used for quantitative real-time PCR, with multiparameter flow cytometry utilised to measure hydrogen peroxide levels, apoptosis, DNA damage and cell proliferation.

Results *DUOX2* is expressed throughout the colonic epithelium, is upregulated in active compared to quiescent ulcerative colitis and also in areas of UC associated dysplasia. In the setting of intestinal inflammation, but not in quiescent disease, 5-ASA enhances *DUOX2* expression in vivo and ex vivo. As expected, 5-ASA was found to suppress cytokine (IL-6 and IL-8) production during an inflammatory flare and to maintain low cytokine levels during remission. The addition of 5-ASA in vitro led to upregulation of *DUOX2* and elevated levels of hydrogen peroxide, DNA damage and apoptosis. These effects were further enhanced in a setting of hypoxia.

Conclusion We have shown that 5-ASA over stimulates *DUOX2* expression in the setting of inflammation and hypoxia, but not in quiescent disease. Importantly, this suggests that during a flare 5-ASA could act as a carcinogen rather than a chemo-preventative drug. Further investigations to confirm the functional relevance of *DUOX2* up-regulation in the colonic mucosa of patients with active UC is indicated.

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PWE-256 INTESTINAL INFLAMMATION REGULATES RETINOIC ACID DEPENDENT IMPRINTING OF GUT TROPISM BY DENDRITIC CELLS INDEPENDENTLY OF RALDH EXPRESSION

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Introduction In the mouse, tissue-specific expression of retinaldehyde dehydrogenase (RALDH) enzymes by CD103+ intestinal dendritic cells (DC) enables them to generate all-trans retinoic acid (RA) and thereby imprint a gut-tropic phenotype on T cells via induction of homing receptors including α4β7 integrin. In health, RA from CD103+ also enhances their generation of Treg, contributing to intestinal homeostasis. In murine models of inflammatory bowel disease (IBD) RALDH expression by CD103+ DC is reduced but little is known about the function of RA in the human intestine. The aim of this study was to determine whether factors present in the healthy and inflamed human intestine regulate RA generation and activity.

Methods Conditioned media (CM) were generated by culture of intestinal biopsies from healthy individuals and IBD patients (inflamed/non-inflamed regions). DC were differentiated from monocytes using GM-CSF and IL-4 in the presence or absence of CM. Expression of RA-generating enzymes was assessed by qRT-PCR and RALDH activity determined using the Aldefluor assay. Induction of α4β7 following activation of naïve allogeneic CD4+ T cells was determined by flow cytometry.

Results Activation of naïve CD4+ T cells by human monocyte-derived DC resulted in RA-dependent upregulation of α4β7. These DC possessed retinal-inhibitable Aldefluor activity and expressed both alcohol dehydrogenase (*RDH10*) and RALDH (*RALDH1,2,3*) enzymes required for the generation of RA from retinol via retinal. Aldefluor activity was regulated by GM-CSF and RA, and reflected predominately the activity of RALDH2 as suggested by qRT-PCR analysis of sorted Aldefluor+ DC. CM significantly suppressed Aldefluor activity ($p<0.0001$) irrespective of whether generated from healthy or IBD tissue (inflamed or non-inflamed). The inhibitory effect of CM generated from healthy tissue could be partially reversed with the prostaglandin E2 (PGE2) EP-2 receptor antagonist AH6809 but this effect was less marked with CM from IBD tissue suggesting the involvement of distinct RALDH regulators. Although the effects of inflamed and non-inflamed CM on Aldefluor activity were similar, DC differentiated in the presence of inflamed CM induced significantly higher ($p<0.05$) levels of CD4 T cell α4β7 expression.

Conclusion Factors generated in the human intestinal mucosa limit RALDH activity in DC and may thereby impact upon their generation of RA. Factors other than PGE2 are involved particularly in inflamed tissue. Intestinal mediators influence the imprinting of gut tropism independently of effects on RA-generating enzymes. Manipulation of RA availability may offer new therapeutic options in IBD.

Competing interests None declared.

PWE-257 THE ROLE OF RDH10 AND RALDH ENZYMES IN RETINOIC ACID-MEDIATED IMMUNE REGULATION BY ANTIGEN PRESENTING CELLS IN THE HUMAN INTESTINE

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Introduction All-trans retinoic acid (RA) has emerged as an important immunoregulatory molecule with specific functions in the intestine. RA is generated enzymatically by the sequential action of alcohol dehydrogenase (ADH) and retinaldehyde dehydrogenase (RALDH) enzymes. In the mouse, RALDH expression is confined to CD103+ intestinal dendritic cells (DC) giving this subset the unique ability to mediate RA-dependent functions such as the imprinting of gut tropism on T cells. RALDH activity is downregulated in mouse models of inflammatory bowel disease (IBD) but little is known about its role in human intestine. The aim of this study was to assess RA-dependent immune regulation by human intestinal antigen presenting cells (APC).

Methods Lamina propria mononuclear cells (LPMC) were extracted from intestinal biopsies by enzymatic digestion and analysed by multicolour flow cytometry. Purified subsets of intestinal APC were obtained by immunomagnetic or FACS sorting. Expression of *ADH* and *RALDH* enzymes was quantified by quantitative RT-PCR and RALDH activity assessed using the Aldefluor assay. The ability to induce the gut homing receptor α4β7 on T cells was assessed by flow cytometry following stimulation of CFSE-labelled naïve CD4+ T cells.

Results Induction of the gut homing receptor α4β7 on human T cells was RA dependent. Intestinal myeloid APC were potent stimulators of naïve CD4+ T cells and induced high levels of α4β7. Both of these activities were attributed to the CD103+ APC fraction. However, analysis of ex vivo intestinal populations revealed RALDH activity in all myeloid APC populations studied: CD103+ and CD103− DC identified as HLA−DR+CD11c+Lin− (Lin=anti-CD3,14,16,19,34) as well as HLA−DR+CD11c+Lin+ monocyte-like cells. Lymphocytes had little or no Aldefluor activity. Comparison of intestinal DC from healthy controls and IBD patients showed similar RALDH activity in inflamed and non-inflamed tissue. RALDH activity was equivalent in CD103+ and CD103− DC. In contrast, the ADH RDH10 was expressed at levels 4.5-fold higher in CD103+ DC.

Conclusion As in the mouse, RA-mediated induction of α4β7 is a property of the CD103+ subset of intestinal DC. However in divergence from murine data, this property is associated with restricted expression of the ADH enzyme RDH10 rather than