Small bowel

**THE PHENOTYPE AND TCR REPERTOIRE OF INTESTINAL CD8+ T CELLS IS ALTERED IN COELIAC DISEASE**

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*10.1136/gutjnl-2020-bsgcampus.43

**Introduction** Coeliac disease (CD) is a common immune-mediated condition driven by aberrant adaptive CD4+ T cell responses to gluten. The cytotoxic CD8+ and γδ+ T cell response in the epithelium is thought to be predominantly cytokine-driven and T cell receptor (TCR)-independent, however recent work has challenged this. We investigated the wider role of intestinal CD8+ and γδ+ T cells in CD using RNA sequencing (RNAseq), single-cell RNAseq, and TCR repertoire sequencing (TCRseq).

**Methods** Intestinal CD8+ and γδ+ T cells were isolated from duodenal biopsies from paediatric and adult patients collected at endoscopy. RNAseq, TCRseq, and single-cell RNAseq were performed on FACS-sorted T cells using the Smartseq2, iRepertoire, and 10x genomics protocols respectively. Flow cytometry was performed on intestinal T cells and peripheral blood mononuclear cells (PBMCs) from subjects with and without CD.

**Results** Bulk RNAseq of intestinal CD8+ and γδ+ T cells from 12 subjects with and without CD were analysed. There were 236 differentially expressed genes (DEGs) between health and active CD in the CD8+ T cells, and 451 DEGs in the γδ+ T cells. Common pathways upregulated in coeliac disease included those involved in the regulation of cell activation and adhesion, and T cell costimulation. Expression of key immune checkpoint molecules differed between CD8+ and γδ+ T cells.

TCRseq of sorted intestinal CD8+ T cells from 20 subjects showed perturbations in the TCR repertoire between health and CD, with particular V-region genes used more frequently in CD. These changes were also seen in the RNAseq dataset, providing validation in a second cohort. The proportion of CD8+ T cells expressing these TCRs was increased in peripheral memory and gut-homing populations in subjects with CD.

Single-cell RNAseq of intestinal CD8+ and γδ+ T cells revealed two transcriptionally distinct clusters of CD8+ T cells that were increased in coeliac disease. These had an activated, cytotoxic transcriptional profile, with high expression of immune checkpoint molecules and associated transcription factors, consistent with a highly regulated phenotype. Similar TCR V-region genes were enriched and clonally expanded in these clusters, suggesting a pathogenic, potentially antigen-driven, role for these cells in coeliac disease.

**Conclusions** This multimodal analysis of cytotoxic T cells in coeliac disease has revealed a population of activated, cytotoxic, and highly regulated CD8+ T cells with clonally expanded and biased TCR repertoires in the intestinal mucosa in CD. These populations may have a previously unappreciated role in CD pathogenesis.