increased rate of tumorigenesis in the colitic bowel, however analysis of a larger patient cohort is needed.

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Disclosure of Interest None Declared.

PWE-087 INTERLEUKIN-17A HOMODIMER REDUCES PRO-INFLAMMATORY CYTOKINE PRODUCTION BY INFLAMMATORY BOWEL DISEASE MUCOSA CULTURED EX VIVO

[1,2]P Biancheri*, [2]A Di Sabatino, [1]R Curciarello, [2]GR Corazza, [3]JO Lindsay, [1]TT MacDonald. *[1]Centre for Immunology and Infectious Disease, Barts and the London School of Medicine and Dentistry, London, UK; [2]First Department of Medicine, Fondazione IRCCS Policlinico S. Matteo, University of Pavia, Pavia, Italy; [3]Centre for Digestive Diseases, Barts and the London School of Medicine and Dentistry, London, UK*

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Introduction Interleukin (IL)-17A, which is up-regulated in inflammatory bowel disease (IBD) mucosal lesions, and IL-17F are normally present as IL-17AA and IL-17FF homodimers and may occasionally form IL-17A/F heterodimers. The role of each IL-17 dimer in IBD is currently unknown. Therefore, we studied the effects of IL-17AA, IL-17FF and IL-17-A/F in ulcerative colitis (UC) and Crohn's disease (CD) mucosa.
Methods Inflamed colonic biopsies from 17 IBD patients (6 UC and 11 CD) were cultured *ex vivo* for 24 h with IL-17AA, IL-17FF or IL-17A/F (1 ng/ml). Mucosal myofibroblasts isolated from the inflamed colon of 4 CD and 4 UC patients were cultured for 24 h with tumour necrosis factor (TNF)-α 20 ng/ml or with increasing concentrations (1–100 ng/ml) of IL-17AA, IL-17FF or IL-17A/F. IL-6 and IL-8 were measured in culture supernatants by ELISA.
Results IL-17AA, but not IL-17FF, significantly reduced both IL-6 and IL-8 production by inflamed IBD biopsies cultured *ex vivo*, whereas IL-17A/F decreased IL-8 release by IBD mucosa. No difference was observed between CD and UC. Neither IL-17AA, nor IL-17FF, nor IL-17A/F exerted any effect on IL-6 and IL-8 production by IBD myofibroblasts. As expected, TNF-α stimulation significantly increased IL-6 and IL-8 production by both CD and UC myofibroblasts *in vitro*. No difference was observed between CD and UC myofibroblasts.
Conclusion IL-17AA exerts an anti-inflammatory action on inflamed IBD biopsies cultured *ex vivo*. The action of IL-17AA is not mediated by myofibroblasts, therefore further studies are underway to ascertain which cell type is the main target of IL-17AA in IBD mucosa.
Disclosure of Interest None Declared.

PWE-088 THE IMPACT OF THIOPURINE HYPERMETHYLATION ON CLINICAL OUTCOMES IN PATIENTS WITH IBD

[1]PA Blaker*, [1]S Fong, [1]V Kariyawasam, [2]AM Marinaki, [1]PM Irving, [1]JD Sanderson. *[1]Gastroenterology, Guys and St Thomas Hospitals' NHS Foundation Trust, London, UK; [2]Purine Research Laboratory, Guys and St Thomas' Hospitals NHS Foundation Trust, London, UK*

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Introduction 15% of patients with inflammatory bowel disease (IBD) prescribed azathioprine (AZA)/mercaptopurine (MP) demonstrate a skewed drug metabolism with low thioguanine nucleotides (TGN) and unexpectedly high methylmercaptopurine (MeMP) levels. This predicts a lack of treatment efficacy and excess adverse events; however studies on the effect of thiopurine hypermethylation (TH) in adult IBD patients are lacking. Importantly, where identified TH can be circumvented with the use of low dose AZA/MP and allopurinol. The aim of this study is to characterise the impact of TH in IBD patients and determine if thiopurine metabolite profiles at week 4 of treatment can predict its occurrence to allow early combination treatment.
Methods 273 patients with IBD, who were anti-TNF-α therapy naïve and matched for red blood cell thiopurine-S-methyltransferase activity, were retrospectively identified. Of these 181 patients demonstrated average MeMP:TGN ratios <11 (at least 2 profiles between weeks 12 – 52) and were used as a control group that was compared to 92 patients with average MeMP:TGN ratios ≥11. Clinical outcomes were recorded for the first 12 months of therapy. A failure of AZA/MP as monotherapy was determined by 3 gastroenterologists with expertise in IBD. Thiopurine metabolite profiles were measured in 139 patients at week 4 of therapy and were compared to average metabolite profiles between 12 – 52 weeks of treatment.
Results The average MeMP:TGN ratios in those with and without TH were 21.0 and 2.52 respectively (p = <0.0001). The normalised dose of thiopurine was higher in patients with TH (1.91 vs. 2.09 mg/ kg; p = < 0.0001). Patients with TH were more likely to fail AZA/MP monotherapy as first line treatment during the first 12 months of therapy, in comparison with patients with normal methylation profiles (p = 0.0088, Gehan-Breslow-Wilcoxon Test). The difference in the number of treatment failures at 12 months was 15.7%. There was no difference in the occurrence of gastrointestinal intolerance, myelotoxicity or pancreatitis between groups, however there was an excess of hepatotoxicity with TH (p = 0.0006; OR = 8.058; 95% CI: 2.188 – 29.670; Fisher's exact). TH was demonstrated in 83.7% of cases by 12 weeks of therapy. A MeMP : TGN level > 6.17 at week 4 accurately predicted TH (ratio ≥11) after 12 weeks of therapy (AUC = 0.839; p = < 0.0001; sensitivity = 75.4%; specificity = 88.4%).
Conclusion TH is associated with an excess of thiopurine treatment failures as monotherapy immunosuppression during the first 12 months of treatment. We also confirm its association with hepatotoxicity. A MeMP:TGN >6.17 may be useful in early identification of patients likely to benefit from combination treatment.
Disclosure of Interest None Declared.

PWE-089 LONG TERM OUTCOMES FROM LEUCOCYTAPHERESIS IN ULCERATIVE COLITIS: A RETROSPECTIVE CASE SERIES

P Harrow*, H Kwok, G Parkes, P Premchand. *Department of Gastroenterology, Queen's Hospital, Romford, UK*

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Introduction Leucocytapheresis, the extracorporeal removal of leucocytes from patient's blood, has conflicting evidence of benefit in ulcerative colitis (UC). Numerous studies have recorded short term clinical remission rates while few have examined the long term end-points of colectomy, death and steroid free remission.

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Queen's Hospital, Romford is one of the largest centres performing leucocytapheresis in the UK. Here we present a retrospective case series of patients treated with the Adacolumn® leucocytapheresis filter column for UC between 2008 and 2012, designed to assess these long term end-points with follow-up to June 2013.

Methods Case notes of all patients who underwent leucocytapheresis for refractory UC were reviewed retrospectively to assess the primary end-points of colectomy and death, and the secondary end-points of clinical remission and steroid-free remission.

Results 34 patients met the entry criteria and relevant outcome data was available in 31/34.

Prior to leucocytapheresis 94% of patients were steroid dependent and 91% had previously failed treatment with a thiopurine. The mean number of leucocytapheresis columns given was 7.7 +/- 0.3.

Following treatment 23% underwent colectomy a median 7 months after the start of this treatment with a mean overall follow-up of 500 days. 1 patient died during the study period (from a sub-arachnoid haemorrhage). 52% experienced an initial clinical response and 32% remained in steroid-free remission at 1 year after treatment.

Conclusion The rate of colectomy after leucocytapheresis compares favourably with other rescue therapies.[1,2] The rate of steroid-free remission with leucocytapheresis is comparable to the response rates seen in randomised controlled trials of anti-TNF therapy.[3]

Given that the patients in this study were steroid dependent and had been refractory or intolerant to thiopurines, these results are similar to sub-group analysis of an earlier sham-controlled trial in which those patients with severe UC were more likely to respond to leucocytapheresis than sham.[4] Leucocytapheresis appears to be a safe and useful option for patients with refractory UC.

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Disclosure of Interest None Declared.

PWE-090 THE EFFECT OF COMMONLY USED IBD DRUGS ON AUTOPHAGY INDUCTION USING AN *IN VITRO* CELL CULTURE SYSTEM

[1]P Henderson*, [2]J Satsangi, [1]DC Wilson, [3]C Stevens. *[1]Child Life and Health, University of Edinburgh, Edinburgh, UK; [2]Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK; [3]School of Life, Sport and Social Sciences, Edinburgh Napier University, Edinburgh, UK*

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Introduction Genome wide association studies and functional experiments in inflammatory bowel disease (IBD) have delineated the importance of autophagy in IBD pathogenesis. We aimed to determine the effect of commonly utilised IBD drugs on autophagy induction and the pathways involved *in vitro*.

Methods Cells naturally expressing (HCT116) and not expressing (HEK293) NOD2, both stably expressing green fluorescent protein-labelled light chain 3 (LC3), were treated with varying concentrations of 6-thioguanine, azathioprine, methotrexate or infliximab at different time points; rapamycin, serum-starvation and bafilomycin A1 served as positive controls. Cells were also treated with ERK (U0126) and autophagy (3-methyladenine) inhibitors where appropriate. For immunofluorescent microscopy images were captured using an Axioskop 2 fluorescent microscope and ImageJ software used to identify cells with >5 punctate foci indicating autophagy induction. For western blot analysis cell lysates were immunoblotted with antibodies to LC3, p62, phospho-rpS6 or total rpS6. All statistical analyses were performed using GraphPad Prism.

Results All four drugs induced significant autophagy in HCT116 cells, with only azathioprine inducing autophagy robustly in both cell lines. Azathioprine induced autophagy in a dose-dependent manner in HEK293 cells with significant autophagy induction at all concentrations (30–90 μM) in HCT116 cells. HCT116 cells treated with 6-thioguanine, azathioprine and methotrexate showed strong LC3-I to LC3-II conversion and a reduction in p62, with 6-thioguanine and azathioprine showing loss of phospho-S6K suggesting autophagy induction through the mTORC1 pathway. Use of U0126 and 3-methyladenine in HCT116 cells treated with azathioprine demonstrated that azathioprine may exert its autophagic effect via mTORC1 through the class I PI3K/Akt pathway.

Conclusion Common IBD drugs effect autophagy induction *in vitro* suggesting that manipulation of the autophagy pathway may be partly involved in the mechanism of action of many of these drugs, most convincingly azathioprine. Further work is now required to replicate these findings and further delineate the pathways *in vivo*.

Disclosure of Interest None Declared.

PWE-091 CROHN'S DISEASE MONOCYTE-DERIVED MACROPHAGES EXHIBIT EQUIVALENT RESPONSES TO INTRAMACROPHAGE BACTERIAL INFECTION RELATIVE TO HEALTHY CONTROLS

[1]PK Flanagan*, [2]S Subramanian, [1]BJ Campbell, [1]JM Rhodes. *[1]Gastroenterology, University of Liverpool, Liverpool, UK; [2]Gastroenterology, Royal Liverpool University Hospital, Liverpool, UK*

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Introduction Patients with Crohn's disease exhibit an attenuated inflammatory response after trauma or *Escherichia coli* infection and have delayed clearance of subcutaneous bacteria compared to healthy controls. Adherent, invasive *E. coli*, increased in Crohn's disease, replicate within macrophages and may have a primary pathogenic role. Crohn's disease patients' macrophages may have a primary defect in bacterial killing, allowing survival of AIEC.

Methods We aimed to assess the relative ability of monocyte-derived macrophages from Crohn's patients to kill intracellular *E. coli* and *Staphylococcus aureus* compared to healthy controls. Peripheral blood monocytes were obtained from consenting adults by centrifugation over Lymphoprep™ followed by 2h adherence to plastic Nuncò tissue culture dishes and subsequent differentiation into macrophages by 5d culture (as per Smith *et al*, J. Exp. Med. 2009, 206: 1883–97). Macrophages were infected with an adherent, invasive *E. coli* HM605, *E. coli* K12 or *Staph. aureus* Oxford strain. Intramacrophage killing was assessed using the gentamicin protection assay. Cytokine release to the culture medium was also determined by sandwich ELISA. Macrophage-mediated chemotaxis of human neutrophils,

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