

The Role of Interleukin 36, a Novel IL-1 Family Cytokine, in Inflammatory Bowel Disease

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Background & Aims: IL-36α, IL-36β, and IL-36γ, which are collectively called IL-36 cytokines, are new IL-1 family cytokines. IL-36 cytokines has recently reported to play important roles as an inflammatory cytokine in chronic inflammatory disorders, such as psoriasis and rheumatoid arthritis. However, the role of IL-36 cytokines remains unknown in colitis. In the present study, we investigated the expression of IL-36 cytokines in the inflamed mucosa of patients with inflammatory bowel disease (IBD) and characterized the function of IL-36 cytokines. **Methods:** The expressions of IL-36 cytokines were examined by real-time PCR. The signal transductions of IL-36 cytokines were evaluated by using chemical inhibitors of MAPKs or JNK, and by siRNA system. The genes induced by IL-36 cytokines were screened by DNA microarray analysis. **Results:** The expressions of both IL-36α and IL-36γ, but not IL-36β, were enhanced in the inflamed mucosa from patients with IBD. IL-36α and IL-36γwere able to induce C-X-C chemokines and this was mediated by the activation of the transcriptional factors, NFκB and AP-1. IL-36α and γ recruited MyD88 adaptor proteins, including MyD88, TRAF6, IRAK1, and TAK1, followed by phosphorylations of MAPKs and JNK in colonic epithelial cells. **Conclusion:** These results suggested that the upregulation of chemokines by IL-36α and IL-36γwas mediated by the recruitment of MyD88 adaptor proteins followed by activating MAPK signaling leading to the activation of NFκB and AP-1. We propose that IL-36α and γplay as pro-inflammatory cytokines to induce C-X-C chemokines in the pathogenesis of inflammatory bowel disease and can be an attractive therapeutic target for IBD.

Mo1723

Role of CCL20-CCR6 Axis and MMP9 in the Mucosal Inflammation and Fibrosis in Inflammatory Bowel Diseases

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Background: Inflammatory bowel diseases (IBD) are characterized by the activation of the innate immune system. Recent data suggest the alteration of the CCL20-CCR6 axis in IBD. Faecal matrix metalloprotease 9 (MMP9) was lately described as a valuable non-invasive biomarker of the disease activity. Our aims were to investigate the pathogenetic role of the C-C Chemokine ligand 20 (CCL20), human beta defensin 2 (hBD2) - Chemokine receptor 6 (CCR6) axis in the inflamed, fibrotic and healthy areas of the mucosa of the patients suffering from UC (ulcerative colitis), CD (Crohn's disease) or IBS (irritable bowel disease), and to observe the MMP9 expression pattern in these samples. Methods: 76 patients in 2 endoscopic centres were recruited. (Healthy control: 29, CD: 25, UC: 14, IBS: 8) Mucosal biopsies were taken from the inflamed, fibrotic and healthy area of the colon and inflamed or healthy ileum. QPCR analysis was performed to determine the CCL20 and MMP9 and hBD2 mRNA expression. Immunohistochemistry was performed from frozen colon samples of active ulcered CD or UC and inactive fibrotic CD or UC mucosal samples to analyze CCR6 receptor protein expression. Results: In healthy patients, ileal and colonic mRNA expression of CCL20 was not significantly different; hBD2 mRNA was not detectable; MMP9 mRNA was significantly (p= 0,0018) more expressed in the ileum compared to the colon. In ileal samples both CCL20 and MMP9 were significantly more expressed in inflamed CD compared to the healthy individuals. There was no change in the colonic CCL20, hBD2 or MMP9 mRNA expression in the IBS patients compared to the control group. In CD or UC significantly higher mRNA expression of CCL20 and MMP9 were observed in inflamed mucosa than in macroscopically intact areas. The CCL20 expression was more prominent in the fibrotic zones than in the active ulcers, conversely, MMP9 mRNA expression was less intense in the fibrotic zones. HBD2 expression was just detectable in certain samples from acute mucosal inflammation and fibrotic areas. Regarding to CCR6 (the only and specific receptor of CCL20 and hBD-2) we found unique apical expression in the cryptal mucosal