Aim To assess the impact of Mbd2 deficiency on the activation status and cytokine production of naïve *Mbd2-/-* leucocytes isolated from murine small intestine (SI) and large intestine (LI) lamina propria (LP).

Methods All mice were bred in specific-pathogen free facilities at the University of Edinburgh. *Mbd2-/-* mice were produced as described previously (1). Single cell suspension of SI and LI LP were isolated as previously described, n = 4 in each group, minimum 2 experiments (2). Controls were wildtype (WT) age and sex matched littermates.

Cells were first stained with LiveDead blue (Life Technologies), FcR-Block and subsequently; CD11c, Ly6C, CD80, B220, CD11b, MHC(II), Ly6G, CD103, F4/80, CD40 and CD45. 4hr incubation with PMA/ionomycin/GolgiStop (BD bioscience) was performed for intracellular cytokine analysis with IFNgamma, IL-4, IL-13, IL-17 and TNFalpha. Samples were acquired using an LSRII and analysed with FlowJo (TreeStar), student t test was used with Prism (GraphPad) in statistical analyses.

Results *Mbd2-/-* mice showed significantly greater IFNgamma, TNFalpha and IL-17 but not IL-13 or IL-4 production in CD4+ and CD8+ lymphocytes isolated from SI and LI LP compared to WT controls. In addition surface activation markers CD80 and CD86 were significantly greater in *Mbd2-/-* CD103+CD11b+CD11c+ Dendritic cells and CD11b+CD11c+F4/80 macrophages from SI LP.

Conclusion These results show for the first time that epigenetic processes can regulate both antigen presenting cell activation status and T cell production of potentially damaging inflammatory cytokines at the mucosal-environmental barrier. They also identify methyl-binding proteins and/or genes that they regulate as exciting new targets for therapeutic modulation of GI inflammation.

Disclosure of Interest None Declared

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OC-090 CORRELATION BETWEEN DISTRIBUTION OF HLA HAPLOTYPES AND SEVERITY OF MUCOSAL DAMAGES

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Introduction Celiac disease is a chronic intestinal inflammation resulting in different degree of intestinal mucosal damages. The disease occurs in genetically predisposed individuals and associated with human leukocyte antigen (HLA)-DQ2/8 heterodimers. The aim of this study is to evaluate the correlation between distribution of HLA haplotypes and the severity of mucosal damages.

Methods Fifty nineIranian celiac disease patients were evaluated to predict the HLA-DQA1 and -DQB1 genes, using tagging SNPs method. All patients had positive tTGA and/or EMA antibodies and histology according to Modified Marsh classification (Marsh I-IIIc) by Rostami *et al*

Results The result of this study show that 86.4% of cases were carriers of HLA-DQ2 and/or HLA-DQ8 heterodimers, either homozygous or heterozygous. Marsh IIIa lesions were seen in 20 cases, Marsh IIIb in 9 and Marsh IIIc in 12 cases. Marsh IIIa is associated with HLA-DQ2 haplotype in 10 cases, IIIb with 4 and IIIc with 10 cases respectively. Marsh IIIa is correlated with HLA-DQ2.5/2.5 in 4 cases followed by HLA-DQ2.5/DQ8 and HLA-DQ2.5/DQX each in 3 cases respectively.

Conclusion According to high prevalence of HLA-DQ2 haplotype in Iranian population, we can conclude that there is a strong link between severity of mucosal damages and presence of HLA-DQ 2 haplotype. Celiac disease cases with Marsh III (a-c) carry more HLA risk alleles compared to those with Marsh I-II and this difference were statistically significant for DQ2.5 haplotype distribution (P = 0.0001).

Disclosure of Interest None Declared

OC-091 ARE THERE CHANGES IN KERATIN EXPRESSION PROFILE IN ACTIVE COLITIS AND IN COLITIS PHENOTYPES ASSOCIATED WITH INCREASED CANCER RISK?

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Introduction Colitis in homozygous mK8$^{-/-}$ mice as a result of a primary epithelial defect and heterogeneous missense mutations in keratin (K) 8 and K18 in IBD suggest a possible association between simple epithelial keratins and IBD. Our previous work using mass spectrometry (MS) and western immunoblotting suggests alterations in the insoluble forms of K8, K18 and K19 in intestinal epithelial cells in colitis phenotypes compared to controls. We have previously shown an increase in insoluble keratin in epithelium from patients with longstanding pancolitis (LSPC) and decreased levels in epithelium from patients with dysplasia (in both the lesion and rectal mucosa), recent onset colitis (ROUC) and PSC associated colitis. There is also a reduction in insoluble keratins in active areas of inflammation compared to inactive areas.

Methods Paired biopsies were taken from patients with active colitis (one from the actively inflamed area and another from a proximal inactive area, n = 10) and patients with dysplasia (biopsies taken from the dysplastic area and an area of rectal mucosa, n = 3). Single rectal biopsies were taken from patients with ROUC (n = 8), LSPC (n = 10) and PSC colitis (n = 7). These tissue sections underwent IHC staining for K8, K18 and K19. Semi-quantitative assessment of keratin expression was performed using a pixel counting algorithm and a manual scoring system, which scored staining intensity at the surface and base of the crypts and extent of crypt staining.

Results There was no difference in K8 expression measured by manual scoring between the groups. K18 showed a significant difference in pixel count scores between the active and inactive inflammation (0.078 p = 0.022) reflecting a shift towards more extensive, moderate and weak staining in the active group. There was also a significant change in the manual scores for the ratio of surface intensity to crypt intensity (p = 0.028) representing a greater surface compared to crypt K18 expression. All other groups were similar in K18 expression and pattern. There was no difference in K19 expression measured by manual and pixel count scoring between the groups.

Conclusion The results suggest a difference between MS and IHC approaches. If the IHC data hold true they may suggest that the changes in keratin expression profile that occur with duration of disease, inflammation and dysplasia may be a result of changes in keratin solubility rather than a change in the total expression of keratins. However, possible explanations include too small sample size for IHC and lack of sensitivity of IHC by comparison with MS.

Disclosure of Interest None Declared