for 5 days with or without metformin. Immunohistochemical staining for phospho-IkB kinase (IKK) was performed in mouse colon tissue. In the colitis-associated tumor model, mice were given a single intraperitoneal injection of azoxymethane, and then three cycles of 2% DSS for 5 days and 2 weeks of free water consumption.

**Results:** Metformin significantly inhibited activated NF-κB signals and the upregulated expression of IL-8 in COLO 205 cells stimulated with TNF-α. Pretreatment with metformin attenuated IkB phosphorylation and NF-κB DNA binding activity induced by TNF-α. In an acute colitis model, administration of metformin significantly reduced the severity of DSS-induced murine colitis, as assessed by the disease activity index, colon length, and histopathology. Immunohistochemical analysis showed that the DSS-induced phospho-IKK activation in IEC was significantly decreased in metformin-treated mice. Finally, metformin significantly reduced the development of colitic cancer in mice.

**Conclusions:** Metformin inhibits NF-κB activation in IEC and ameliorates DSS-induced acute murine colitis and colitis-associated tumorigenesis, which suggest that metformin is a potential therapeutic agent for the inflammatory bowel disease.

**P048**

Metallothionein, an emerging danger signal during experimental colitis

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**Background:** Danger signals have been postulated as regulators of gut mucosal immunity. During intestinal inflammation, the epithelium is compromised and signals, alerting adjacent cells of tissue damage, are released. Hence, we were interested in metallothioneins (MTs), small proteins which have been identified at inflammation sites. We explored triggers releasing MTs from colon epithelial cells and identified its role as extracellular danger signal during experimental colitis.

**Methods:** HT29 cells were subjected to the following treatments: 200 ng/ml LPS, 200 microM H2O2, hypoxia (-1% oxygen), 100 ng/ml TNF-alpha + 300 ng/ml IFN-gamma to induce apoptosis, and repeated freeze/thaw cycles to mimic necrosis. Supernatant was analysed for MT levels using western blot and for lactate dehydrogenase activity (LDH). A Boyden chamber was used to explore chemotactic potential of extracellular MT and the capacity of monoclonal anti-MT antibody (100 μg/ml UC1MT) to abolish this. The role of MT as chemo-attractant was further explored using dextran sulphate sodium (DSS)-induced colitis in MT knockout (MT-/-), transgenic (MT+/+) and wild type mice (WT). The therapeutic use of monoclonal therapy was tested in DSS- and 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis. Inflammatory cell infiltrate was evaluated in all experiments together with standard inflammation markers.

**Results:** Necrosis and TNF-induced apoptosis resulted in detectable MT levels in supernatant of HT29 cells, which was not the case for LPS, H2O2 or hypoxia treatment. LDH activity was not increased after stimulation with TNF, ruling out an uncontrolled release of MT from TNF-treated cells. Increased leukocyte migration towards this MT-containing supernatant was detected, whereas the addition of UC1MT was able to overcome this chemo-attraction (p < 0.05). Significantly less neutrophil infiltration was observed in MT-/- mice compared to MT+/+ and WT mice in DSS colitis (p < 0.05). Complementary, i.p. UC1MT treatment reduced the amount of F4/80-positive macrophages in DSS- and TNBS-induced colitis (p < 0.05). Less inflammatory infiltrate was associated with reduced histological inflammation in all three colitis experiments.

**Conclusions:** We characterized metallothionein as a danger signal released from HT29 cells after necrotic and TNF-induced apoptotic cell death. Inhibiting MT function by monoclonal therapy reduces leukocyte infiltration and represents a novel therapy dampening experimental colitis.

**P049**

Longitudinal assessment of epithelial and immune cell changes following ileostomy closure in patients with ulcerative colitis

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**Background:** The interactions between microbiota, epithelial barrier and innate immune responses are important in the pathogenesis of IBD. The ileo-anal pouch offers a unique opportunity to study these inter-relationships before the onset of disease. There are few data regarding tight junction expression or dendritic cell (DC) characterisitcs following restorative proctocolectomy (RPC) for ulcerative colitis (UC). We aimed to assess the relationship between changes in epithelial tight junction expression, dendritic cell phenotype and mucosal cytokine production over the first year following RPC for UC.

**Methods:** Mucosal biopsy samples were taken from UC patients undergoing RPC, from the ileostomy afferent loop, the pouch pre-ileostomy closure (P0) and the pouch 6 and 12 months post-ileostomy closure (n = 5). Epithelial cells and lamina propria DC were isolated from biopsy tissue. Epithelial cells were identified as pancytokeratin positive and DC were identified as an HLA DR+, lineage (CD3-, CD14-, CD16-, CD19+, CD34+) population. Epithelial cell expression of zona occludens (ZO)-1, claudin 1 and claudin 2 and DC expression of TLR 2 and 4, CCR9, β7 and CD40 were measured by multicolour flow cytometry. Cytokines were assessed by multiplex ELISA of biopsy supernatants. The paired t-test was used for statistical analysis.

**Results:** Epithelial expression of claudin 2 was increased (p = 0.04) at 6 months and remained elevated at 12 months. No changes were seen in ZO-1 or claudin 1 expression. There was a significant increase in β7 expression on lamina propria DC (p = 0.02), but no differences in DC TLR or CD40 expression were seen at 6 months. DC expression of β7 was further elevated (p = 0.005) as well as significantly increased TLR 4 and CD40 expression (p = 0.04). No cytokines were found to be elevated at 6 months, but at 12 months there was a trend towards increased IL6 (p = 0.05) and IL10 (p = 0.06).

**Conclusions:** In patients with UC, altered tight junction expression with increased epithelial expression of the "pore-forming" tight junction claudin 2 was an early event after ileostomy closure that preceded the onset of mucosal inflammation. In parallel, more lamina propria DC expressed gut homing markers possibly in response to increased exposure to the changing microbial signals and a more permeable epithelial barrier. These changes in parallel may lead to increased microbial stimulation of DC with increased TLR and co-stimulatory molecule expression that could predispose to the development of inflammation.