388: THE HIV RESERVOIR RESIDES MAINLY IN CD32– /CD4+ T CELLS IN PERINATAL INFECTION

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Introduction

- The latent HIV reservoir in resting memory CD4+ T cells is not amenable to targeting by immune mechanisms or antiretroviral drugs due to viral quiescence.
- Recently, the low affinity Fc receptor CD32a, not usually present on T cells, was shown to be highly enriched for inducible proviral genomes in CD4+ T cells of HIV-infected, ART-suppressed adults[1]. This marker has potential for targeting therapies aimed at eradicating CD4+ T cell reservoirs towards HIV remission and cure[2].
- The presence of the CD32a reservoir in perinatal infection is not defined.

Objectives

- Determine the expression of CD32 on CD4+ T cells in perinatally-infected, ART-suppressed participants.
- Determine the size of the induced viral reservoir in CD32+ and CD32-/CD4+ T cells using the quantitative viral outgrowth assay (QVOA).
- Assess differences in growth kinetics and replication competence in the induced proviral genomes harbored in CD32+ and CD32-/CD4+ T cells.

Methods

- PBMCs were isolated from whole blood by Ficoll density gradient centrifugation (GE Healthcare) and cryopreserved in 10% DMSO until further processing for cell sorting (Becton Dickinson MoFlo) into CD32+ and CD32-/CD4+ T cell populations using the following methods:
- **Method 1**: PBMCs were enriched for CD4+ T cells, using negative bead selection (Miltenyi Biotec) prior to cell sorting into CD3+/CD4+/CD32+ and CD3+/CD4+/CD32- cell populations (Figure 1A). The following antibody panels were used for cell sorting: CD4-PE, CD3-FITC (BD Biosciences) and CD32-APC (Sony Biotechnology).
- **Method 2**: PMBCs were stained with the following antibody panel: CD4-PE, CD3-FITC, HLA DR-BV605, CD19-PE-Cy7, CD14-BV421 (BD Biosciences) and CD32-APC (Sony Biotechnology), and sorted into CD3+/CD4+/CD32+ and CD3+/CD4+/CD32- populations (Figure 1B).
- Sorted cell populations from both methods were cocultured in the QVOA and carried to 14 days.
- The size of the reservoir, expressed as infectious units per million cells (IUPM), was determined using maximum likelihood methods[3] based on the detection of p24 antigen concentrations in the culture supernatant on day 14 of coculture using the ultrasensitive p24 SIMOA (Quanterix). The kinetics of virus replication between the two cell populations were characterized by examining differences in p24 in the culture supernatant between days 7 and 14 of coculture.

Results

Table 1: Participant Demographics and HIV Virologic Profiles

ID	Sex	Age (yrs)	Race	Duration of Virologic Suppression (yrs)	HIV-1 DNA (copies per million PBMCs)	%CD32+ CD4+ T cells
Method 1: Sorting of Enriched CD4+ T cells						
0301.1	F	21	AA	4.8	132.1	0.312
0302.1	M	18	Mixed	17.3	106.7	0.055
0115.2	M	17	AA	3.9	861.4	0.150
0116.2	F	18	AA	4.6	2.2	0.022
Method 2: Sorting Directly from PBMCs						
0301.2	F	21	AA	5.3	81.1	0.207
0302.2	M	19	Mixed	17.6	127.9	0.068
0304.1	F	15	AA	12.5	206.2	0.058

AA: African American

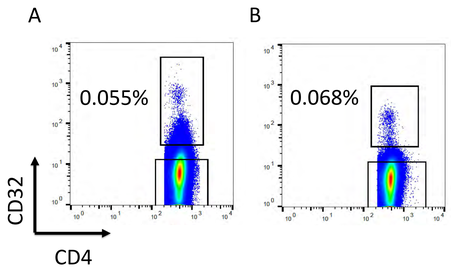


Figure 1: Representative populations of CD32+ and CD32- cells in CD3+/CD4+ cells sorted using Method 1 **(A)** and Method 2 **(B)** for coculture.

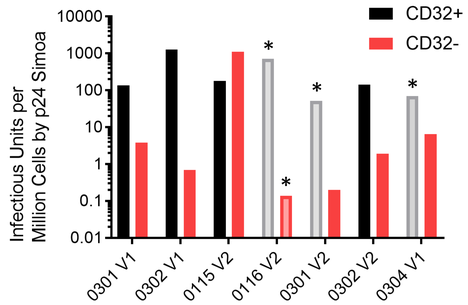


Figure 2: Detectable infectious proviral genomes in both CD32+ and CD32-/CD4+ T cells. *p24 concentrations below the LOQ at day 14, bars represent maximum likelihood estimates.

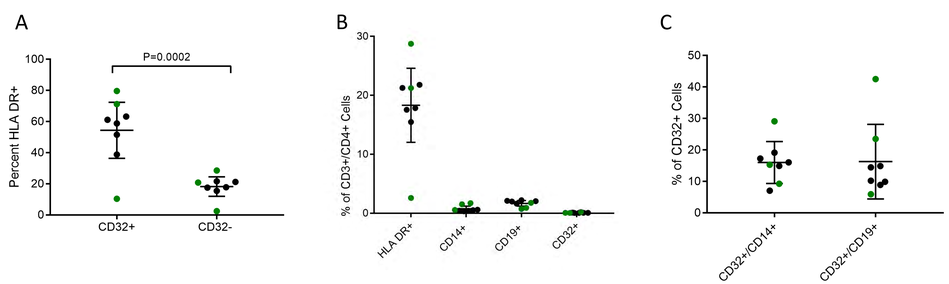


Figure 3: (A) Significantly higher proportion of CD32+ cells express HLA DR compared to CD32-/CD4+ T cells. **(B)** Distribution of HLA DR, CD14, CD19 and CD32 expression in sorted CD3+/CD4+ T cells. **(C)** Expression of CD14 and CD19 on CD3+/CD4+/CD32+ T cells. Green dots represent sorted and cultured cells, black dots represent staining analysis only.

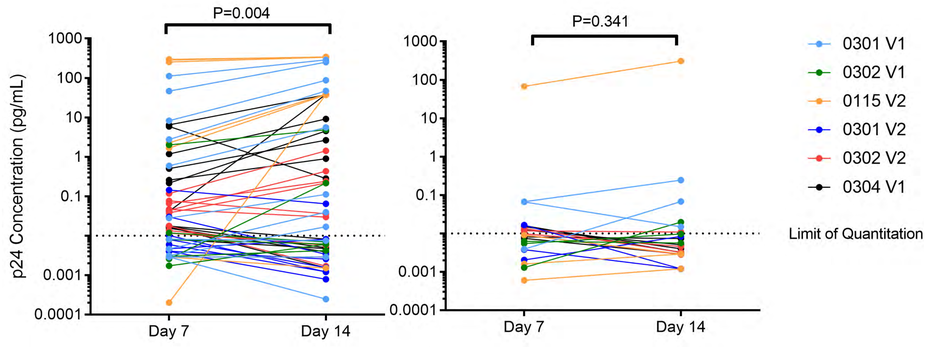


Figure 4: Detectable p24 concentration in coculture of CD32+ and CD32-/CD4 T cells. There was a significant increase in p24 from day 7 to 14 in CD32- cells only.

Conclusions

- A CD32+/CD4+ T cell population is present in perinatal infection with a mean of 0.12%.
- A significantly higher proportion of CD32+/CD4+ T cells express HLA DR, as compared to CD32-/CD4+ T cells, suggesting a relationship between the presence of CD32+ and immune activation.
- Induced proviral genomes are detectable in CD32+ and CD32-/CD4+ T cells. However, ultrasensitive p24 Simoa was required for detection in CD32+ cells.
- p24 concentrations in CD32-/CD4+ T cells significantly increase from day 7 to 14 (P=0.004) but not in CD32+/CD4+ T cell cocultures (P=0.341).
- Combined with the low frequency and lower replication potential of induced proviral genomes in CD32+/CD4+ T cells, targeting of these cells will have a negligible effect on depletion of highly replication competent HIV reservoirs in perinatal infection.

References and Acknowledgements

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