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GUT HOMING DEFECTS AND EXHAUSTION OF BLOOD TH17 AND TH1 CELLS IN HIV INFECTION

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BACKGROUND

- HIV-1 infection induces a breakdown in intestinal homeostasis linked, in part, to the depletion of protective gut mucosal CD4 T helper (Th) cells, resulting in microbial translocation (MT) and inflammation.
- We have previously reported a greater depletion of Th17 than Th1 cells in colon tissue of chronic HIV-1 infected persons not receiving anti-retroviral therapy (ART).[1, 2]
- Numerous mechanisms likely contribute to gut Th cell depletion including altered expression of molecules necessary for circulating Th cells to home to the gut and/or altered expression of gut-specific chemokines necessary for their recruitment into the gut.
- Circulating CD4 T cells display features of increased activation and exhaustion during HIV-1 infection.
  - Immune dysfunction of specific circulating Th subsets and their potential link to gut pathogenesis has not been extensively studied.

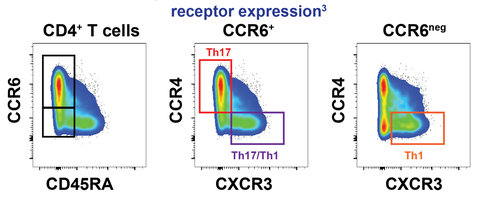
OBJECTIVE

To evaluate gut-homing potential, activation and exhaustion states of peripheral blood Th subsets during untreated, chronic HIV-1 infection and determine colonic levels of gut-homing chemokines.

STUDY DESIGN

- Cross-sectional study: Chronic HIV-1-infected adult participants who were not receiving ART and uninfected adult control participants.
  - Stored peripheral blood mononuclear cells (PBMC) and colonic tissue samples and systemic biomarker measurements were obtained from a previously completed clinical study.[1,2]
- Multi-color flow cytometry: Th17, Th17/Th1 and Th1 subsets were identified in PBMC as memory (CD45RA-) $CD4^+$ T cells expressing:
  - **Th17:** $CCR6^+$ $CCR4^+$ $CXCR3^-$
  - **Th17/Th1:** $CCR6^+$ $CCR4^-$ $CXCR3^+$
  - **Th1:** $CD45RA^-$ $CCR6^-$ $CCR4^-$ $CXCR3^+$
  - Regulatory T cells ($CD25^+CD127^-$) were excluded

Identification of Th17, Th17/Th1 and Th1 cells based on chemokine receptor expression[3]



Phenotypic evaluation:

- Gut-homing: Percentage of cells expressing $\alpha4^+\beta7^+$
- Activation: Percentage of cells expressing $CD38^+HLA\text{-}DR^+$
- Exhaustion: Expression levels of PD1

- RNAseq: Gene transcripts levels of CCL20 (CCR6 chemokine) and CXCL10 (CXCR3 chemokine) were measured in study-participant matched colonic tissue samples.
- Statistical analysis: Non-parametric tests were performed.

Study Participant Characteristics. (values shown as median (range).	Uninfected study participants (N=13)	HIV-1 infected study participants (N=16)
Age (yrs)	29 (23-54)	32 (22-52)
Male/Female	8/5	12/4
CD4 count (cells/µl)	720 (468-1071)	410 (238-782) $^{P=0.002}$
Plasma viral load (HIV-1 RNA copies/ml)	-	66,600 (2,880-207,000)

ACKNOWLEDGEMENTS

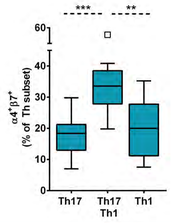
We gratefully acknowledge and thank the physicians, staff and patients in the Infectious Diseases Group Practice at the University of Colorado Hospital and the uninfected study participants for their assistance and involvement in our clinical study. We also thank the staff of the Endoscopy Lab, University of Colorado. This work was supported by the National Institutes of Health Grants R01 DK088663, R01 AI108404 and, in part, by NIH/NCATS Colorado CTSI Grant Number UL1 TR000154.

References:

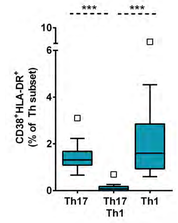
1. Dillon *et. al.* 2014 (PMID: 24399150). **2.** Dillon *et. al.* 2016 (PMID 25921339). **3.** Wacleche *et. al.* 2017 (PMID 29048384)

1. Phenotypic profiles of blood Th cell subsets

Gut-homing: $\alpha4^+\beta7^+$



Activation: $CD38^+HLA\text{-}DR^+$



Exhaustion: PD1

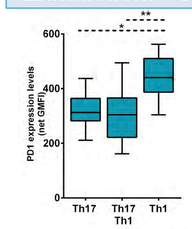
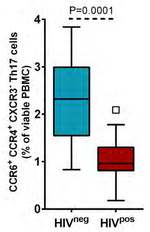


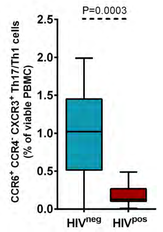
Figure 1. Blood Th subsets exhibit differential phenotypic profiles. Expression was evaluated in uninfected study participants.

2. Frequencies of blood Th cells during HIV-1 infection

Th17



Th17/Th1



Th1

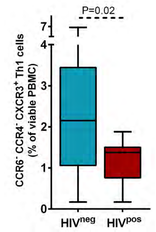
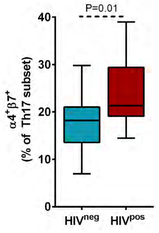


Figure 2. Frequencies of Th17, Th17/Th1 and Th1 cells are decreased in PBMC during untreated, chronic HIV-1 infection.

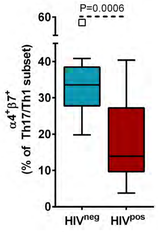
3. Alterations in gut-homing by blood Th cells during HIV-1 infection

A) α4β7 expression

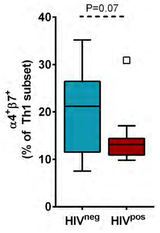
Th17



Th17/Th1

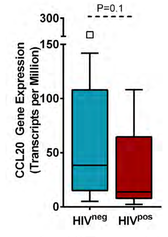


Th1



B) Colonic chemokine gene expression

Colonic CCL20



Colonic CXCL10

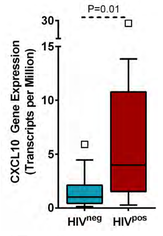
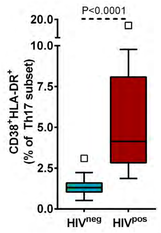


Figure 3. Expression of α4β7 was higher on blood Th17 cells and lower on blood Th17/Th1 and Th1 cells in keeping with decreased colonic expression of CCL20 (CCR6-specific chemokine) and increased gene expression of CXCL10 (CXCR3-specific chemokine).

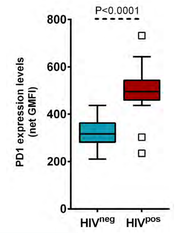
4. Activation and exhaustion levels of blood Th cells are increased during HIV-1 infection

Th17

$CD38^+HLA\text{-}DR^+$

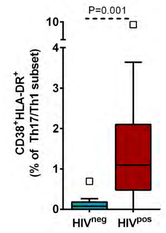


PD-1

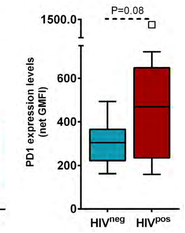


Th17/Th1

$CD38^+HLA\text{-}DR^+$

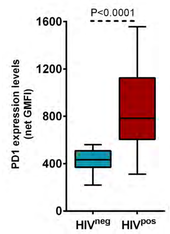


PD-1



Th1

$CD38^+HLA\text{-}DR^+$



PD-1

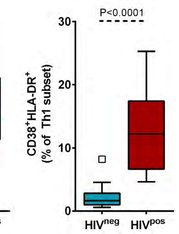
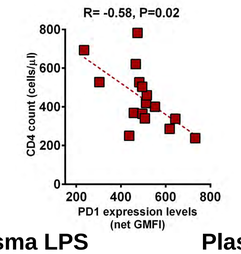


Figure 4. Blood Th17 and Th1 cells display features of increased activation and exhaustion during untreated, chronic HIV-1 infection. Alterations in Th17/Th1 cell phenotypes are less pronounced.

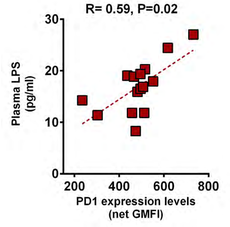
5. Expression of exhaustion and activation markers on Th cells correlate with indicators of HIV-1 disease progression and MT

Th17 cells: PD1 expression

CD4 count

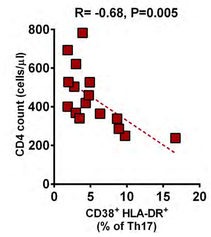


Plasma LPS

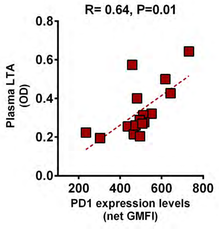


Th17 cells: activation

CD4 count



Plasma LTA



Th17/Th1 and Th1 associations:

- $CD38^+$ $HLA\text{-}DR^+$ Th17/Th1 cells inversely correlate with CD4 count: R= -0.65, P=0.01
- PD1 expression levels on Th17/Th1 positively correlate with plasma LPS: R= 0.62, P=0.046
- PD1 expression levels on Th1 cells positively correlate with plasma LTA: R= 0.63, P=0.01

Figure 5. Levels of PD1 expression inversely correlate with CD4 count and positively correlate with plasma levels of LPS and LTA. PD1 expression levels on Th1 cells and Th17/Th1 cells positively correlated with levels of plasma LTA or LPS respectively. Percentages of activated Th17 and Th17/Th1 cells inversely correlated with blood CD4 count. Dotted line is a visual representation of direction and strength of correlation.

SUMMARY & CONCLUSIONS

- An accumulation of blood Th17 cells expressing α4β7 may result from decreased colonic expression of CCR6-specific chemokine necessary for their homing to the gut.
- Blood Th17, Th17/Th1 and Th1 cells display features of increased activation and exhaustion which are variably associated with indicators of disease progression and microbial translocation **suggesting a link between dysfunctional circulating Th cells, in particular Th17 cells, and HIV-associated gut pathogenesis and disease progression.**