Germinal Centre T and B Cells in Lymph Node Fine Needle Biopsies during HIV Infection

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Introduction

- Measurement of HIV-1 reservoirs is usually studied in peripheral blood (PB), but 98% of CD4 T cells are in lymphoid tissues and other organs.
- Histology has shown characteristic hyperplastic germinal centres (GC) in lymph nodes, recently associated with increases in the T follicular helper cell (Tfh) subset of CD4 T cells.
- There is now recognition that GC and Tfh are an important anatomic and cellular reservoir of HIV-1.
- Tfh are infected with HIV-1 DNA and RNA, and follicular dendritic cells retain on their surface, within GC, the most number of virions of any anatomical location in the body

Aim

- We investigated whether ultrasound-guided fine needle biopsies (FNB) from peripheral lymph nodes (LN) can provide a minimally invasive means of accessing HIV-1 reservoirs in GC.

Methods

- Fine needle biopsies were performed using 5 passes of ultrasound guided 25 gauge needles, from 10 healthy controls (HC) and 21 HIV + subjects (10 on antiretroviral therapy (ART), and 11 were ART-naïve), without adverse events.
- Cells were washed once and stained with monoclonal antibodies for 10-colour immunophenotyping, analysed on a 4-laser LSR II.
- Accurate cell counts were performed using TruCount tubes

- Tfh were first gated on CD4 memory T cells, then as PD-1high CXCR5+ICOS+ CD127dim
- Germinal centre B cells were first gate on CD19+ B cells, then as CD38high Ki-67+CD20high Bcl-6+

- CD4 T cells, from FNB and PB, respectively, were purified by cell sorting, from 19 patients
- HIV DNA and cell-associated (CA) HIV RNA were quantified by real-time PCR

- Results for patient groups are shown as medians and interquartile ranges
- Non-parametric Mann-Whitney tests between patient groups were done on PRISM 6

Results

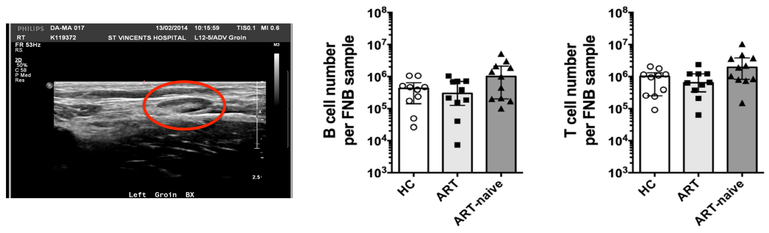


Fig. 1 - Ultrasound image of inguinal LN and recovery of T and B cells

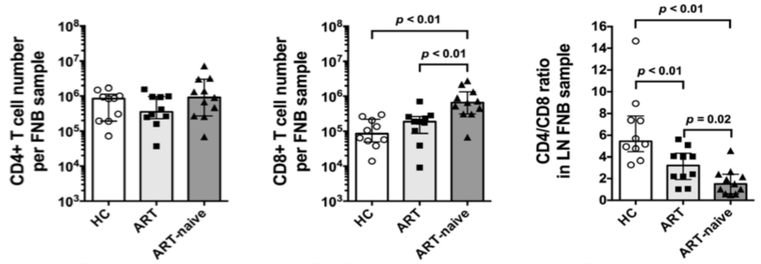


Fig. 2 - Recovery of CD4 and CD8 T cells and decreased CD4:CD8 ratio in HIV+ subjects

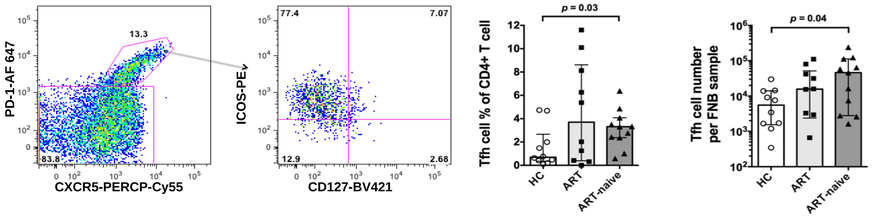


Fig. 3 - Recovery of Tfh: representative flow plots gated on memory CD4 T cells from an HIV+ subject, and summary data for all subjects

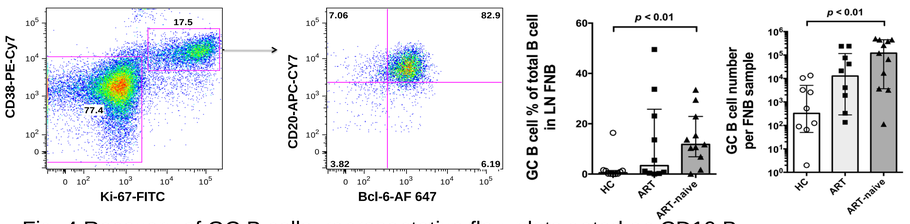


Fig. 4 Recovery of GC B cells: representative flow plots gated on CD19 B cells from an HIV+ subject, and summary data for all subjects

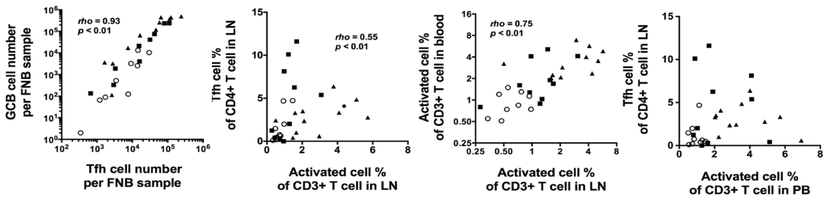


Fig. 5 – Correlations of GC B cells, Tfh and activated T cells (CD3+CD38high Ki-67+) in FNB and with activated T cells in peripheral blood (PB). Circles: HC; Squares: HIV+ ART; Triangles: HIV+ ART-naive

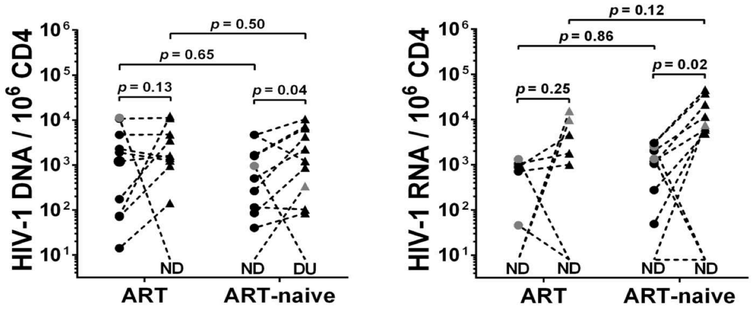


Fig. 6 – Total HIV-1 DNA (left) and unspliced HIV-1 RNA (right) copies per 10^6 CD4+ T cells purified from PB (circles) and LN FNB (triangles) specimens. Grey symbols indicate PBMC or LN FNB samples that were paired with a sample that was not detected (ND) or detected but unquantifiable (DU). Paired WB and LN FNB samples were compared using Wilcoxon signed rank tests. Unpaired WB or LN FNB samples from ART and ART-naïve groups were compared using Mann-Whitney tests.

Conclusions

- Ultrasound guided FNB of unenlarged LN was well-tolerated, and provided sufficient CD4+ T cells and B cells for high-dimensional flow cytometry and cell sorting for PCR
- Tfh cells in inguinal LN were clearly elevated in HIV+ ART-naïve subjects and 4/10 HIV+ subjects on ART
- GC B cells were also elevated in the same HIV+ subjects
- ART does not always normalize Tfh and GC activity in inguinal LN
- Elevated GC activity correlates with T cell activation within LN, but is not necessarily reflected by activated T cells in blood
- CD4+ T cells were sorted from FNB samples from HIV+ subjects and HIV DNA and RNA successfully measured by real-time qPCR
- FNB thereby provide access to Tfh and GC B cells for HIV reservoir, pathogenesis, therapy and vaccine studies.

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