1 Colonic mucosal expression of barrier genes in patients with inflammatory bowel disease before and after first infliximab treatment

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Introduction and Aim: Intestinal epithelial barrier function is impaired in inflammatory bowel disease (IBD), but it remains unclear whether this dysfunction is a primary event of IBD or a consequence of mucosal inflammation. This study investigated the impact of anti-inflammatory therapy with infliximab on the colonic mucosal expression of genes related to intestinal epithelial barrier function in IBD patients using oligonucleotide arrays.

Methods: The expression of 121 genes related to intestinal epithelial barrier function, including IBD candidate susceptibility genes, mucins, trefoils, E-cadherin, genes involved in the apical junction complex function, were investigated in colonic mucosal biopsies obtained at endoscopy from 43 IBD patients with active colitis (24 ulcerative colitis (UC) and 19 Crohn’s disease (CD)) before and 4.6 weeks after first infliximab infusion. The patients were classified for response to infliximab based on endoscopic and histologic findings: 20 responders (12 CD and 8 UC) and 23 non-responders (7 CD and 16 UC). 6 control patients undergoing colonoscopy for screening were included. Total RNA was isolated, labelled and hybridized to Affymetrix Human Genome U133 Plus 2.0 Array. Array data was analyzed using Bioconductor software. Moderated t-statistics were used for comparative data analysis. A false discovery rate <5% combined with a >2 fold-change were considered statistically significant.

Results: There was no significant difference between UC and CD at baseline. In UC and CD, the mRNA expression levels of MUC1, MUC4, MUC5B, MCAM, TFF1, CLDN1, CLDN2, JAM2 and TLR4 were all upregulated at baseline compared to controls, while the mRNA expression levels of MUC20, CLDN23, CLDN8, MPP5, MPP7, CDH1, SLCl2A4, SLC22A5, ABCB1 and PDZD3 were all downregulated at baseline compared to controls. In responders to anti-TNFalpha treatment, the expression levels of TFF1, TFF2, CLDN2, CLDN8, SORBS1, ABCB1 and DSG3 in UC and CLDN1 in UC and CD normalized after treatment. In CD (not in UC) responders, MUC1 and MUC4 mRNA expression remained increased and CXADR and SLC22A5 mRNA expression remained decreased after complete healing in comparison with controls.

Conclusion: Our data demonstrate that the expression of many barrier genes were dysregulated in the colon of active IBD. After controlling for inflammation with infliximab therapy, the expression of most of these genes was restored in UC, while in CD the expression of some barrier genes remained dysregulated. We do not find arguments for a primary defect in barrier in UC whereas in Crohn’s disease some barrier defects persist in healed colonic mucosa.

2 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: Prolonged Ca2+ entry through Ca2+-sensitive channel, Ca2+-sensitive transcription factor, nuclear factor of activated T cells, responsible for directing T cell proliferation and cytokine gene expression. To establish whether targeting CRAC might counteract intestinal inflammation, we evaluated the in vitro effect of a selective CRAC inhibitor on Th1 cytokine production and T-bet expression by lamina propria mononuclear cells (LPMCs) and biopsies from inflammatory bowel disease (IBD) patients.

Material and Methods: The selective inhibitory activity of the CRAC blocker was investigated through patch-clamp experiments on rat basophilic leukaemia (RBL) cells and fluorometric imaging plate reader intracellular Ca2+ assays using thapsigargin-stimulated Jurkat T cells using thapsigargin-stimulated Jurkat T cells. LPMCs stimulated with anti-CD3/CD28 antibodies and biopsies from 40 IBD patients were cultured with a range of CRAC inhibitor concentrations (0.01–10 μM). IFN-γ, IL-2, IL-8, and IL-17 were analysed by ELISA. T-bet was determined by immunoblotting. T cell activation genes were analysed by microarray.

Results: We found that the CRAC blocker dose-dependently inhibited CRAC current in RBL cells and thapsigargin-induced Ca2+ influx in Jurkat T cells, and down-regulated the expression of a large number of genes associated with T cell activation. A dose-dependent reduction in T-bet expression and production of IFN-γ, IL-2, IL-17, but not IL-8, was observed in IBD LPMCs and biopsies treated with the CRAC inhibitor.

Conclusions: We here provide evidence that the suppression of CRAC channel function may dampen the increased Th1 response in the inflamed gut, thus suggesting a promising role for CRAC inhibitor drugs in the therapeutic management of IBD patients.

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