Single-cell TCR repertoire analysis reveals highly polyclonal composition of human intraepithelial CD8+ αβ T lymphocytes in untreated celiac disease

Celiac disease (CeD) is a chronic inflammatory disease driven by exposure to dietary gluten. The mucosal changes in the small intestine are characterized by infiltration of intraepithelial lymphocytes (IELs) that resolves on a gluten-free diet [1]. In CeD, CD8+ αβ IELs are considered to be central in killing of enterocytes and contribute to tissue destruction [2]. Thus, we wanted to investigate the clonal contribution of these T cells in gut biopsies of CeD affected and non-affected individuals. Early studies in mice showed that the TCRαβ repertoire in the small intestine is oligoclonal [3,4]. Further studies in germ-free rats demonstrated that the repertoire is surprisingly broad but transitioned into a more oligoclonal repertoire upon microbial colonization [5]. In humans, it has been reported that the CD8+ αβ repertoire of IELs is oligoclonal in adults but polyclonal during infancy [6–8], suggesting that similar mechanisms influence human repertoires.

By high-throughput paired single-cell TCRαβ sequencing, we compared the CD8+ αβ IEL repertoire from untreated CeD patients (UCeD, n = 5, 1032 cells), treated CeD patients (TCeD, n = 5, 370 cells) and healthy controls (n = 9, 1499 cells). We found that the IEL repertoire of untreated CeD patients was highly diverse and polyclonal compared to both treated patients and healthy controls. In contrast to previous reports, we found that the IEL infiltration in untreated CeD is characterized by a polyclonal expansion of diverse CD8+ αβ T-cell clonotypes.

Due to the large diversity in TRAV and TRBV gene segments, we next focused on the top 10 expressed TRAV and TRBV genes. The top 10 V genes accounted for approximately 50% of the total V-gene usage in all groups (Fig. 1A). The top 10 TRBV genes used were similar to what have been described for healthy individuals previously [9]. We wanted to look into the TRAV/TRBV gene pairing, as this could differ despite similar gene segment usage. Here we included all available V-gene pairs and found that there were no obvious preferences in V-gene pairing in any group (Fig. 1B). We also compared the relation between expanded clonotypes by dividing each participant group into three; the 10 most expanded clonotypes that occupied a smaller percentage of the total repertoire, and had less dominating clonotypes that on average accounted for more than 50% of the total repertoires (Fig. 2D). Interestingly, the clonal distribution in the controls, previously reported to be oligoclonal [6,7], varied in a broad range between donors. Importantly, the UCeD group was hallmarked by the contribution of many distinct T-cell clonotypes that occupied a smaller percentage of the total repertoire, and had less dominating expanded clonotypes overall. This feature likely reflects an influx of new CD8+ IEL clonotypes in the active disease state, leading to a polyclonal repertoire that revert to a more oligoclonal repertoire when patients are on a GFD.

In conclusion, our study of single-cell TCRαβ sequencing demonstrates that there is a highly diverse TCR repertoire in CD8+ αβ IELs of untreated CeD without presence of any dominating clonotypes.
Figure 1. TRAV and TRBV gene usage and pairing in CD8⁺ αβ IELs in CeD. (A) Top 10 TRAV (left) and top 10 TRBV (right) gene segment used in CD8⁺ αβ IELs. (B) TRAV/TRBV chain pairing in CD8⁺ αβ IELs. The number of distinct clonotypes in subject category analyzed were: Controls (n = 494), UCeD (n = 740), and TCeD (n = 227). In the circos plots a forward slash (/) (e.g. TRBV5-4/4 denotes a possibility of two undistinguishable V genes. Colon (:) denotes a number of V-genes, e.g. TRBV13:15 includes TRBV13, 14, and 15. Period (.) includes different V genes, e.g., 10–2,3 includes both TRBV10-2 and TRBV10-3.

We also demonstrate that healthy individuals possess more of a mix between oligoclonal and polyclonal IEL repertoires than what has previously been appreciated. This picture is also seen in treated CeD patients. Notwithstanding, the repertoire in CeD patients with active disease is significantly more diverse than what is seen in treated patients and controls. Altogether, these data support the notion that the infiltration of IELs in active CeD is not coming from some selected clones but rather induced by the inflammatory conditions in the small intestine.

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Figure 2. Analysis of clonality and diversity in CD8$^+$ αβ IELs. (A) The degree of clonality for each patient in each group is depicted as three groups of clonal proportions, 1:10 (blue), 1:100 (light blue), and 1:1000 (dark blue). 1:10 indicates the 10 most expanded clonotypes. (B) The frequency of the top 10 clonotypes per group is depicted as boxplots and each dot in the plot indicates each donor. The boxplot shows the median and the whiskers indicate data spread. (C) The degree of clonal diversity is estimated using Shannon entropy (normalized by subsampling down to the smallest cell number across all 19 donors). The median is depicted in the boxplot and the range of data indicated by the Whiskers. (D) The clonal size for each clonotype is denoted by the number within the squares, for each patient and group. The total percentage of expanded clonotypes (>2 cells) is shown by the length of each bar. The blank space above the bars up to 100% is the portion of clonotypes with only 1 cell. The total number of cells and clonotypes per donor are denoted below each bar. Red lines indicate the top 10 clonotypes in each donor. 

$P$ value ** = $P < 0.01$, ns not significant, determined using an unpaired Mann-Whitney test.
L.M.S. analyzed the data. L.M.E., L.F.R., and L.M.S. wrote the manuscript. R.S.N. developed the bioinformatic tools used and revised the manuscript. KEAL provided donor material and revised the manuscript.

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Abbreviations: CeD: celiac disease · UCeD: untreated CeD · TCeD: treated CeD · GFD: gluten-free diet · IEL: intraepithelial lymphocyte

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