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

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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

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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,