been proposed for this: induction of IL-13Rα2 expression on the surface membrane by signalling of IL-13 through the IL-13Rα1 receptor in the presence of TNF-α, followed by IL-13 signalling through IL-13Rα2. Signalling via this route in macrophages induced production of TGF-β, potentially leading to collagen synthesis and fibrosis. Therefore, the aim of this study is to determine whether this two-step process occurs in human intestine and particularly if it occurs in collagen-producing intestinal fibroblasts.

Using intestinal tissue taken from patients with CD, ulcerative colitis (UC) or cancer, the preliminary immunohistology data suggests that both IL-13 receptors are expressed in the intestinal muscle and mucosa of all patients, and appear to be co-expressed on the same cells which, phenotypically, are stromal or epithelial cells. There seems to be little difference in either the number of IL-13Rα2 cells present, or the appearance of the cells, between cancer and UC tissue. However, in the muscle layer of fibrotic areas of CD tissue, IL-13Rα1/IL-13Rα2 double positive cells appear to be enlarged. Fibroblast lines generated from all tissue samples retain the expression of IL-13Rα1 but Ro2 expression was variable, as determined by immunofluorescence. These receptors are able to signal since induction of phosphorylated stat 6 was detected by Western blotting in cell lysates generated from cell lines treated with IL-13, with maximum stimulation at 60 minutes, while there was no change in the level of total stat 6 over the same period. Initial studies in cell lines treated with IL-13 indicate increased collagen synthesis and decreased TIMP-1 and TIMP-2, as detected by ELISA on cell culture supernatants. Up-regulation of IL-13Rα2 was observed in cell lines treated with IL-13 and TNF-α, in comparison with untreated cells, quantified by Western blotting. In summary, our data are consistent with the hypothesis that signalling through IL-13 receptors in mesenchymal cells contributes to the fibrotic process in CD. We propose, therefore, that IL-13 and its receptors may be considered as targets for future therapy.

P272 Abnormal TLR4-mediated interferon response in ulcerative colitis
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Introduction: Although the aetiology of ulcerative colitis (UC) or cancer, the preliminary immunohistology data suggests that both IL-13 receptors are expressed in the intestinal muscle and mucosa of all patients, and appear to be co-expressed on the same cells which, phenotypically, are stromal or epithelial cells. There seems to be little difference in either the number of IL-13Rα2 cells present, or the appearance of the cells, between cancer and UC tissue. However, in the muscle layer of fibrotic areas of CD tissue, IL-13Rα1/IL-13Rα2 double positive cells appear to be enlarged. Fibroblast lines generated from all tissue samples retain the expression of IL-13Rα1 but Ro2 expression was variable, as determined by immunofluorescence. These receptors are able to signal since induction of phosphorylated stat 6 was detected by Western blotting in cell lysates generated from cell lines treated with IL-13, with maximum stimulation at 60 minutes, while there was no change in the level of total stat 6 over the same period. Initial studies in cell lines treated with IL-13 indicate increased collagen synthesis and decreased TIMP-1 and TIMP-2, as detected by ELISA on cell culture supernatants. Up-regulation of IL-13Rα2 was observed in cell lines treated with IL-13 and TNF-α, in comparison with untreated cells, quantified by Western blotting. In summary, our data are consistent with the hypothesis that signalling through IL-13 receptors in mesenchymal cells contributes to the fibrotic process in CD. We propose, therefore, that IL-13 and its receptors may be considered as targets for future therapy.

Results: Serum IP-10 levels were significantly elevated 48 (2.5-fold, p < 0.01) and 72 (8.6-fold, p < 0.05) hours after HkEc injection in UC, the timepoints when blood flow was raised in these patients [1]. Analysis of TLR pathway-associated gene expression following HkEc stimulation showed that macrophages from patients with UC significantly over-express the interferon (IFN) family-related genes: IFN-γ, IFN-α (both 6-fold, p < 0.02), CD80 (8-fold, p < 0.01) and CD86 (4.5-fold, p < 0.05) (T cell co-stimulatory molecules). After stimulation with HkEc (p < 0.01) and LPS (p < 0.001), but not Pam3-CSK4 and Flagellin, macrophage IP-10 secretion was significantly elevated in UC.

Conclusions: Increasing evidence highlights the importance of IP-10, a potent T cell chemoattractant, in the pathogenesis of UC. Raised levels have been reported locally [2] and systemically [3] in patients with active disease and its inhibition has been shown to ameliorate inflammation in mouse models of colitis [4]. Here we demonstrate that abnormally elevated IP-10 levels in UC occur in response to bacterial stimulation. The underlying defect appears to lie at the level of the macrophage, and specifically relates to dysregulated TLR4 signalling. Over-expression of IFN family-related and T cell co-stimulatory genes during the acute inflammatory response may eventually in the T cell-mediated chronic inflammation characteristic of UC. To work to define the precise molecular defects underlying dysregulated TLR4 signalling in UC is ongoing and may offer novel therapeutic targets in the future.

Reference(s)

P273 Mannose-binding lectin (MBL) deficiency is associated with complicated Crohn’s disease in patients, and more severe colitis in mice
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Background: Mannose-binding lectin (MBL) represents a first line of defense mechanism against yeasts and certain bacteria. Even though we have observed an association of MBL-deficiency with anti-Saccharomyces cerevisiae antibodies (ASCA) in Crohn’s disease (CD), the role of MBL in the pathogenesis of CD is unclear. A possible correlation with distinct disease phenotypes as well as consequences of MBL-deficiency in experimental colitis have not been addressed so far.

Methods: Serum concentrations of functional MBL were measured by ELISA in 164 CD patients, 32 patients with ulcerative colitis (UC) and 51 healthy controls (HC). CD patients were grouped according to the Montreal classification. MBL was classified as deficient (<100 ng/mL), low (100–500 ng/mL) and normal-to-high (>500 ng/mL). MBL was further quantified in samples collected during endoscopy and from full thickness small bowel specimens. DSS administration was used to induce experimental colitis. Wild type and MBL-deficient mice were immunized with yeast to assess experimental ASCA generation.

Results: Low MBL levels were positively associated with complicated CD (P < 0.001), while negatively associated with pure inflammatory disease (P < 0.0001). MBL protein as well as mRNA was rarely detected and only at very low levels in intestinal specimens. Experimental ASCA had a prolonged half-life in MBL-deficient compared to wild type mice. Furthermore, MBL-deficient mice showed more severe DSS colitis especially when simultaneously challenged with an adhesive and invasive strain of E. coli, originally isolated from the ileum of a CD patient.

Conclusions: Serum MBL, but not locally produced MBL in the intestine, plays a role in CD phenotype. Sustained ASCA titers in MBL-deficient mice, together with the increased susceptibility to pathogen-amplified DSS colitis suggest, that prolonged persistence of invasive gut pathogens in the absence of MBL may play a role in intestinal inflammation.