**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 assessed 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 associated with HIV DNA, figure (iii-vi).

**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.