PTU-114 EXPRESSION OF TOLL-LIKE RECEPTOR (TLR)-2 AND -4 IN THE INTESTINAL CRYPT EPITHELIAL CELLS IN INFLAMMATORY BOWEL DISEASE (IBD)

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Introduction TLRs are pattern recognition receptors which detect conserved molecular patterns on commensal and pathogenic bacteria. Studies in mice have shown that TLR signalling protects against the development of colitis but may exacerbate established colitis. Epithelial stem cells and their progeny are located in crypts of the small and large intestine. We have investigated TLR2 and TLR4 expression in ulcerative colitis (UC) and Crohn's disease (CD) crypt intestinal epithelial cells (IEC).

Methods Ileal and colonic mucosal samples were obtained from operation resection specimens and were histologically normal (>5 cm from cancer; normal controls) or inflamed (CD or UC). Additionally, paired samples from histologically normal (proximal colon) and inflamed (distal colon) mucosa were obtained from five colectomy specimens with isolated left-sided UC. Crypt IECs were isolated using EDTA and pancreatin. Expression of mRNA transcripts was studied by conventional and real-time RT-PCR. Cell surface TLR protein was studied by flow cytometry (expressed as median fluorescent intensity, MFI). TLR expression was also studied by immunofluorescent (IF) staining of tissue sections. Data are expressed as median (IQR).

Results Relative to normal controls, TLR2 mRNA expression in IEC was significantly elevated in inflamed colonic UC [3.18-fold (1.03–10.40), p=0.003] and inflamed colonic CD [3.45 (0.80–5.40), p=0.012]. TLR4 mRNA was significantly elevated in normal colonic UC [1.90 (1.69–4.31], p=0.017), inflamed colonic UC [2.33 (1.15–4.45), p=0.024], inflamed colonic CD [1.71 (1.00–4.32), p=0.042] and inflamed ileal CD [1.84 (1.43–4.66), p=0.03]. In paired analyses, IEC TLR2 and TLR4 mRNA expression was not significantly different in normal UC mucosa compared to inflamed UC mucosa. Compared to IEC from normal control colon, IEC from colonic CD showed significantly greater expression of cell surface TLR2 protein [MFI 10.1 (2.4–29.0) vs 33.1 (17.7–107.5), p=0.05] and TLR4 protein [MFI 12.1 (4.9–25.3) vs 40.9 (23.2–103.5), p=0.04]. IF staining confirmed TLR2 and TLR4 protein expression on the luminal surface of crypt IEC.

Conclusion IEC expression of TLR4 mRNA was up-regulated in inflamed and un-inflamed colonic and ileal IBD. TLR2 mRNA was up-regulated in inflamed colonic IBD. Interestingly, TLR2 and TLR4 mRNA expression was similar in IECs isolated from un-inflamed, histologically normal mucosa as it was in IECs isolated from inflamed mucosa in UC. This may suggest TLR2 and TLR4 up-regulation is a primary rather than secondary event in UC. Moreover, TLR2 and TLR4 protein was expressed in greater amounts on the surface of IECs in CD.

Competing interests None declared.

PTU-115 EFFICACY OF MYCOPHENOLATE MOFETIL THERAPY IN THE MANAGEMENT OF INFLAMMATORY BOWEL DISEASE

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Introduction The role of Mycophenolate mofetil (MMF) as an immunomodulatory drug in managing inflammatory bowel disease is yet to be fully defined. We reviewed our experience of the efficacy, safety and tolerability of MMF in treating patients with refractory inflammatory bowel disease.

Methods A retrospective analysis was performed of the case records of all patients treated with MMF for inflammatory bowel disease at our institution between 2003 and 2011. Remission was assessed by reviewing clinical, endoscopic and laboratory indices.

Results We identified 36 patients, 23 male (64%) with a median age 46 yrs (range 19–75). Disease was classified as Crohn's disease in 19, ulcerative colitis 16, indeterminate colitis.[1] 33 patients (92%) had previously received azathioprine; 32 discontinued this due to side-effects. Five patients had undergone surgery for small bowel Crohn's disease. The starting dose of MMF was between 500 mg and 2 g daily, titrated to a dose of 2 g daily as tolerated. 26 patients (72%) were concurrently taking oral corticosteroids, and 18 (50%) were taking an oral 5-aminosalicylate. At 8 weeks, 29 patients (81%) had either achieved acute remission or maintained previous remission. Drug side-effects were experienced by eight patients (22%)—these symptoms were managed successfully by dose reduction in six patients (75%), with discontinuation in two patients. There were no serious haematological or other adverse drug effects. After 6 months of treatment, 33 patients continued to take MMF of which 19 patients (58%) had achieved sustained steroid-free remission. Median length of MMF treatment observed was 21.5 months (IQR 9.7–31.6). At the end of the observation period, 29 patients (81%) remained on MMF. 13 patients (36% of the original treatment group; UC 7, Crohn's 5) remained in steroid-free remission with median time of remission 21.4 months (IQR 11.0–30.0). A further 13 patients achieved sustained remission with the addition of corticosteroids and/or anti-TNF therapy. Five patients (14% overall) were refractory to all medical therapy and underwent surgery: colectomy for UC 3, right hemicolectomy for Crohn's.[2]

Conclusion From our experience, MMF may represent a promising alternative treatment for inducing and maintaining remission in patients intolerant of or unable to receive thiopurines. It appears well tolerated with a good safety profile in thiopurine intolerant subjects.

Competing interests None declared.

PTU-116 EXPRESSION OF NLRP3 INFLAMMASOME PROTEINS IN NORMAL AND CROHN'S DISEASE COLON

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Introduction The pathogen recognition receptor, NLRP3, and its inflammasome are implicated in the innate immunity.[1] Both intracellular and extracellular stressors induce its activation including the bacterial protein muramyl dipeptide,[2] also recognised by NLRC2 (NOD2) which is known to be associated with Crohn's disease (CD). Little is known about expression of the NLRP3 inflammasome in normal gastrointestinal tract (GIT) or in CD, therefore the expression profile of four inflammasome proteins in the GIT has been investigated in this study.

Methods Paraffin embedded colonic tissue from healthy controls (HC) and CD patients were cut and fixed on slides. Protein antigens were exposed by pressure cooker heating in 0.01% antigen retrieval solution and water. Following application of a primary antibody (anti-NLRP3, anti-ASC, anti-pyrin, anti-caspase-1) the proteins were observed by washing in DAB/chromogen solution, following incubation with a Horseradish peroxidase-labelled secondary antibody. Degree of staining was assessed using a validated Quick score model[3] where intensity of staining is scored 0–3, and proportion of

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