Small bowel

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

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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.

044 TIME TO REDEFINE SERONEGATIVE COELIAC DISEASE? THE LARGEST EXPERIENCE FROM TWO INTERNATIONAL CENTRES OVER 19-YEARS

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Introduction Seronegative coeliac disease (SNCD) is a poorly defined clinical entity. The aim of this study was to assess the prevalence of SNCD among patients with CD and to compare clinical features and long-term outcomes between SNCD and seropositive CD (SPCD).

Methods Clinical notes of SNCD patients attending two centres (Sheffield, UK and Pavia, Italy) between Jan-2000 and Jan-2019 were retrospectively reviewed. SNCD was diagnosed in immunocompetent HLA-DQ2/DQ8 positive patients on a normal gluten-containing diet with duodenal villous atrophy (VA) and the following: negative IgA endomysial (EmA) and tissue transglutaminase (tTG) antibodies, no alternative causes for VA, and clinical/histological response to a gluten-free diet (GFD). CD+IgA deficiency was diagnosed in patients with VA and positive IgG EmA/tTG. Patients on immunosuppressants and those already on a GFD at time of initial diagnosis of VA were re-investigated thoroughly to confirm/exclude SNCD. Baseline demographics, presenting symptoms, HLA, onset of complications, date and cause of death were collected and statistically compared between SNCD patients and SPCD controls diagnosed in the same timeframe.

Results 104 SNCD patients and 1066 SPCD controls were diagnosed over 19 years. 61 of these 104 patients (59%) had true SNCD, whereas 21 patients (20%) had IgA deficiency and positive IgG EmA/tTG, thus confirming CD+IgA deficiency. Finally, SNCD was definitely excluded in 22 (21%), thanks to a gluten challenge showing either normal biopsies or positive IgA EmA/tTG (having had an initial biopsy with VA). Since 17 of the 61 patients with true SNCD were referred from other centres, prevalence of true SNCD was 44/1210 (3.64%, 95%CI 2.65–4.85). When compared to SPCD, true SNCD patients were older at diagnosis (53±19 vs 42 ±16 years, $p<0.001$) and presented more frequently with weight loss ($p< 0.001$), diarrhoea ($p<0.001$), and HLA-DQ8 ($p<0.001$). Risk of complications (HR 9.81, 95%CI 4.19–22.99, $p<0.001$) and mortality (HR 2.65, 95%CI 1.15–6.14, $p=0.02$) were higher in true SNCD than in SPCD.

Conclusions This is the largest study assessing SNCD. True SNCD is rare and characterised by a more aggressive disease phenotype, a higher risk of complications and mortality than conventional SPCD. Misdiagnoses of SNCD can occur in greater than 20% of cases with a previous diagnosis of SNCD. Strict follow-up is mandatory in true SNCD patients.