Phenotype and cytokine expression of peripheral and gut-derived γδ T cell subsets from HAART-treated HIV+ individuals and healthy controls

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Introduction: Despite successful treatment, alterations in gut immunity are still observed in highly active antiretroviral therapy (HAART)-treated HIV+ patients. These changes are associated with elevated mucosal permeability which contributes to the chronic immune activation observed in these individuals. γδ T cells are important cells in the maintenance of homeostasis in the gut, however, the impact of HIV infection on the various γδ T cell subsets remains to be established.

Methods: Peripheral blood and pinch biopsies from the rectum were collected from HAART treated HIV+ individuals (CD4 count of 772 ± 194 cells/ul, ART suppression of 4.4 ± 1.6 years) and healthy controls. Phenotypic analysis of γδ T cell populations was performed using flow cytometry. γδ T cell population functionality was evaluated based on their expression of IFN-γ, TNF-α, IL-17, and IL-22 following stimulation with PMA and ionomycin.

Results: In the periphery, the overall proportion of γδ T cells among the $CD3^+$ population did not differ between HIV+ and healthy individuals, however the Vδ1:Vδ2 ratio was inverted in HIV+ individuals. In the gut, the proportion of γδ T cells among $CD3^+$ cells was not significantly different between the two groups. When evaluating cytokine expression, a similar γδ T cell cytokine profile was observed in the periphery between the two groups, however, there was a higher proportion of IFN-γ^+ cells in the gut of HIV+ compared to healthy individuals.

Conclusion: Despite effective therapy, the proportion of IFN-γ^+ γδ T cells in the gut remains higher than that seen in the HIV uninfected individuals and might play a role in the impaired gut immunity and chronic immune activation that persists in treated HIV+ individuals.

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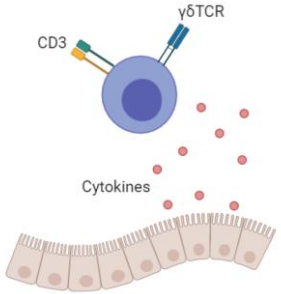
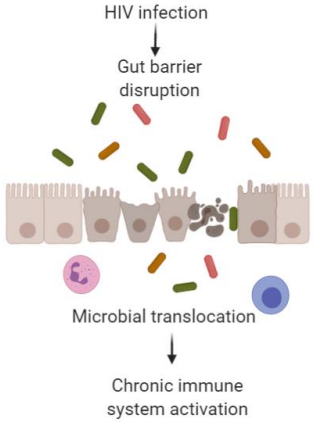
Background

- γδ T cells: innate-like lymphocytes
  - Express a unique T cell receptor (TCR) with limited antigen specificity
  - Recognize microbial metabolites and signals of cell stress [1]
  - 2 subtypes: Vδ1 and Vδ2, more predominant in the gut and periphery, respectively [1,2]

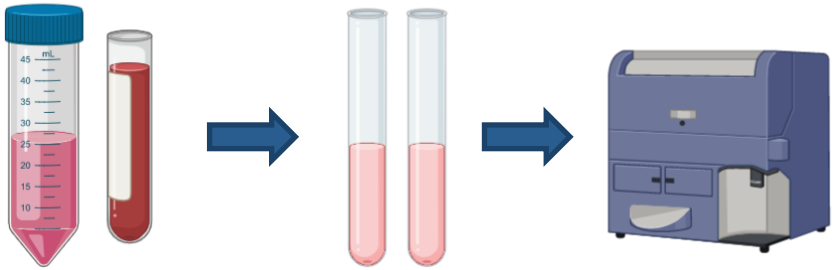


- Role of γδ T cells in the gut
  - Help maintain epithelial function and integrity [3]
  - Secrete IFN-γ, TNF-α, IL-17, and IL-22 [4,5]

- HIV infection and γδ T cells
  - HIV infection disrupts gut barrier integrity and highly active antiretroviral treatment (HAART) is not able to restore it [6]
  - The impact of HIV infection on γδ T cells subsets in the periphery and in the gut is still poorly understood



Methodology



Isolation of peripheral blood mononuclear cells (**PBMC**) and rectal mononuclear cells (**RMC**)

Stimulation with **PMA/IO for 5h, with Golgi inhibitor** (to induce accumulation of the secreted proteins inside the cell)

γδ T cells phenotype and **cytokine expression** evaluation using **Flow Cytometry**

Results

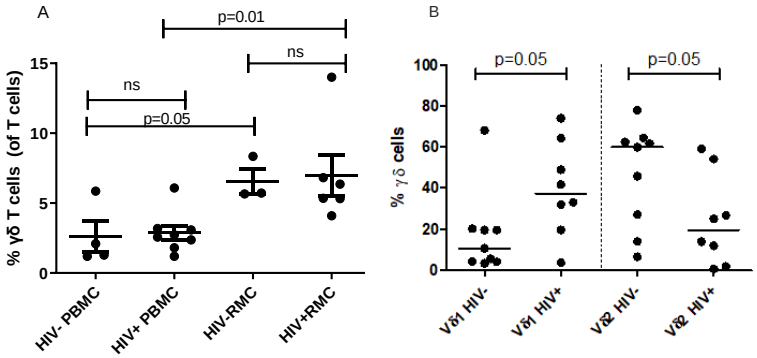


Figure 1. Analysis of γδ T cells in PBMC and RMC from healthy and HIV-seronegative individuals. Peripheral blood mononuclear cells (PBMC) and rectal mononuclear cells (RMC) were isolated from HIV seronegative and HAART-treated HIV+ individuals and γδ T cells were assessed by flow cytometry. **(A)** Proportion of γδ T cells in the CD3+ T cell population from HIV seronegative and HIV infected individuals. **(B)** Proportion of Vδ1 and Vδ2 subsets within the γδ T cell population in the blood from HIV-seronegative and HIV-infected individuals (PBMC: healthy n=8, HIV n=8; RMC: healthy n=3, HIV n=6). Significant differences are shown in the graph.

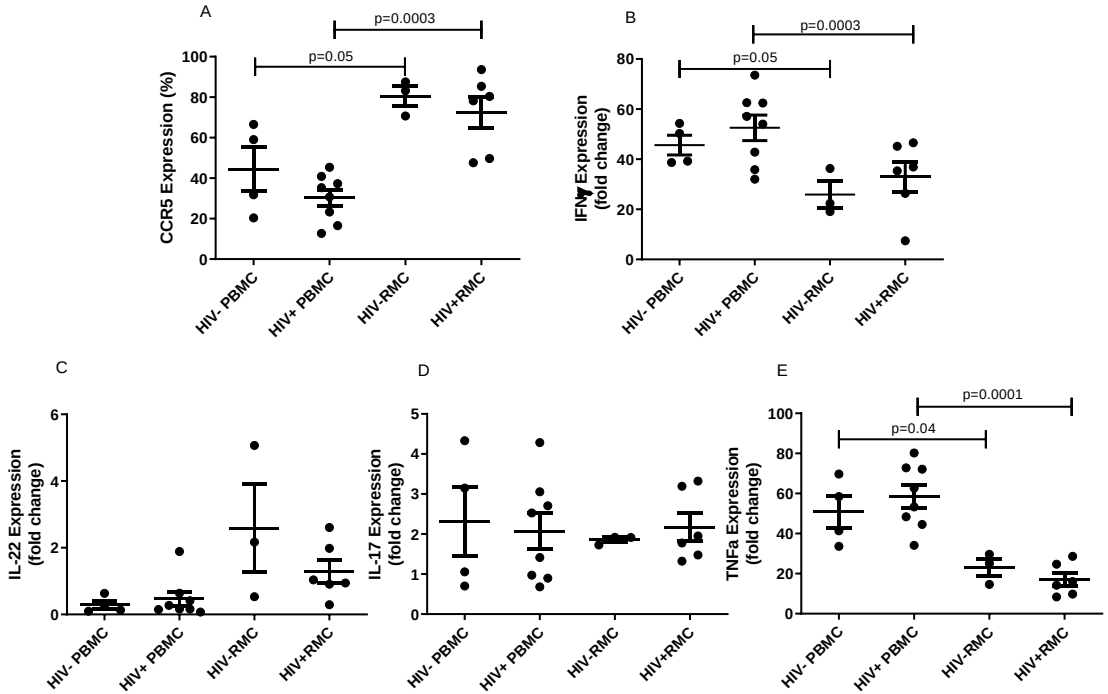


Figure 2. Analysis of different γδ T cells subsets in PBMC and RMC from healthy HIV-seronegative individuals. Peripheral blood mononuclear cells (PBMC) and rectal mononuclear cells (RMC) were isolated from HIV seronegative and HAART-treated HIV+. γδ T cell expression of CCR5 was evaluated before stimulation **(A).** Following stimulation with PMA/IO for 5h, expression of IFN-γ **(B)**, IL-22 **(C)**, IL-17 **(D)**, TNFα **(E)** were evaluated by flow cytometry and fold change is shown in the graph (PBMC: healthy n=8, HIV n=8; RMC: healthy n=3, HIV n=6). Significant differences are shown in the graph.

Results

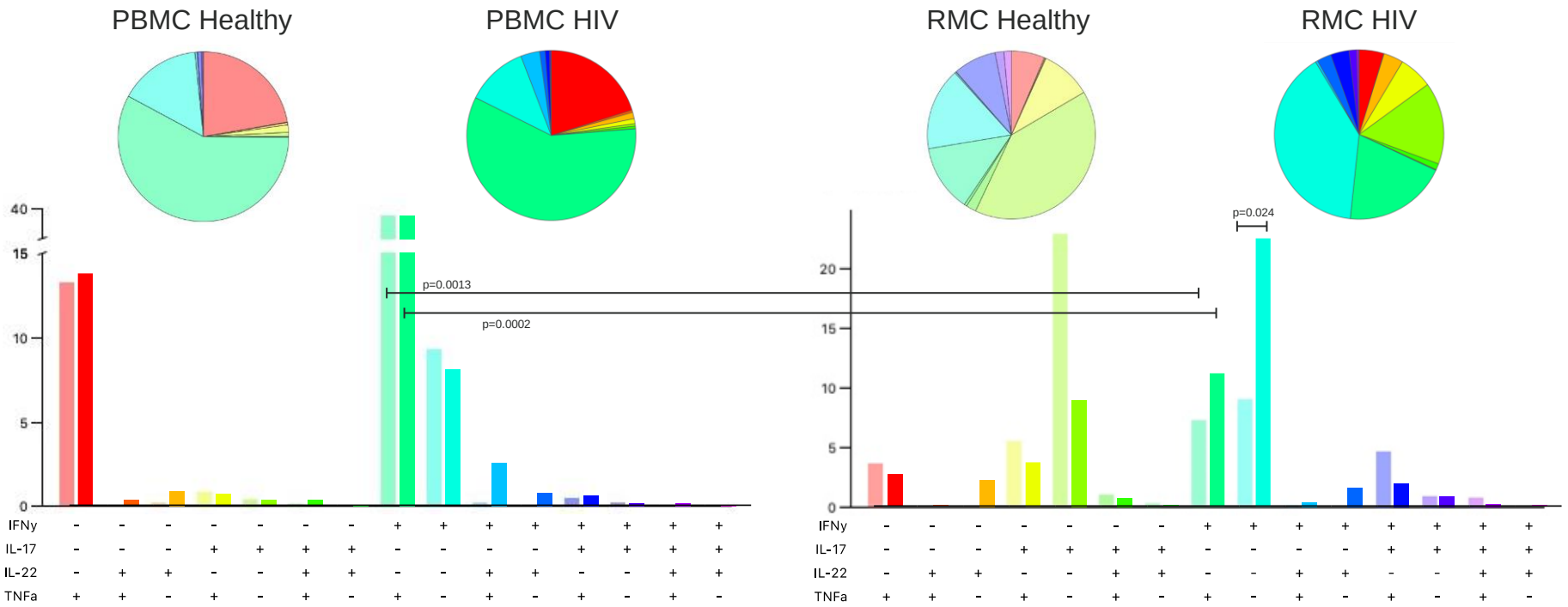


Figure 3. Polyfunctional γδ T cells subsets in PBMC and RMC from HAART-treated HIV+ individuals and healthy HIV-seronegative controls. Peripheral blood mononuclear cells (PBMC) and rectal mononuclear cells (RMC) were isolated from HIV seronegative (pale colors) and HAART-treated HIV+ individuals (vivid colors). Cells were stimulated with PMA/IO for 5h and analyzed by flow cytometry (PBMC: healthy n=8, HIV n=8; RMC: healthy n=3, HIV n=6). Significant differences are shown in the graph.

Conclusion

- In the periphery, HIV infection impacts γδ T cells by inducing inversion in the Vδ1:Vδ2 ratio, as previously demonstrated [7], however, there was no difference in the cytokine production within γδ T cells when comparing healthy and HIV+ individuals.

- In the gut, a higher proportion of IFNγ+ γδ T cells was observed in cells derived from HIV+ individuals. Interestingly, there was a trend for higher proportion of IL-17+ γδ T cells in the healthy controls when compared to the HIV+ individuals (p=0.11). This observation could suggest a protective role of IL-17 in the gut in this context [8], however, this possibility needs to be evaluated in a bigger cohort.

- Furthermore, in the gut, a higher percentage of TNFα+IFNγ+ γδ T cells was observed in both groups when compared to the peripheral blood.

- In conclusion, phenotypical and functional differences in the various γδ T cells subsets might play a role in the gut immunity impairment that persist in HIV individuals, despite sucessful treatment.

Conflict of Interest Disclosure

We have no conflicts of interest

Acknowledgments

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