levels. LILRB1 mRNA was highly expressed in intestinal-like macrophages, when compared with classically differentiated macrophages or peripheral blood monocytes. Conversely, LILRA5 mRNA expression was lower in cultured cells than blood monocytes, without a significant difference between classically differentiated and intestinal-like macrophages. Stimulation of classically differentiated macrophages with LPS, IFN-γ or IL-10 resulted in higher levels of LILRB1, but not of LILRA5. LILRB1 expression, however, was not increased in intestinal-like macrophages in response to cell stimulation.

Conclusions: Using colonic stromal derived conditioned media, a model of intestinal-like macrophages has been developed. These cells demonstrate a blunted TNF-α responsiveness to LPS stimulation, suggesting that colonic stromal factors are likely to hold a key to the immune tolerant state of the intestine. Of importance is the over-expression of LILRB1 in this intestinal macrophage model that correlates with its presence in colonic lamina propria macrophages. This immunoregulatory molecule is also more prevalent in inflammatory bowel disease, and our colonic macrophage model enables further determination of its role, whether protective or pro-inflammatory, in this condition.

P027 Proteomic analysis of serum and tissue proteins in patients with intestinal Behcet’s disease by MALDI-TOF/TOF mass spectrometry

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Background: Although intestinal Behcet’s disease (BD) has an unpredictable disease course with exacerbations and remission like inflammatory bowel disease (IBD), data concerning diagnosis, treatment, and prognosis are yet to be determined and the pathogenesis of intestinal BD are poorly understood. Therefore, we aimed to investigate the differentially expressed proteins both in serum and tissue from patients with intestinal BD and search for biomarkers associated with disease pathogenesis using proteomics analysis.

Methods: Serum samples from 15 intestinal BD, systemic BD, and normal control were pooled and tissues were obtained from surgical specimens of intestinal BD patients who underwent surgery due to disease exacerbation. Two-dimensional electrophoresis (2-DE) technology was performed to characterize the total proteins of serum and tissues from intestinal BD patients. Candidate protein spots were identified using matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry (MALDI-TOF/TOF-MS) and bioinformatics analysis. Enzyme-linked immunosorbent assay (ELISA) was applied to validate the results of 2-DE and MS.

Results: Proteomic profiles of serum pooled samples were compared, and approximately 400 protein spots were observed in intestinal BD patients. Mass spectrometric analysis identified that expression levels of 3 of the protein spots in intestinal BD were significantly higher than those in the normal control and systemic BD, including serum amyloid A, apolipoprotein A-IV, and fibrin. ELISA revealed that serum amyloid A was overexpressed in intestinal BD (median 12.17 ng/ml, range 5.67–38.67) but not in systemic BD (median 10.57 ng/ml, range 6.15–58.34) but not in systemic BD (median 10.57 ng/ml, range 5.67–38.67). The 2-DE protein expression profile of 575 protein spots from the intestinal BD tissues also shows a definite difference from that of the normal controls. Seven individual protein spots were identified, of which 4 proteins were up-regulated including heat shock protein 27, transgelin, manganese superoxide dismutase, and calprotectin, and 3 were down-regulated including heat shock protein 60, selenium binding protein, and galectin-3.

Conclusions: A distinct proteomic profile of serum and tissue in intestinal BD patients was found that 7 up-regulated and 3 down-regulated proteins were identified. Our data might be helpful for understanding the pathogenesis of intestinal BD and providing potential biomarkers for the early diagnosis and treatment of intestinal BD.

P028 Protein kinase D1 mediates CREB activation and increased SOCS3 expression induced by PGE2 in colon epithelial cells

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Background: Ulcerative colitis (UC) is a chronic disease associated with long periods of quiescent disease followed by fulminant exacerbation. Imminent relapse in UC is associated with high mucosal expression of suppressor of cytokine signaling 3 (SOCS3). Hence, knowing mechanisms underlying mucosal SOCS3 expression may provide important clues as to rational therapy for ulcerative colitis. However, the molecular mechanisms that regulate SOCS3 expression and signal transduction pathway remain poorly understood. Here we report that protein kinase D (PKD), a newly described serine/threonine kinase that has been implicated in inflammatory response, contributes to CREB signaling and increased SOCS3 expression in the colonic epithelial cells, activation of PKD1 induced by pro-inflammatory cytokine prostaglandin E2 activates CREB phosphorylation and up-regulates SOCS3 expression.

Methods: The colon epithelial cell line Caco-2 & HT-29 and the intestine cell line IEC-6 were stimulated with interleukin (IL)-6 or prostaglandin E2 (PGE2) to allow correlations between SOCS3 expression with signal transducer and activator of transcription 3 (STAT3), and adenosine 3’,5’-cyclic monophosphate (cAMP) signaling, respectively, and the phosphorylation of PKD and downstream signaling molecules were detected by Western blot. We also transfected the plasmids and the small interference RNA of PKD1 into Caco-2 & HT-29 to evaluate its effect on related molecular phosphorylation and SOCS3 expression with PGE2 treatment. Immunofluorescence and Co-immunoprecipitation were made to prove the relevance between PKD1 and cAMP response element-binding protein (CREB).

Results: Overexpression of wild type or constitutive active mutant of PKD1 increased SOCS3 expression, whereas overexpression of dominant negative of PKD1 significantly reduced SOCS3 expression in the colonic epithelial cells. Furthermore, Knockdown of PKD1 by siRNA transfection or specific PKD inhibitor KB-NB142–70 treatment remarkably inhibited CREB (ser133) phosphorylation and SOCS3 expression induced by PGE2 but not IL-6 in the colon epithelial cell line. There is no effect of siRNA transfection on PKA and STAT3 phosphorylation with or without PGE2 treatment. In addition, ser744/748 phosphorylation of PKD1 and interaction of PKD1 with CREB were also enhanced with PGE2 treatment, and PKD1 activation induced by PGE2 promoted phosphorylated CREB translocation into nucleus and subsequently upregulated SOCS3 expression.

Conclusions: Our data suggest that PKD1 may contribute to inflammatory response of ulcerative colitis through CREB phosphorylation and increased SOCS3 expression, which may be a potential target for anti ulcerative colitis.