

Characterizing TCR $\gamma\delta$ T cells in tissues and disease

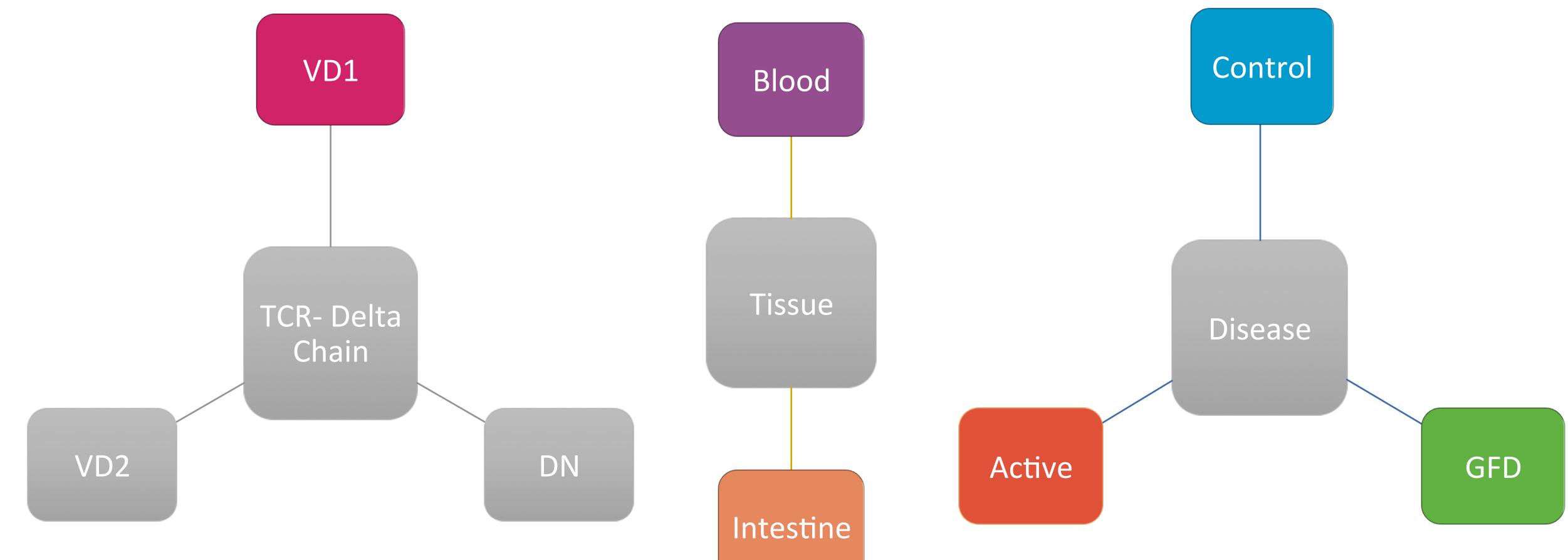
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Abstract

Gamma delta T cells are innate like lymphocytes that have been hypothesized to play a role in lymphoid stress surveillance. In this study, we investigate the hypothesis that the small intestinal microenvironment uniquely selects the repertoire and functional profile of TCR V δ 1 T cells under physiological and pathological conditions. To that end, we choose to use celiac disease (CD) as a model. In accordance with the literature, our data shows TCR $\gamma\delta$ intraepithelial lymphocytes (TCR $\gamma\delta$ IEL) are increased in frequency of CD3 $^+$ lymphocytes in the small intestine of patients with Active CD and treated CD (gluten free diet) when compared with controls. Our results indicate that the TCR repertoire of TCR V δ 1 IEL is unique when compared to PBL and overlap between individuals is dominated by the γ -chain. More specifically, we find Vy4 is highly enriched in TCR V δ 1 IEL of controls when compared to CD patients suggesting a turnover in disease. Interestingly, only TCR V δ 1 IEL of Active CD patients have the capacity to produce IFN- γ when stimulated *ex vivo*. Therefore, we believe the intestinal microenvironment selects for a unique TCR V δ 1 IEL repertoire and imprints a unique functional profile on TCR V δ 1 IEL under different tissue states. Alterations to this repertoire and core program may give insights into the role of these cells in homeostasis and disease.

Approach



Methods

Human samples

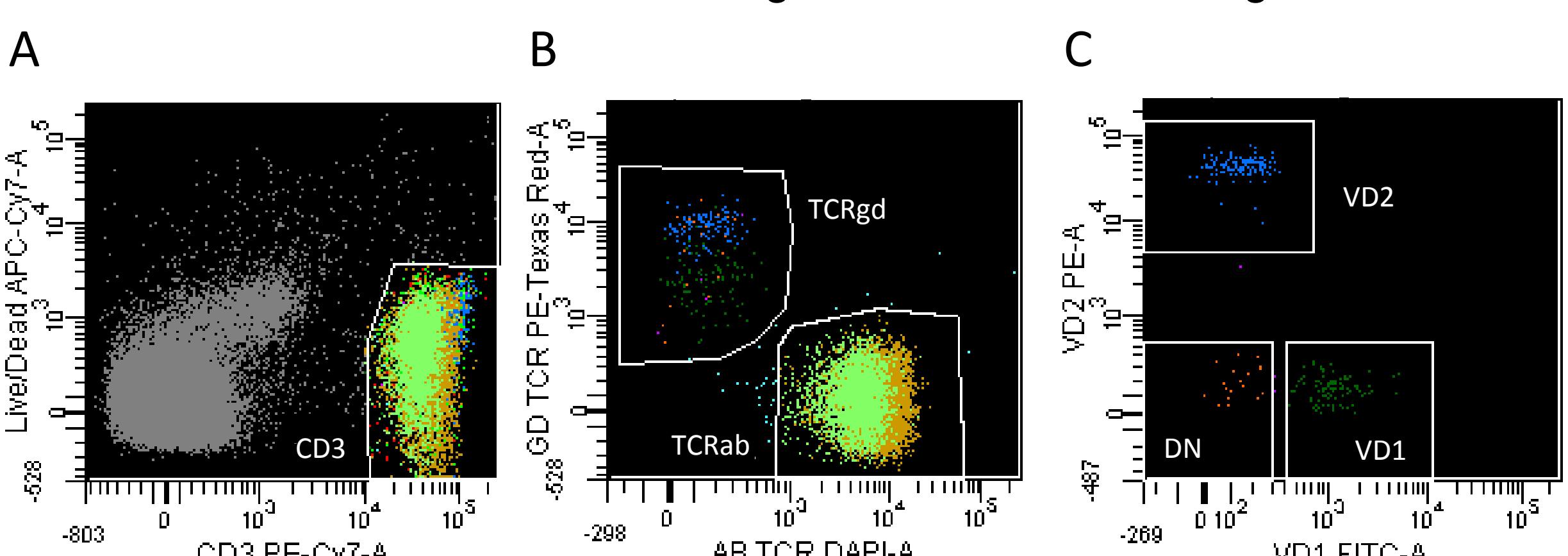
Paired peripheral blood and duodenal biopsies were obtained from individuals that were then categorized as either Control, Active CD, or Gluten Free CD (GFD) based on serology reports and histological scoring of the duodenal biopsy (Data not shown).

Cell Isolation

Lymphocytes were isolated from peripheral blood (PBL) using ficoll separation, while intraepithelial lymphocytes (IEL) were isolated from duodenal biopsies using mechanical disruption coupled with EDTA.

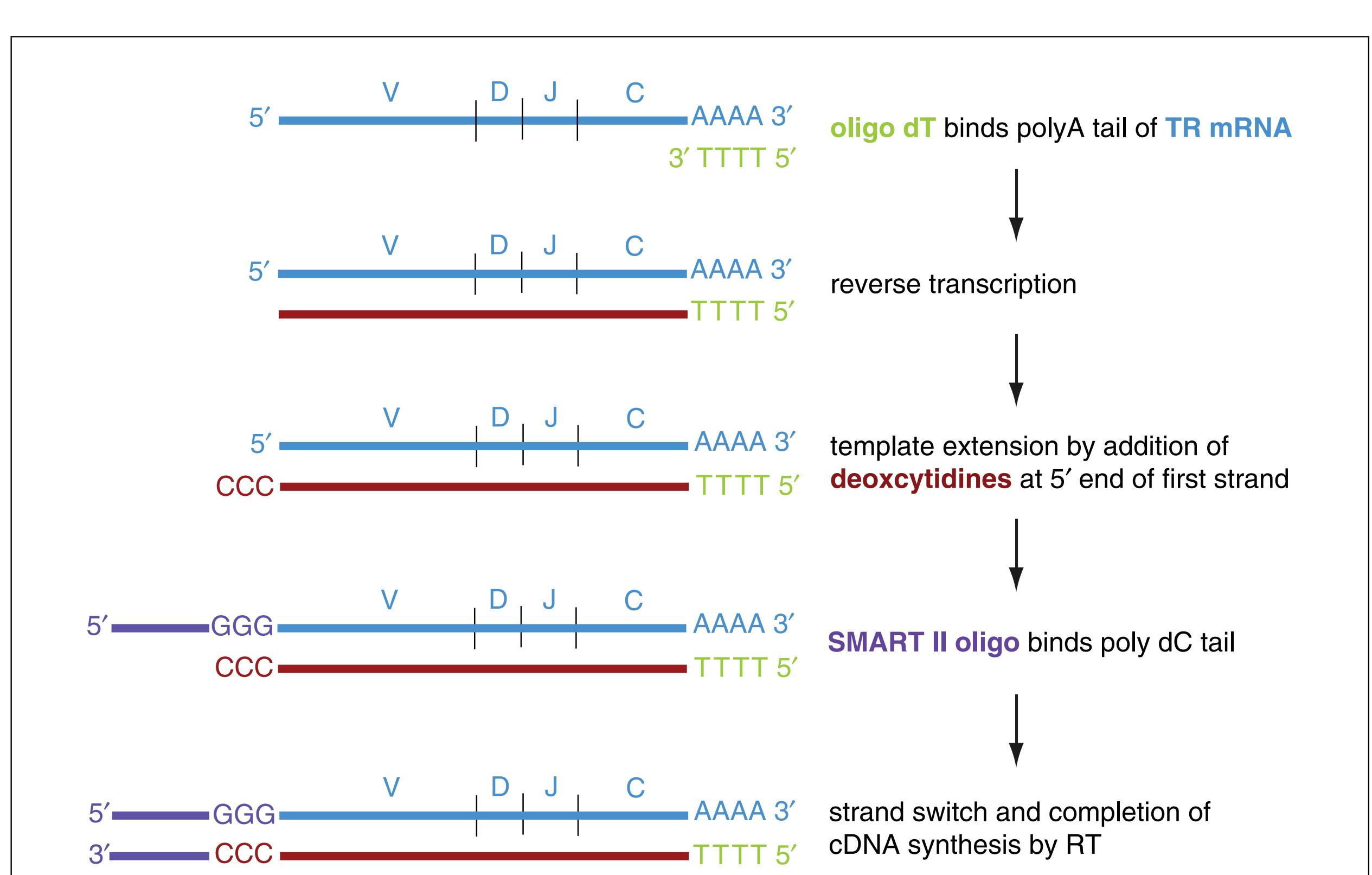
Sorting strategy

TCR $\gamma\delta$ T cells were isolated as above, stained with relevant antibodies and sorted based on the expression of particular delta chains as shown in panel C below. This method was used to generate the data in Figures 1 and 2.



TCR sequencing

TCR $\gamma\delta$ T cells expressing the V δ 1 receptor (500-5,000 cells) were sorted as above and processed for TCR sequencing by template-switch anchored RT-PCR (see below) followed by high throughput sequencing. Sequences were analyzed with Sequencher software and the IMGT database.



Results

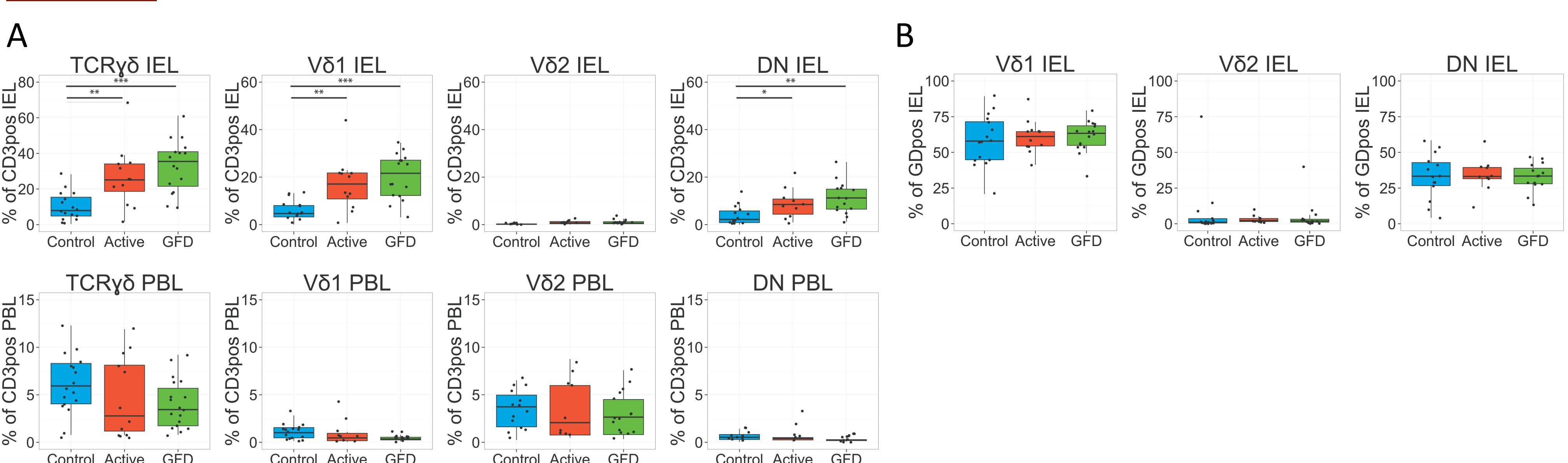


Figure 1. Frequency of TCRgd T cell subsets based on expression of particular delta chains varies across tissues and disease states
Intraepithelial lymphocytes (IEL) and peripheral blood lymphocytes (PBL) were isolated from individuals and stained for the surface markers CD3 (UCHT1), TCR $\gamma\delta$ (SA6.E9), TCR $\alpha\beta$ (IP26), V δ 1 (TS8.2) and V δ 2 (B6). A) Analysis of relevant populations shown as a % of total CD3 positive lymphocytes reveals a significant enrichment of TCRgd T cells in the duodenum of Active and GFD celiac patients. B) Further analysis of the IEL compartment shows no significant overrepresentation of a particular delta chain in disease when looking at the % composition of TCRgd T cells on a per chain basis. Box plots display the first and third quartiles and **p<0.01; ***p<0.001, using unpaired t test.

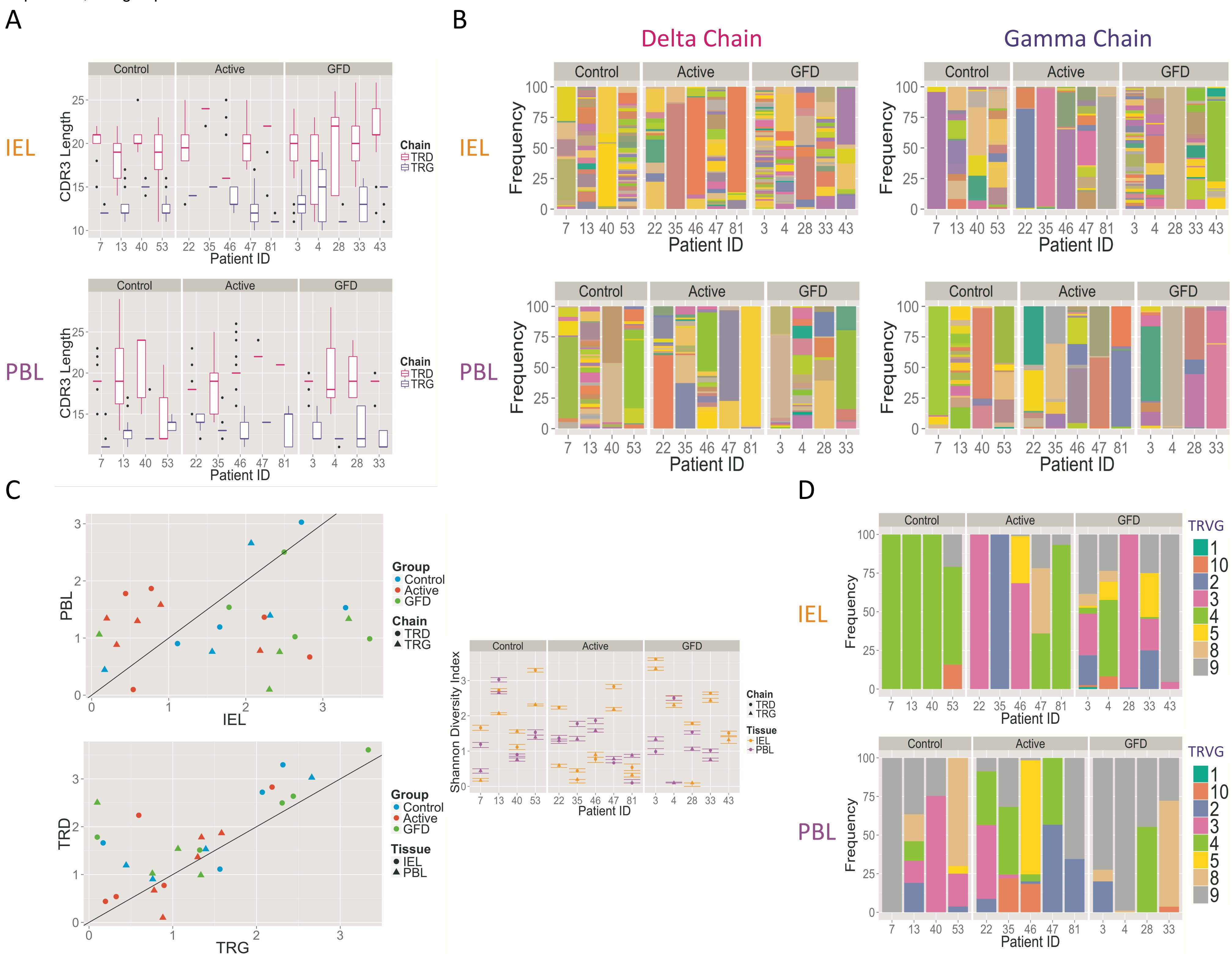


Figure 2. TCR γ and δ chains exhibit different patterns of diversity in tissues and disease
V δ 1 TCR $\gamma\delta$ T cells were *ex vivo* sorted and sequenced as described in the methods section. A) CDR3 lengths are displayed as box plots on a per individual basis for the corresponding tissue and chain. B) CDR3 diversity for the VD1 chain (left) and all corresponding gammas (right) are shown on frequency plots where every color corresponds to a unique CDR3 and the length of the color corresponds to the frequency of that given CDR3. Similar colors do not correspond to overlaps in CDR3 sequence. C) Shannon diversity indexes for each individual were calculated for each chain by subsampling 50 CDR3s (right) and plotted in two dimensions to look at how tissues segregate disease states (top) as well as how the chains compare across disease (bottom). D) Analysis of the variable gamma region (TRVG) usage shown as a frequency plot where each gamma chain is assigned a color and the length of the colored bar corresponds to the frequency of that given gamma in a given individual.

Figure 3. TCR $\gamma\delta$ T cells exhibit a unique cytokine profile in the intestine of Active CD patients

Cells were isolated from peripheral blood and duodenal biopsies as described in the methods section. Cells were then treated with PMA/ionomycin for 3 hrs and subsequently stained for the cytokines IFN- γ and TNF- α . A) Representative plot of IFN- γ staining for V δ 1 T cells from one Control and one Active patient. B) The frequency of IFN- γ positive cells (top) and TNF- α positive cells (bottom) is shown for relevant cell subsets from the IEL compartment. C) The frequency of IFN- γ positive cells (top) and TNF- α positive cells (bottom) is shown for relevant cell subsets from the PBL compartment. Box plots display the first and third quartiles and **p<0.01, using unpaired t test.

Conclusions

- V δ 1 T cells bearing the Vy4 chain are selected in the small intestine of controls suggesting homeostasis in the small intestine is driven by a ligand that involves recognition by Vy4.
- The shift in gamma chain usage and low diversity in Active CD coupled with a unique cytokine profile suggests the gamma chain may be implicated in recognition of a CD-associated ligand.

Future Directions

- Further characterize V δ 1 T cells in the different disease states using *ex vivo* RNA-seq on small cell numbers
- Go after the ligand by cloning tissue specific TCRs from different disease states and using TCR tetramer based approaches as well as computational approaches to model TCR structures.

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