Gut integrity, CD4 T follicular helper cells and IgA+ B cells in GALT following ART

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Introduction

- Disruption of the gastrointestinal tract (GIT) epithelial and immune barriers is believed to contribute to microbial translocation, systemic inflammation and progression of HIV-1 infection.
- ART may lead to reconstitution of gastrointestinal-associated lymphoid tissue (GALT), but its impact on the integrity of the epithelial barrier is unclear.
- We hypothesized that incomplete reconstitution of CD4 T cells in the GALT following ART commenced in chronic HIV-1 infection, compared with commencing in primary HIV-1 infection, may be associated with reduced numbers of CD4+ T follicular helper cells (Tfh).
- This in turn may result in lower levels of locally resident B cells switched to IgA production, loss of first line defense against luminal microbial products, and reduced GIT epithelial integrity.

Aim

- We investigated whether HIV-1+ subjects on ART had decreased numbers of Tfh, GC B cells or IgA+ B cells, in single cell suspensions from gut biopsies, compared to samples from healthy adult controls.

Methods

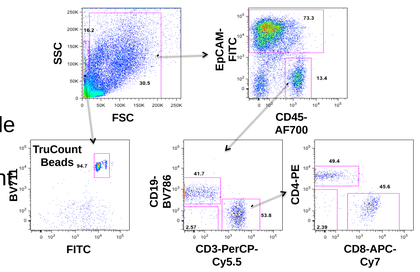
- Gut pinch biopsies were obtained from left colon (LC), right colon (RC) and terminal ileum (TI) from subjects undergoing routine endoscopy: 23 HC and 29 HIV+ subjects on ART, commenced either in primary HIV-1 infection (PHI; n=4); or in chronic HIV-1 infection (CHI; n=25).
- Ten biopsy pieces were pooled from each site, minced and digested for 1 hr with collagenase and DNase I, pushed through a 70 µm cell strainer and carefully washed to avoid cell loss.
- Single cell suspensions were analysed by 9-colour flow cytometry, on a 4-laser LSR II. Accurate cell counts were performed using TruCount tubes
- Initial gating used CD45+ lymphocytes vs EpCAM+ epithelial cells.
- Tfh were then gated on CD4 memory T cells, then as PD-1high CXCR5+
- Germinal centre B cells were first gated on CD19+ B cells, then as CD38high Ki-67+CD20high Bcl-6+.
- IgA+ B cells were gated on CD20+CD27+ B cells

- Confocal endomicroscopy was used to assess gut integrity in vivo in 5 subjects from each group. An Optiscan CIS-2 prototype confocal laser endomicroscope was used to obtain serial microscopic images of the terminal ileum captured at scanning depths 25-70µm during and after IV administration of 5mL 10% fluorescein sodium contrast.

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

Results

Fig. 1 – Gating of CD45+ lymphocytes versus EpCAM+ epithelial cells in single cell suspensions for counting in TruCount tubes



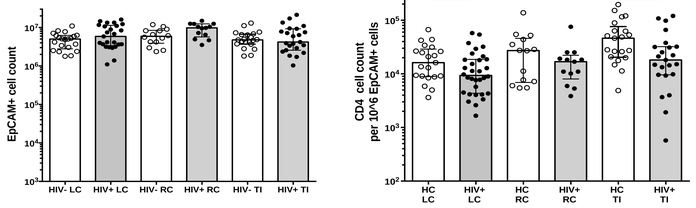


Fig. 2 – Recovery of EpCAM+ epithelial cells (left) and CD4 T cells normalized to epithelial cell count (right)

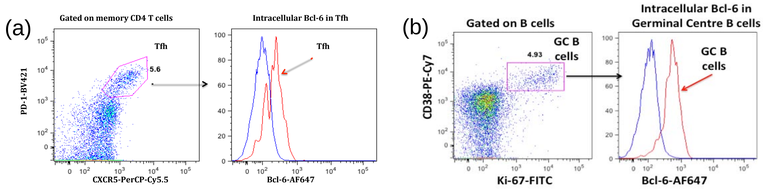


Fig. 3 – Gating of Tfh (a) and germinal centre B cells (b)

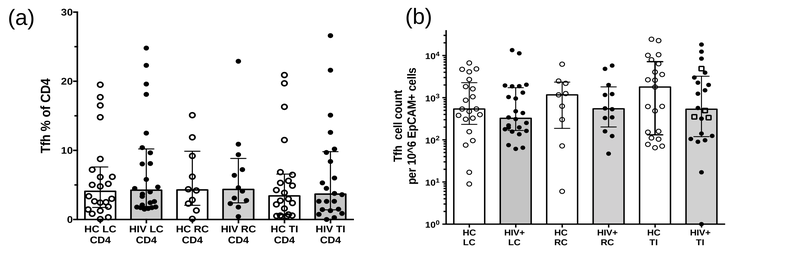
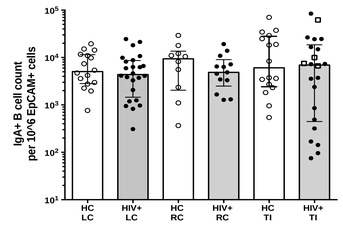


Fig. 4 – Recovery of Tfh cells as % of CD4 T cells (a) and cell numbers (b)
Circles: Healthy controls (HC); Filled circles: HIV+ commenced ART in chronic infection; Squares: HIV+ commenced ART during primary infection



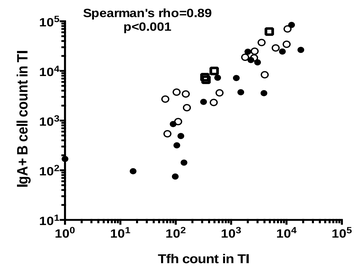


Fig. 5 – Recovery of IgA+ B cells (CD20+CD27+IgA+) as cell numbers (left), and correlation of IgA+ B cells with Tfh (right)
Circles: Healthy controls (HC); Filled circles: HIV+ commenced ART in chronic infection; Squares: HIV+ commenced ART during primary infection

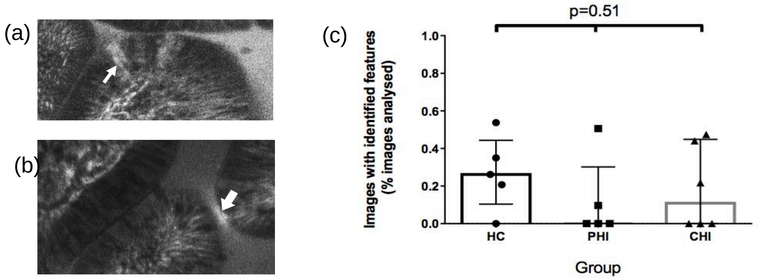


Fig. 6 – Confocal endomicroscopy showing (a) representative area of increased fluorescence from the basal to the apical surface between 2 epithelial cells; (b) Fluoroscein leak, characterised in the epithelial cell layer and a distinct plume of contrast leakage into the lumen; (c) Summary of percent of images with identified features, as shown in (a) and (b), for 3 subject groups, from a median of 889 images per patient.

Conclusions

- We found lower CD4 counts in the TI and LC in HIV+ subjects on ART, when compared with a large number of healthy controls, and using epithelial cell count to account for differences in sampling and digestion of biopsies
- There was no overall significant reduction in Tfh or IgA+ B cell counts compared with healthy controls, although 5 subjects had IgA+ B cell counts in TI below the observed normal range
- IgA+ B cells were highly correlated with Tfh cell numbers
- Whether ART was commenced in PHI or CHI did not appear to be a major factor in this patient group
- In contrast to the current paradigm, confocal endomicroscopy did not find visual evidence of disrupted GIT integrity in treated HIV-1 infection, using features identified in other gastrointestinal pathologies, including inflammatory bowel disease, coeliac disease and gastrointestinal metaplasia

Acknowledgements: This study was funded by the Australian Government Department of Health and Ageing, the Australian NHMRC and a grant from St Vincent's Hospital. The views expressed in this publication do not necessarily represent the position of the Australian Government.