sion of Paneth cell α-defensins. This link provides a new mechanism for small intestinal CD pathogenesis. Wnt signaling promotes expression of several factors inhibiting the pathway e.g. DKK-1, Axin2. Regarding those embedded negative feedback mechanisms, we hypothesized an influence on Tcf-4 gene expression via Wnt signaling. We aimed to investigate possible Tcf-4 auto-regulation.

Methods: Potential Tcf-4 binding sites (WWC2A4WG) were analyzed by gel shift, using recombinant Tcf-4 peptide in vitro. Transient transfection of HEK293 cells and luciferase assay experiments were performed to study the promoter activity. 2 different length of Tcf-4 reporter constructs were cotransfected: pGL-[1900] Tcf-4Luc and pGL-[1306] Tcf-4Luc together with expression vectors: Full-length Tcf-4 or α and constitutively active form of β-catenin. Additionally chemical activation of the Wnt pathway, via 20μM GSK3 inhibitor Li was performed. TopFlash reporter plasmid, an indicator of Wnt pathway activity, was transfected as a positive control. Finally the transcriptional activity of related reporter constructs was quantified by luciferase assay.

Results: Among the 3kb 5’ flanking upstream region of Tcf-4, 8 potential Tcf-4 binding sites were identified. Gel shift experiments identified the binding site closest to the transcription start (+127 to +121) has the highest binding activity for Tcf-4 in vitro. Obtained data indicated that over expression of Tcf-4 alone can not activate the Wnt pathway. As expected Tcf-4 serves as an inhibitor both for itself and TopFlash transactivation activity in this inactivated status. After Wnt pathway activation via co-transfection of β-catenin with Tcf-4 or stimulation with LiCl, the transcriptional activity of Tcf-4 was further decreased while the TopFlash activity highly increased (about 20-fold), hinting towards a Tcf-4 negative-feed back under Wnt signaling.

Conclusion: Multiple Tcf-4 binding sites exist in the promoter region of Tcf-4. A negative auto-regulatory loop for Tcf-4 gene regulation and its influence on Wnt pathway activity. This novel negative feedback of Tcf-4 and its target genes might be an important mechanism in balancing the pathway function and therefore interesting regarding the pathogenesis on ileal CD

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THE ENDOCANABINOID SYSTEM IN INFLAMMATORY BOWL DISEASE
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Aim: Activation of cannabinoid receptors 1 (CB1) and 2 (CB2) by endocannabinoids impacts on a number of gastrointestinal functions. Recent data indicate that treatment of mice with a CB1 receptor agonist reduces LPS-induced colitis, thus suggesting a role for the endogenous cannabinoid ag-

Materials and methods: In biopsy specimens from inflamed colonic mucosa of 20 patients with active Crohn’s disease or ulcerative colitis and from normal mucosa of 15 controls, endocannabinoid levels were determined by high performance liquid chromatography-tandem mass spectrometry. Activity of FAAH and NAAA was assessed by a radiolochromatographic method. Biopsies or anti-C3D/CD28-stimulated lamina propia mononuclear cells were cultured for 48h with increasing concentrations of Δ(9,11) -THC in the presence or absence of CB1 and CB2 receptor antagonists. Released cytokines, i.e. interferon (IFN)-γ and tumor necrosis factor (TNF)-α, were measured in cell supernatants by ELISA. Results: Anandamide and PEA, but not 2-AG, were significantly decreased in IBD inflamed mucosa. De-

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AN ANTIBODY TO SCLEROSTIN INHIBITS AND REVERSES INFLAMMATION INDUCED BONE LOSS
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Low bone mineral density (BMD) has been noted in many inflammatory bowel disease (IBD) patients with around 30% developing osteoporosis (Lichtenstein et al., 2003, Ann J Gastroenterol, 98, 524-530). The mechanism for reduced BMD in IBD patients is unclear but direct links have been postulated between demineralisation and the inflammatory process (Bjarnason et al., 1997, Gut, 40, 226-233). The CD4+ CD58Rhi T cell transfer model of colitis is a well established animal model of IBD and association with osteopenia has recently been demonstrated by Byrne et al. (2005, Gut, 54, 78-86) with inflammatory mechanisms being cited as the most likely cause for this low BMD. Sclerostin (scl.) is a negative regulator of bone formation, antagonizing the wnt sig-

Patients and Methods: The study population included 95 patients, 15 of them and CB2 receptors plays a crucial role in dampening the Th1-mediated im-

Poster Presentations