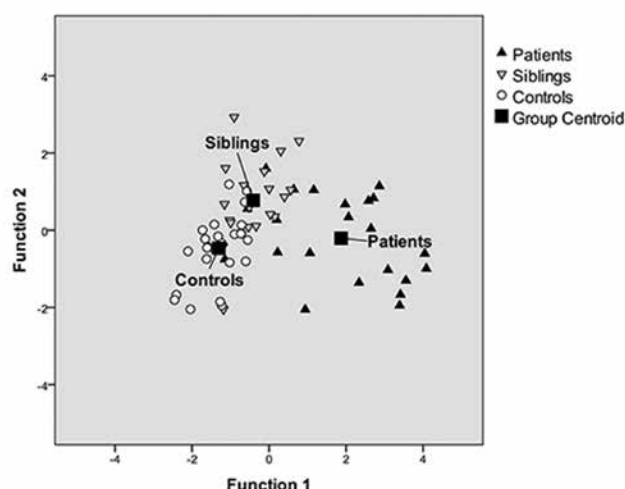


Aims Delineate the genetic, immune and microbial phenotype of patients, siblings and healthy controls (HC); identify factors associated with CD that discriminate siblings from HC.

Methods Faecal microbiota, FC, blood T-cell phenotype, IP and genotype risk over 72 CD risk loci, were measured by qPCR, ELISA, flow cytometry, sugar permeability and Illumina Bead Array respectively, in 22 patients with inactive CD, 21 of their healthy siblings and 25 HC.

Results In addition to genotype risk, siblings shared aspects of the phenotype of CD patients, distinct from HC, as previously reported.¹ Direct discriminant function analysis revealed that the variables maximally separating siblings from HC (Function 2) were: increased $\beta 7$ integrin expression by circulating naïve CD4⁺ T-cells and an increased proportion of memory CD4⁺ T-cells as well as reduced faecal *Roseburia* spp. (Image 1). In contrast, the variables differentiating CD patients from HC (Function 1) were: elevated FC and altered faecal microbiota (reduced *Faecalibacterium prausnitzii*, Cluster IV *Ruminococcus* spp. *Bacteroides-Prevotella* and Clostridial cluster IV).



Abstract OC-017 Figure 1

Conclusion Healthy siblings of CD patients manifest immune and microbiological abnormalities associated with CD, distinct from their genetic risk. Unaffected siblings of CD patients are an excellent model in which to investigate early CD pathogenesis.

Disclosure of Interest None Declared

REFERENCE

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OC-018 LIMITED LONG TERM TOLERANCE OF METHOTREXATE FOR INFLAMMATORY BOWEL DISEASE: 11 YEARS EXPERIENCE FROM A SINGLE CENTRE

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Introduction Methotrexate (MTX) is used as an immunosuppressive treatment in inflammatory bowel disease (IBD). The aim of the study was to evaluate the long-term tolerability of MTX in adults with IBD from a large, single, tertiary referral centre.

Methods IBD patients who had received MTX at a single centre between 2000 – 2011 were identified from the IBD service database and clinical records reviewed.

Results 137 patients received MTX (Crohn's Disease 105 (77%); ulcerative colitis (UC) 32 (23%)); mean age 44 (range 18–77). The

median duration of MTX treatment was 13 months (range 1–127) with an initial dose of 25mg/week (range 10–25 mg) in 127/137 (93%) with 94% commencing MTX intramuscularly. The proportion who continued MTX for $\geq 3, 5, 10$ years were 24% (33/137), 10% (14/137) and 1.5% (2/137) respectively.

89/137 (65%) discontinued MTX during the 11 year follow-up. The cessation rate due to lack of effectiveness was 17/137 (12%) by the end of the 2nd year of MTX, after which there were no further discontinuations for this reason. 77/137 (57%) reported ≥ 1 side-effect (SE) attributed to MTX, which was the commonest reason for discontinuing MTX (61/89; 69%). SEs leading to MTX cessation were most frequent during induction (defined as 0–3 months): 22/61 (36%); followed by 3–12 months following initiation of MTX: 16/61 (26%). Specific SEs resulting in cessation during the 1st year of MTX were due to ≥ 1 of the following; 15 (40%) gastrointestinal (GI), 12 (32%) neurological (NS), 5 (13%) nonspecific malaise, 5 (13%) abnormal hepatic aminotransferase levels (2-fold increase up to or over the upper limit of normal), 2 (5%) hair loss, 5 other (1 each of sore throat, rash, infection, low platelets, injection site reaction). The incidence of discontinuation due to SEs in successive MTX years fell from commencement of MTX: 10/61 (16%) in the 2nd year, 5/61 (8%) in the 3rd year, 3/61 (5%) in the 4th year and 5 (8%) in total between the 5th–10th years. The most frequent SEs attributed to late discontinuation (>1 year) were GI and NS.

Over 11 years there were 12/137 (9%) reported cases of presumed MTX induced abnormal hepatic aminotransferase levels, of which 8 (67%) resulted in MTX cessation with biochemical resolution. Hepatotoxicity occurred during induction in 7/12 cases (58%) and was stopped in 5 of these (71%). In 5 patients with late abnormal hepatic aminotransferase levels, the median time to detection was 36 months (range 10–100 months). MTX was discontinued in 3 of these.

Conclusion In the largest single-centre experience to date, MTX in IBD is limited by high withdrawal rates with a 28% discontinuation rate due to SEs within the 1st year. Late hepatotoxicity highlights the need for long term monitoring in maintenance therapy.

Disclosure of Interest None Declared

OC-019 ADAMDEC1: A NOVEL MOLECULE LINKED TO CROHN'S DISEASE, IS ASSOCIATED WITH AN INCREASED SUSCEPTIBILITY TO CITROBACTER RODENTIIUM COLITIS IN THE KNOCK OUT MOUSE

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Introduction Innate immunity is attenuated in patients with Crohn's disease (CD), with impaired neutrophil recruitment, delayed clearance of *E. coli*, and defective secretion of pro-inflammatory cytokines from macrophages^{1,2}. This primary macrophage defect may result in failure to eradicate bacterial flora entering the tissues and lead to the chronic granulomatous inflammation characteristic of CD. To discover the molecules responsible, transcriptomic profiles were obtained from cultured human macrophages from CD patients and controls. ADAMDEC1 a Disintegrin and Metalloprotease was under-expressed in ~10% of CD patients. This protein is almost exclusively found in macrophages and dendritic cells in the small and large bowel lamina propria. Here we describe the response of *Adamdec1*^{-/-} mice to an enteric bacterial infection with *Citrobacter rodentium*.

Methods *Adamdec1*^{-/-} and wild type mice were administered $\sim 10^8$ or 10^9 *C. rodentium* by oral gavage and body weight monitored for three weeks. At intervals mice were sacrificed and samples of serum, stool, colon and spleen were collected. Serum cytokine levels were measured and bacteria counted, in stool and spleen. Bowel inflammation was assessed histologically. Neutrophil and immune cell recruitment to the colon were measured by MPO assay and qPCR respectively.

Results During infection, control mice experienced a mild self-limiting colitis, with minimal weight loss. Expression of *Adamdec1* was up-regulated in the colon and this normalised with resolution. *Adamdec1*^{-/-} mice were more susceptible to *C. rodentium* infection: they demonstrated dramatic weight loss ($p < 0.001$), a more severe colitis and a reduced survival at the higher dose (67% vs 0%, $p = 0.009$). Serum levels of TNF, IL-1 β and IL-6 were significantly lower in the knock-out mice ($p < 0.05$). Impaired survival was associated with positive cultures of the organisms from the spleen ($p = 0.02$).

Conclusion By analysing the transcriptome of macrophages from CD patients we have identified a novel molecule involved in mucosal immunity. Further work is underway to elucidate the precise role of ADAMDEC1 in the immune response. Individuals with grossly attenuated expression levels may be at an increased risk of developing CD as a consequence of an impaired ability to handle enteric bacterial pathogens.

Disclosure of Interest None Declared

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Gastroduodenal free papers

OC-020 THE MURINE GASTRIC MICROBIOME IS INFLUENCED BOTH BY *HELICOBACTER FELIS* INFECTION AND SOMATIC DELETION OF NF-KAPPAB FAMILY MEMBERS

doi:10.1136/gutjnl-2013-304907.020

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Introduction The development of gastric atrophy in C57BL/6 mice infected with *Helicobacter felis* is differentially regulated by signalling involving NF- κ B1 and NF- κ B2. After infection, more severe atrophy developed in *Nfkb1*^{-/-} mice than wild-type (WT), whilst *Nfkb2*^{-/-} mice were protected from atrophic gastritis. Previous studies have also shown that the development of *H. pylori* induced gastric pathology was delayed in INS-Gas mice maintained in germ free, compared to conventional animal house conditions. Consequently we hypothesised that the different phenotypes observed in *H. felis* infected mice lacking specific NF- κ B proteins may be influenced by altered gastric microbiomes. We have therefore quantified the abundance of specific bacterial phyla in *Nfkb1*^{-/-}, *Nfkb2*^{-/-} and C57BL/6 mice with and without *H. felis* infection.

Methods Groups of 3 C57BL/6, *Nfkb1*^{-/-} and *Nfkb2*^{-/-} mice aged 6 weeks were infected with *H. felis* by gavage. Animals were euthanased at 12 weeks and gastric antral DNA was extracted. Total bacterial load and relative abundance of α -Proteobacteria, γ -Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria were determined by qPCR of 16S rDNA. *H. felis* colonisation was quantified by qPCR for *Flaa*, samples were normalised to murine *Gapdh*.

Results Untreated WT mice had 3.4 and 2.6 fold greater universal bacterial transcripts than *Nfkb1*^{-/-} and *Nfkb2*^{-/-} mice respectively. Actinobacteria abundance was 6.0 fold greater in untreated *Nfkb1*^{-/-} mice *cf* WT and 7.0 times greater in *Nfkb2*^{-/-} mice. α -Proteobacteria were 9.0 times more abundant in untreated *Nfkb1*^{-/-} mice *cf* WT. *H. felis* infection of WT mice resulted in 5.6 and 16.7 fold increases in α -Proteobacteria and γ -Proteobacteria respectively *cf* uninfected mice. γ -Proteobacteria were more abundant in all infected groups, but significantly more so in *Nfkb2*^{-/-} mice than others. This correlated with a 30 fold higher abundance of *H. felis* (Phylum: γ -Proteobacteria) in infected *Nfkb2*^{-/-} compared to WT mice. Infected *Nfkb2*^{-/-} mice also had a 3.3 fold greater abundance of Actinobacteria *cf* infected WT mice. No statistically significant differences were observed in the abundance of Firmicutes or Bacteroidetes.

Conclusion Constitution of the murine gastric antral microbiome is influenced both by *Helicobacter felis* infection and somatic deletion of NF- κ B family members. Since deletion of NF- κ B1 and NF- κ B2 have been reported to alter susceptibility to *H. felis* induced gastric atrophy, these data support the hypothesis that specific differences in microbiota may be responsible for, or reflect this altered susceptibility. Further studies are needed to determine whether specific organisms influence the development of gastric pathology either individually or within complex communities.

Disclosure of Interest None Declared

OC-021 A RETROSPECTIVE AUDIT OF PNEUMOCOCCAL & INFLUENZA VACCINATION IN COELIAC DISEASE

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Introduction Patients with coeliac disease (CD) are at increased risk of mortality from sepsis. In particular, the risk of sepsis with encapsulated organisms such as Pneumococci is higher¹. This may be a consequence of hyposplenism. Current Department of Health Green Book guidelines as well as Coeliac UK recommend vaccination against Pneumococci and influenza. We aimed to investigate whether or not patients with CD received vaccination against Pneumococci and influenza.

Methods CD patients were identified through a register of 1000 ICD-10 classified patients currently under follow up at Royal Liverpool and Broadgreen University Hospitals NHS Trust. A random sample of 250 patients was selected from the register. Electronic case notes were interrogated for demographic details as well as the most recently confirmed comorbidities. Patients of all ages were included in the study. Patients aged over 65 and patients with certain comorbidities (e.g. chronic obstructive airways disease, ischaemic heart disease, liver cirrhosis etc) were analysed separately as these patients should already be immunised against pneumococci and influenza regardless of their coeliac disease diagnosis. Immunisation status was obtained by contacting individual patient's general practitioners. The proportion of vaccinated patients were analysed according to the age groups < or > 65.

Results A total of 250 patients were included out of which we were able to obtain records for 198 patients. Of the 198 patients, 32% of patients had osteopenia and 13% of patients had one or more concurrent autoimmune illness (e.g. thyroid disease, pernicious anaemia, Sjogren's syndrome). 129 patients were < 65 years of age (M: F ratio 1: 2, median age-50, range 19–64) and 69 were > 65 (M: F ratio 1: 2, median age-73, range 65–84).

In the < 65 category, we found 10 with no comorbidities necessitating vaccination which left 119 patients in the main study arm; 23 (19.3%) were vaccinated against Pneumococci and 42 (35%) against influenza.

Abstract OC-021 Table 1 Vaccination uptake in < and > 65 age groups.

Age	Percentage of patients who had received the vaccination	
	Pneumococcal vaccine	Influenza vaccine
< 65	19.3%	35%
> 65	72.5%	82.6%

Conclusion Very few patients with CD are vaccinated against preventable causes of sepsis such as Pneumococci. Gastroenterologists, GPs and patients need to be aware of the increased risk of sepsis and the need to administer vaccines against preventable infections in CD patients. Vaccination status should be routinely obtained during follow up visits. Due to the lack of a simple clinical test for