STAT3 regulates cytotoxicity of human CD57+ CD4+ T cells in blood and lymphoid follicles

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Running title

Cytotoxic CD57+ CD4+ T cells

Supplementary figures legends

Fig. S1. PD-1 high cells have effector phenotype both in blood and tonsil

A. Representative flow cytometric analysis of level of PD-1 expression by different CD4+ T cells in blood and tonsils from the same donor according to expression of CXCR5 and CD45RA. Colours of the overlay histogram are derived from the quadrants in the dot plot. **B**. Flow cytometric analysis of expression of CXCR5 and PD-1 (right) in blood and tonsil gated on total CD4+ T cells (left panel) or according to CD57 status. **C**. Flow cytometric analysis of CXCR5 MFI on different PD-1 CD57 subsets in the tonsil, with summary plots from independent analyses. **D**. Flow cytometric analysis of CCR7 and CD57 by CD4+ CD45RA- T cells in a blood and a tonsil.

Fig. S2. Analysis of cytokine and proliferative responses of tonsil CD4+ T cells defined by PD-1 and CD57 expression

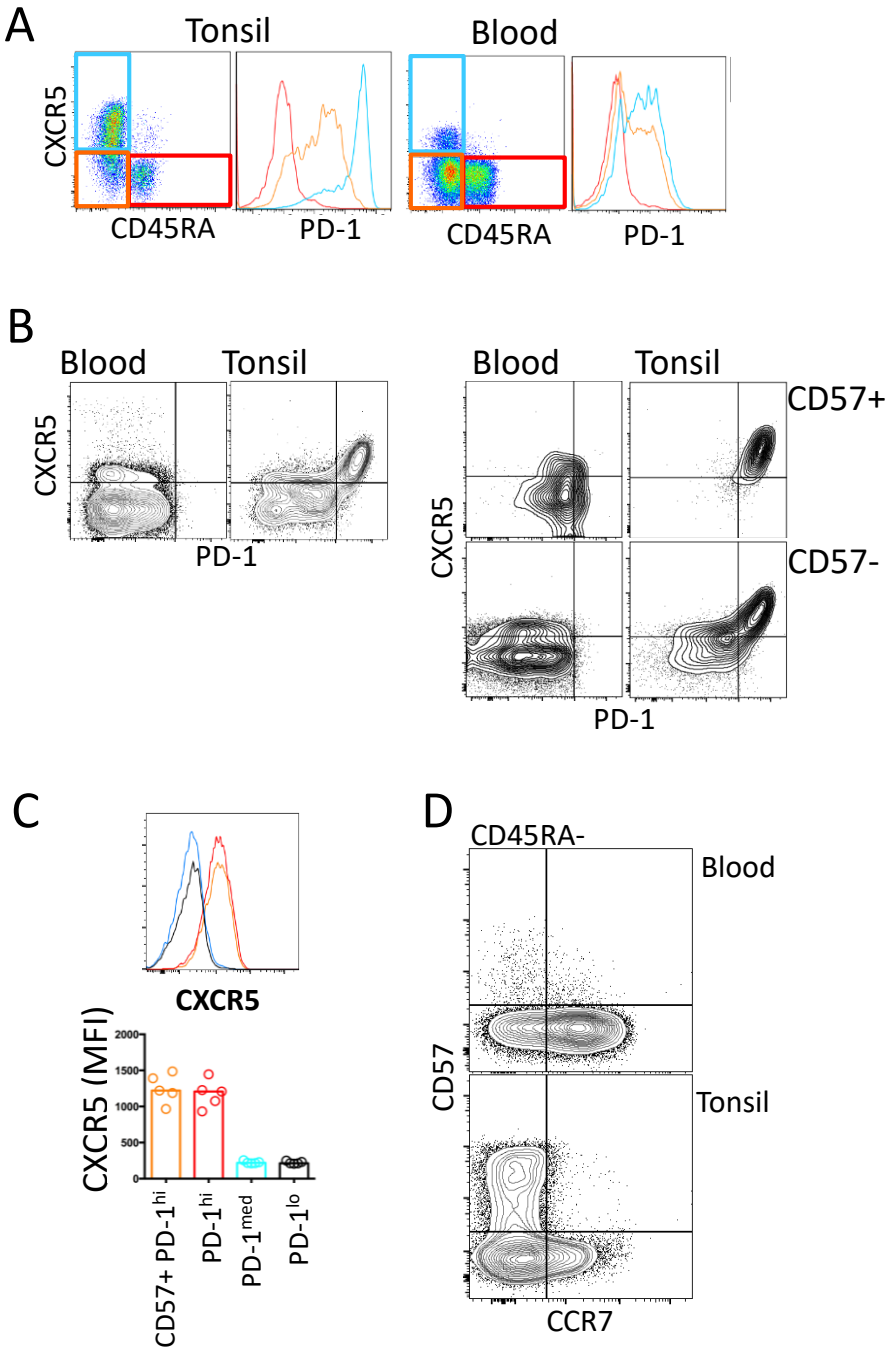
Representative analysis of intracellular IL-10 and IL-4 expression by tonsils sorted according to CD57 and PD-1, either immediately ex vivo, or after activation with CD2/3/28 and either IL-6 or IL-21 for two days.

Fig. S3. Induction of granzyme expression by IL-6

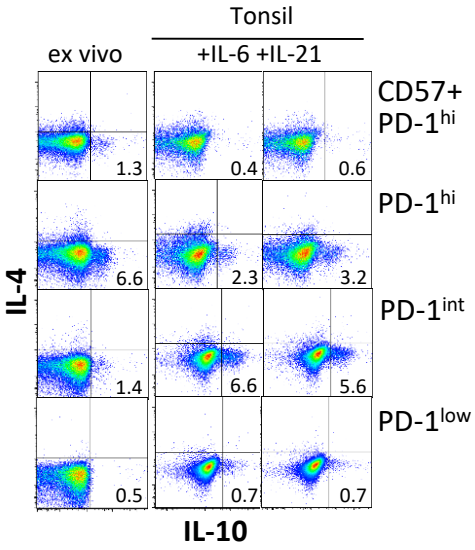
Purified CD57+ and CD57- subsets of tonsils all Pd-1hi were isolated by FACS sorting and stimulated with CD3/28 +/- IL-6 for 1 day then analysed for granzyme expression. Each pair of connected circles indicates the results from a single donor.

Fig. S4. Analysis of IL-21 receptor expression in CD4+ T cell subsets.

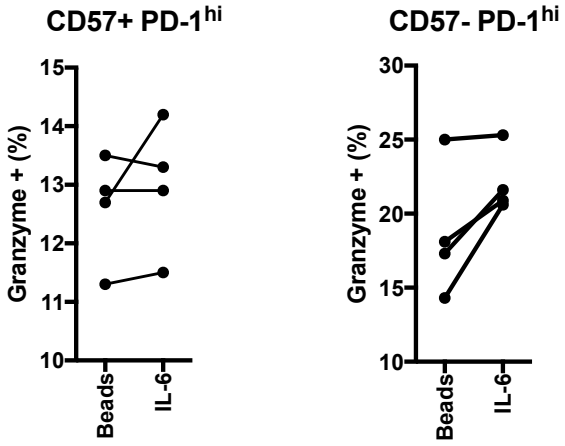
A. Representative histograms of IL-21R expression by tonsil subsets defined according to CD57+ PD-1^{hi} (orange), CD57- PD-1^{hi} (red) and CD57- PD-1^{lo} (black), and presence or absence of CXCR5 (upper and lower panels). Right panels show results from identical analysis as left panels, with the exception of omission of the IL-21R-specific antibody (fluorescence minus one, FMO). B Summary of IL-21R analysis exemplified in **A**, for CD4+ T cells from blood and tonsils, with subsets defined by CD57 and PD-1 expression.



Supplementary fig. 1

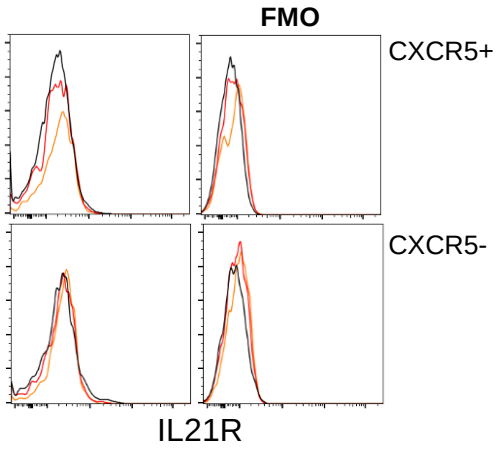


Supplementary fig. 2

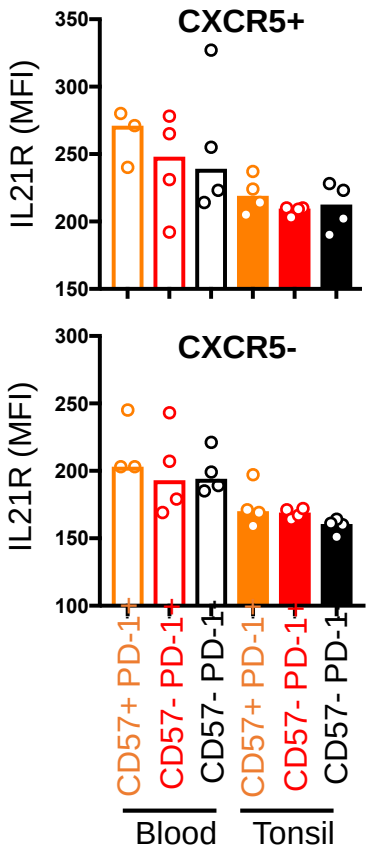


Supplementary fig. 3

A



B



Supplementary fig. 4