# Abstract 389: CD4⁺ T Cells Expressing CD32 From HIV-1-infected Patients Are Not Enriched for Proviral DNA

Adam M. Spivak[1], Racheal A. Nell[1], McKenna L. Coletti[1], Luis J. Montaner[2], Vicente Planelles[1]

[1]*University of Utah School of Medicine, Salt Lake City, UT, USA* and [2]*The Wistar Institute, Philadelphia, PA, USA*

**Contact Information**
Adam M. Spivak M.D.
Division of Infectious Diseases
University of Utah School of Medicine
30 North 1900 East 4B319 SOM
Phone: 801 585 7433
Fax: 801 585 3377
adam.spivak@hsc.utah.edu

## Background

• Combination antiretroviral therapy (ART) has rendered HIV-1 infection a manageable illness, however ART is not curative due to the presence of long-lived cells in which the virus remains dormant (known as the latent reservoir). Latently infected CD4⁺ T cells do not express viral antigens and at present cannot be distinguished from uninfected CD4⁺ T cells, hindering efforts to identify and target this reservoir.

• A recent publication described enrichment of the latent reservoir in resting CD4⁺ T cells (rCD4) expressing the low-affinity Fc receptor CD32 [1]. Identification of a cell surface marker that distinguishes the minority of cells harboring latent HIV-1 provirus from the larger pool of circulating, uninfected CD4⁺ T cells would represent a crucial scientific advance.

• Using cells obtained from aviremic HIV-1-positive participants, we hypothesized that CD32 expression would identify a CD4⁺ T cell population enriched for proviral DNA.

## Methods

• Cell isolation: we isolated rCD4 cells from peripheral blood mononuclear cells (PBMCs) obtained from aviremic, HIV-1-infected individuals on ART and healthy uninfected controls using magnetic bead negative selection (StemCell Technologies).

• Proviral DNA frequency: rCD4 cells from patients (n=6) underwent CD32 antibody-magnetic bead pull-down to purify CD32+ and CD32- populations. Cellular DNA was isolated from total rCD4 cells, CD32+ and CD32- cells and quantitative PCR was performed using primers and probes specific to the HIV-1 5'LTR per published protocol that quantifies copies of the CD3 gene in parallel to normalize cellular DNA input [2].

• HIV-1 RNA expression: rCD4 cells from patients (n=3) were stained with fluorophore-conjugated CD32 antibodies (CD32-PE, FUN-2 clone, Biolegend Inc.). Florescence activated cell sorting (FACS) was used to purify CD32+ and CD32- rCD4 populations. Cells were then cultured in the presence or absence of CD3/CD28 antibodies. After 72 hours, cell-associated HIV-1 mRNA transcripts were quantified via qPCR using published methods [3,4].

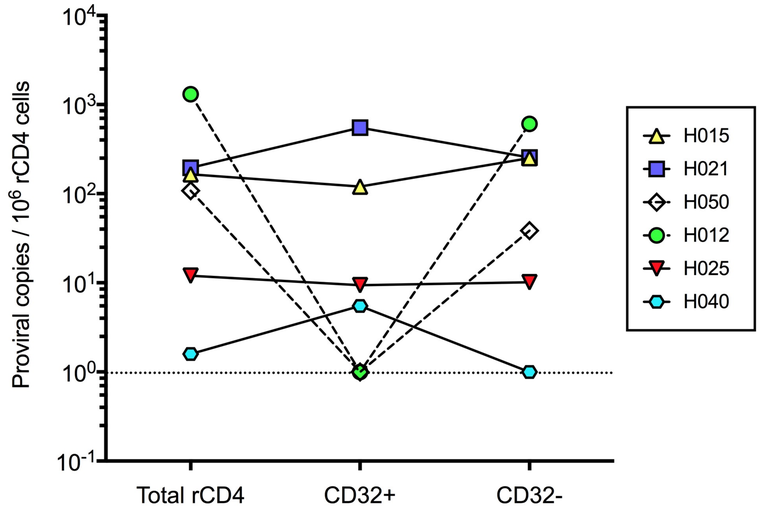
• CD32 expression and dynamics *in vitro*: total CD4⁺ T cells (resting + activated T cells) were isolated from PBMCs obtained from healthy, uninfected donors. A subset of total CD4⁺ T cells were activated via exposure to CD3/CD28 antibodies and subsequently infected using a replication-defective psuedotyped NL4-3 virus expressing green florescent protein (GFP) upon active infection. Expression of GFP, CD32 and HLA-DR (a cell-surface marker of T cell activation) was quantified by multi-color flow cytometry.

## References

[1] Descours Nature 2017
[2] Vandergeeten J Virol 2014
[3] Shan J Virol
[4] Spivak AAC 2015

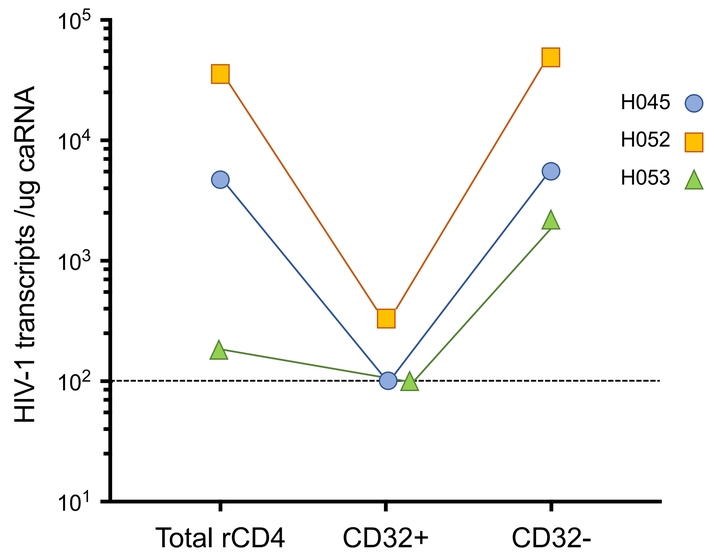
## Results

### CD32 expression does not enrich for rCD4 cells harboring HIV-1 proviral DNA



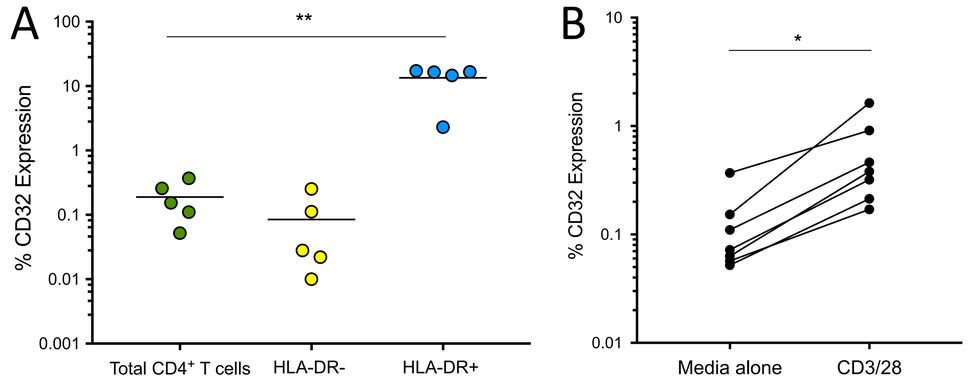

Resting CD4⁺ T cells from aviremic HIV-1-positive donors (n=6) expressing CD32 did not contain significantly more proviral DNA than the CD32-negative population or total rCD4 cells (repeated measures one-way ANOVA, P=0.48). Proviral DNA was undetectable in CD32+ cells from two donors, H012 and H050.

### HIV-1 RNA expression is decreased among CD32+ rCD4 cells from aviremic patients



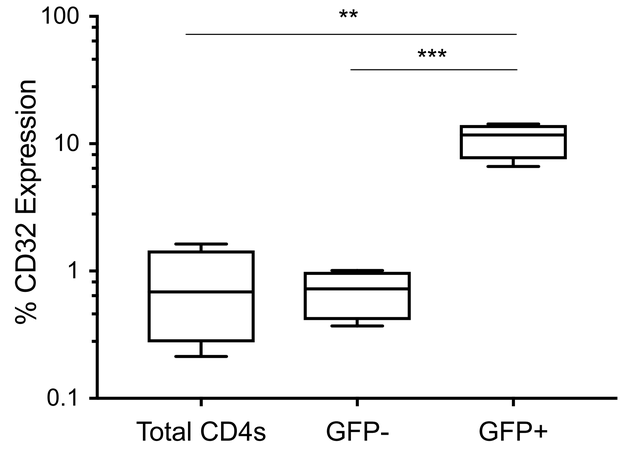

rCD4 cells from HIV-1-positive donors (n=3) underwent flow sorting to isolate pure CD32+ and CD32- populations. After T cell receptor stimulation for 72 hours, cell-associated RNA transcripts were quantified via qPCR. Two of three CD32+ cultures had no detectable HIV-1 mRNA cell-associated transcripts. Total rCD4 and CD32- cultures had similar HIV-1 expression.

### CD32 expression is associated with activated CD4⁺ T cells




**Panel A**: CD32 expression is low among total CD4⁺ T cells isolated from peripheral blood (n=5). Cells expressing the activation marker HLA-DR are significantly enriched for CD32 expression compared to the total CD4 population (P=0.0015). **Panel B**: CD32 expression increases on CD4⁺ T cells after 72 hour exposure to CD3/28 antibodies compared to cells maintained in medium alone cultures (P=0.015; n=7).

### CD32 expression is enriched in productively infected CD4⁺ T cells *in vitro*




Healthy donor (n=4) CD4⁺ T cells were activated and infected with a GFP-expressing NL4-3 psuedotyped virus. CD32 and GFP were quantified 48 hours post-infection by flow cytometry. CD32 expression increased approximately 10-fold on productively infected CD4⁺ T cells (GFP+) compared to uninfected cells (GFP-; P=0.0009) or all cells in culture (Total CD4s, P=0.001).

## Conclusions

- CD32 expression on rCD4 cells from aviremic HIV-1-positive participants did not identify a population of cells enriched for proviral DNA.
- CD32+ CD4 cells from aviremic HIV-1-positive donors expressed less HIV-1 mRNA upon cell activation than CD32-negative or unsorted rCD4 cells.
- CD32 expression was associated with T cell activation and productive HIV-1 infection *in vitro*.

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