B CELL – T CELL DOUBLETS IN GUT ASSOCIATED LYMPHOID TISSUE ARE ENRICHED FOR TFH CELLS BUT NOT FOR HIV DNA

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Introduction

Gut-associated lymphoid tissue (GALT) is a key HIV reservoir site and may play a role in HIV persistence on ART. T Follicular helper (TFH) cells and CD32+CD4+ T cells have been proposed to be enriched for HIV DNA. Here, we show that CD32+CD4+ T cells in GALT are B cell-T cell (B:T) doublets and that sCD40 (a soluble marker shed after B:T cell interaction through CD40/CD154 signalling) but not CD32 is associated with HIV DNA in GALT.

Methods

GALT from the terminal ileum (TI), rectum & tonsil tissue (n=1) was obtained from consenting individuals treated during primary HIV infection (PHI). HIV DNA was quantified in GALT biopsies by qPCR.

CD32 expression on GALT CD4 T cells was measured by flow cytometry (n=19) and imaging cytometry assessed CD19, CD3, CD4, ICOS, HLA-DR & CD32 expression in healthy control GALT and HIV+ tonsil. Associations between HIV DNA & CD32 were tested by Spearman's correlation.

Concurrent plasma samples were used to measure IL-4, IL-5, IL-6, IL10, IL-15, MCP-1, MIP-1α, MIP-1β, IP-10, sCD163, CD40 & CD40L by Luminex (two timepoints measured 1 year apart were available for 9 individuals). LASSO regression analyses & Spearman's correlation were used to test for associations between GALT HIV DNA & plasma variables.

Results

23 PHI individuals were studied; median (IQR) HIV DNA was significantly higher in TI compared to rectum [2.82 (2.58-3.05) vs 2.73 (2.42-2.96) log CPM gut T cells, p=0.03].

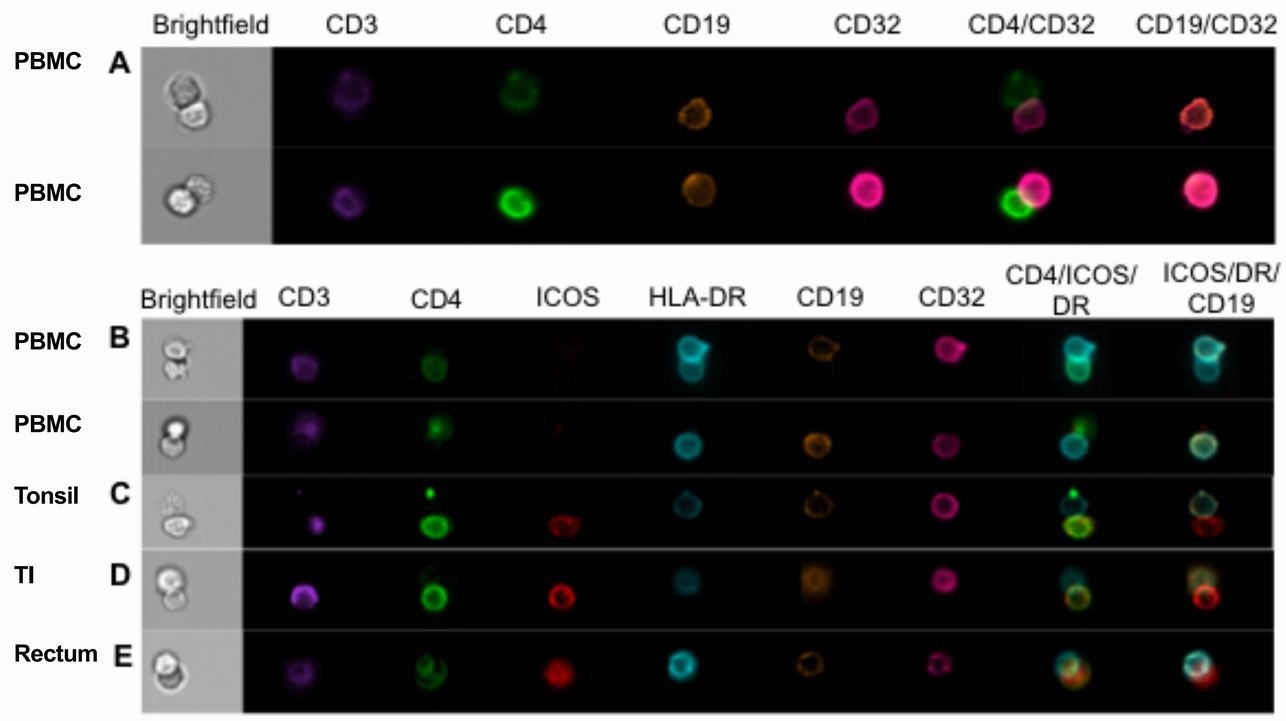
Imaging cytometry analysis showed that CD32 expression on CD4 T cells in GALT (n=1) & HIV+ tonsil (n=1) was consistent with B:T cell doublets with CD32 expression primarily from B cells, while associated CD4+ T cells expressed ICOS, figure (i).

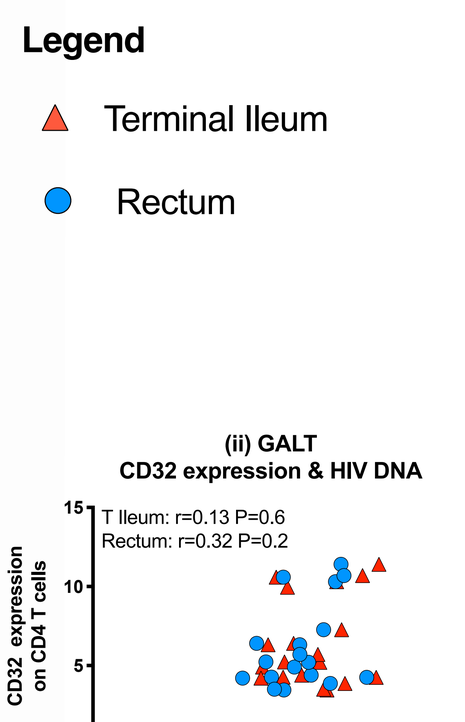
CD32 expression on GALT CD4 T cells was not associated with HIV DNA, figure (ii).

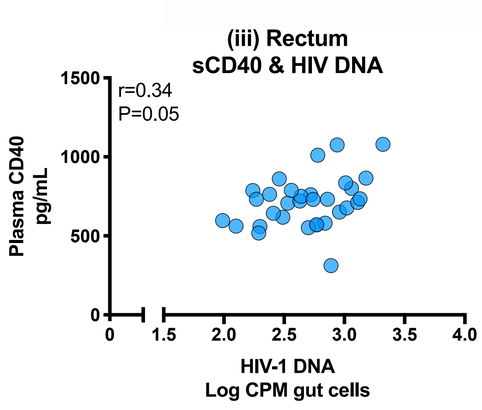
Plasma (n=32) sCD40 (TI: r=0.36 P=0.04, rectum: r=0.34 P=0.05) and sCD14 (TI: r=0.39 P=0.04, rectum: r=0.44 P=0.01) were the variables most strongly association with HIV DNA, figure (iii-vi).

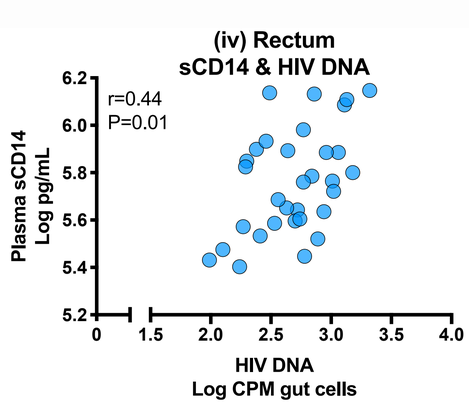
FIGURE (i) Image stream data; showing brightfield, CD3, CD4, CD19, CD32, ICOS & HLA-DR expression on PBMCs (A & B), GALT (D &E) and Tonsil cells (C). (ii) shows GALT CD4 T cell CD32 expression vs. HIV DNA. Rectal HIV DNA levels vs (iii) plasma sCD40 and (iv) sCD14 are shown in blue circles. Terminal ileum HIV DNA levels vs. (v) plasma sCD40 and (vi) plasma sCD14 are shown in red triangles. Abbreviations: PBMC, peripheral blood mononuclear cell; TI, terminal ileum, GALT, gut-associated lymphoid tissue; s, soluble. P values calculated using Spearman's correlation.

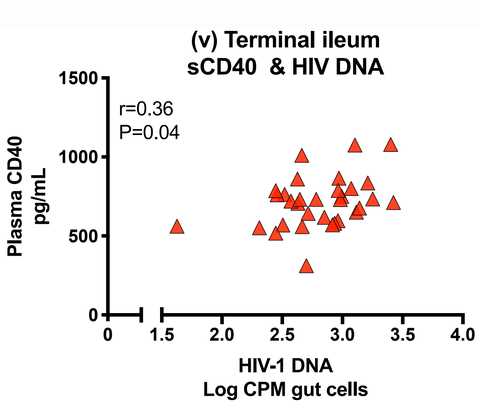
(i)

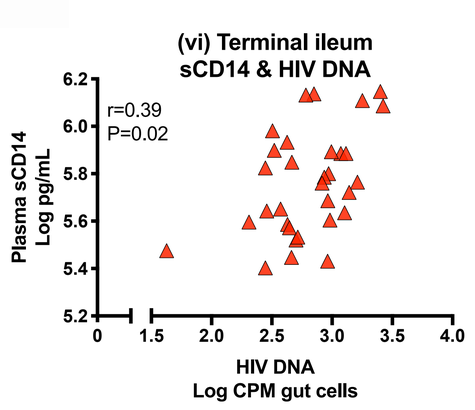












Conclusions

CD32 expression on CD4 T cells in GALT and tonsil when gated as singlets using standard methodology is due to B cell-TFH cell doublets, with CD32 expression primarily on B cells. The enrichment for TFH cells within these doublets raises the issue of whether they are artefactual or physiological.

Plasma sCD40, a marker of the B:T cell interaction, & sCD14, a marker of bacterial translocation, were the factors most associated with HIV DNA, while CD32 expression was not. This suggests that the B:T cell interaction & microbial translocation in GALT may be supporting HIV persistence while CD32 is a surrogate marker of this interaction.

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