**IBD: PRECLINICAL**
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### T.65. Reversal of Established Colitis by Adoptive Transfer of *ex vivo*-generated, Gut-homing Regulatory T Cells

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Naturally-occurring regulatory T cells (nTregs; CD4+Foxp3+) are known to suppress the development of chronic gut inflammation *in vivo*. A major limitation in defining the molecular and cellular mechanisms by which nTregs exert their suppressive activity is the relative paucity of these regulatory T cells. We have developed a relatively simple and rapid *ex vivo* method to generate large numbers of Foxp3-expressing Tregs that can be used to evaluate their therapeutic efficacy in mouse model chronic colitis. We have found that polyclonal activation of naïve/conventional CD4+CD25- Foxp3- in the presence of small amounts of IL-2, TGFβ and all trans retinoic acid (RA) induces >90–95% conversion of these conventional T cells to Foxp3-expressing Tregs as well as a 5-fold increase in proliferation following a 4 day incubation period *in vitro*. This protocol generates as many as 30–40 million "induced" Tregs (iTregs) from one mouse spleen. Furthermore, we observe enhanced surface expression of the gut-homing adhesion molecule α4β7 suggesting tissue-specific imprinting of these iTregs by this conversion/expansion protocol. Finally, we demonstrate that these iTregs are significantly more potent at suppressing T cell activation *in vitro* and are as effective (or more effective) than nTregs at reversing established colitis *in vivo*. We conclude that this protocol generates sufficient numbers of iTregs that can be used *in vitro* and *in vivo* to assess the therapeutic efficacy and immunological mechanisms responsible for suppression of inflammation in different models of autoimmune disease (Supported by PO143785).

### T.66. Resolvin E1, an Endogenous Lipid Mediator Derived from Eicosapentaenoic Acid, Prevents Dextran Sulfate Sodium Induced Colitis

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Background: Resolvin E1 (RvE1), an endogenous lipid mediator derived from eicosapentaenoic acid (EPA) has been identified in local inflammation during the healing stage. RvE1 reduces inflammation in several types of animal models including peritonitis and retinopathy, and blocks human neutrophil transendothelial cell migration. The RvE1 receptor ChemR23 is expressed on myeloid cells such as macrophages and dendritic cells. The aim of this study was to determine whether RvE1 regulates colonic inflammation when the innate immune response of macrophages plays a key role in the pathogenesis and tissue damage. Methods/Results: RvE1 receptor, ChemR23, was expressed in mouse peritoneal macrophages as defined by flow cytometry. Peritoneal macrophages were pretreated with RvE1, followed by lipopolysaccharide (LPS) stimulation whereupon of the transcriptional levels of proinflammatory cytokines were analyzed. RvE1 treatment led to the inhibition of proinflammatory cytokines including TNF-α and IL-12p40. In HEK293 cells, pretreatment with RvE1 inhibited TNF-α-induced nuclear translocation of NF-κB in a ChemR23 dependent manner. These results suggested that RvE1 could regulate pro-inflammatory responses of macrophages expressing ChemR23. Therefore, we investigated the beneficial effects of RvE1 in dextran sulfate sodium (DSS) induced colitis. RvE1 treatment led to amelioration of colonic inflammation. Conclusions: These results indicate that RvE1 suppresses pro-inflammatory responses of macrophages. RvE1 and its receptor may therefore be useful as therapeutic targets in the treatment of human inflammatory bowel disease (IBD) and other inflammatory disorders.

### T.67. Targeting Gut T cell Ca2+ Release-activated Ca2+ Channels Inhibits Th1 Cytokine Production and T-box Transcription Factor T-bet in Inflammatory Bowel Disease

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Background and Aims: Ca2+ entry through Ca2+ release-activated Ca2+ channels (CRAC) up-regulates nuclear factor of activated T cells, a transcription factor promoting T cell proliferation and cytokine production. Inflammatory bowel diseases (IBD) are characterised by increased number and activation of gut T cells. Here we evaluate the *in vitro* effect of a CRAC inhibitor on pro-inflammatory cytokine response in IBD. Methods: CRAC blocker selectivity and specificity was investigated by patch-clamp experiments on rat basophilic leukaemia (RBL) cells and fluorometric-imaging-plate-reader intracellular Ca2+ assays on thapsigargin-stimulated Jurkat T cells. Anti-CD3/CD28-stimulated lamina propria mononuclear cells (LPMCs) and biopsies from 40 IBD patients were cultured with increasing concentrations of the CRAC inhibitor GSK1349571A (0.01–10 microM). Interferon (IFN)-gamma, interleukin (IL)-2, IL-8, IL-17 were analysed by ELISA. T-bet was determined by immunoblotting. T cell activation genes were also analysed by microarray. Results: GSK1349571A dose-dependently inhibited CRAC current in RBL and Jurkat T cells, and down-regulated the expression of a number of T cell activation genes. GSK1349571A also dose-dependently reduced T-bet expression and production of IFN-gamma, IL-2, IL-17, but not IL-8, in IBD LPMCs and biopsies. Conclusions. The suppression of activated Ca2+ channels potently dampens mucosal immune responses in the

inflamed gut, indicating CRAC as a promising therapeutic target in IBD.

### T.68. Rapid Migration of Thymic Emigrants to the Colon in Ulcerative Colitis Patients

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Rapid migration of thymic emigrants to the colon in Ulcerative colitis patients

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The output of naïve T cells from the thymus was estimated by analyses of T cell Receptor Excision Circles (TRECs) in peripheral blood lymphocytes, intraepithelial lymphocytes (IELs) and lamina propria lymphocytes (LPLs) from both colon and small intestine of Inflammatory Bowel Disease (IBD) patients, compared to healthy volunteers and colon cancer patients. Patients with IBD displayed similar levels of TRECs in the peripheral blood compared to healthy volunteers. Sorted integrin $\alpha_4\beta_7^+$ peripheral blood lymphocytes demonstrated decreased TRECs levels in IBD patients compared to healthy controls, albeit not statistically significant. In strong contrast to peripheral blood, increased levels of TRECs were found in both IELs and LPLs from the colon from patients with active and inactive ulcerative colitis compared to colonic IELs and LPLs from colon cancer or Crohn´s disease patients. This could not be explained by increased extrathymic maturation of T lymphocytes *in situ* in the intestinal mucosa, as judged by unaltered frequencies of $CD16^-CD19^-CD3^+CD5^+CD7^+$ T cell precursors and undetectable levels of RAG1 and pTCRα transcripts in intestinal lymphocytes from IBD patients. Thus, our results demonstrate a marked migration of newly produced T lymphocytes from the thymus straight into the colonic mucosa of ulcerative colitis patients.

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