# Productive HIV-1 Infection Upregulates CD32 *in vitro* and *in vivo*

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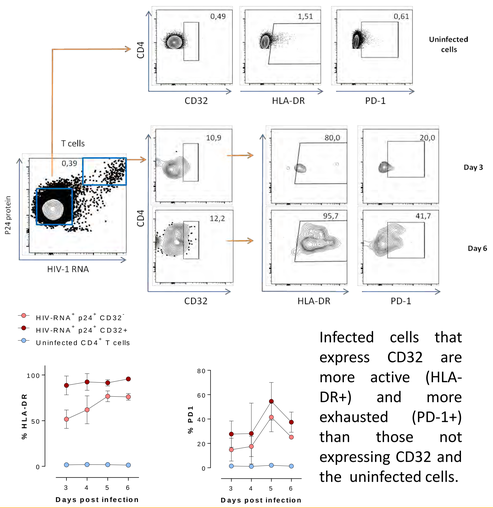
**Background:** The cell surface receptor protein CD32a has recently been postulated as a marker of a CD4+ T-cell HIV reservoir harboring replication-competent proviruses in ART-suppressed subjects. The expression of CD32 in different CD4+ T-cell subsets in HIV-infected patients and expression in persistent infected cells retained within lymphoid tissue after ART are important questions that still need to be evaluated.

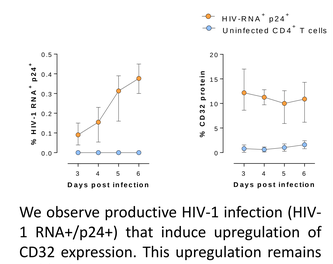
**Methods:** The expression of CD32 in different CD4+ T-cell subpopulations was measured in 9 ART-treated patients by fluorescence activated cell sorting (FACS) immunophenotyping using antibodies that recognized CD3, CD4, CD27, CD45RO, CD32 and HLA-DR. HIV and CD32a RNA expression in tissue was measured by fluorescent in situ hybridization (FISH) in lymph node sections from 2 aviremic and 4 viremic HIV-infected patients. Ex vivo HIV-1 infection kinetics was measured by the RNA/flow technique during 6 days after the initial infection of healthy donor unstimulated PBMCs, and in a cervico-vaginal tissue explant model of HIV infection. Wilcoxon signed rank and spearman's rank correlation tests were used for statistical analysis.

**Results:** Terminally-differentiated CD4+ T cells expressed significantly higher proportions of CD32 receptor compared to other CD4+ T subpopulations ($p<0.05$ for all comparisons). The majority of CD4+CD32+ cells had a naïve phenotype (CD45RO–CD27+) followed by central memory (CD45RO+CD27+) and terminally-differentiated phenotype (CD45RO–CD27–). In vivo, the vast majority of productively infected cells in lymph node tissues from HIV aviremic and viremic patients also co-expressed the CD32a marker (90% of all infected cells), while most of CD32a single positive cells were absent from the B cell follicle, one of the major reservoirs for HIV. HIV infection of unstimulated PBMCs and cervico-vaginal histocultures upregulated the expression of CD32 in approximately 10-20% of all infected cells. CD32+ infected cells expressed more frequently the activation marker HLA-DR compared to CD32- infected cells (92% vs. 77%) and the immune check-point PD-1 (46% vs 25%).

**Conclusion:** CD32 is preferentially detected in HIV transcriptionally active cells in tissues after ART and was identified to be co-expressed with activation markers in in vitro infected cells suggesting its expression is also associate with active HIV-infected cells

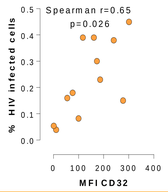
## ✓ Productive HIV-1 infection upregulates CD32 in unstimulated PBMCs



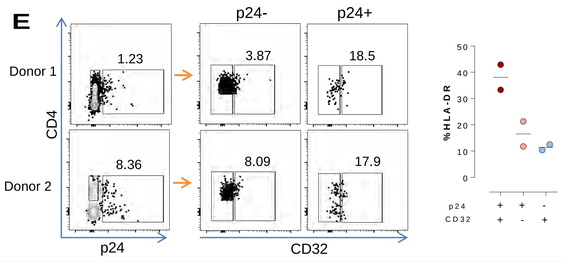

We observe productive HIV-1 infection (HIV-1 RNA+/p24+) that induce upregulation of CD32 expression. This upregulation remains stable during time.

Infected cells that express CD32 are more active (HLA-DR+) and more exhausted (PD-1+) than those not expressing CD32 and the uninfected cells.




Exist a positive correlation between percentage of infected cells and MFI of CD32

## ✓ Productive HIV-1 infection upregulates CD32 in cervical tissue

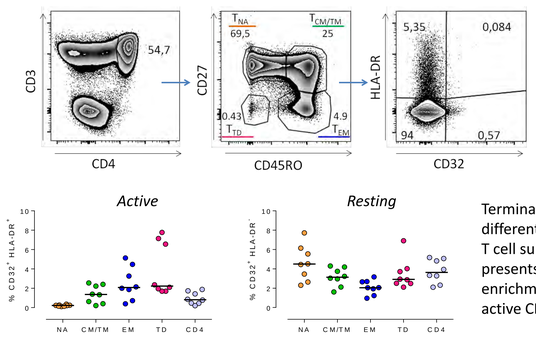


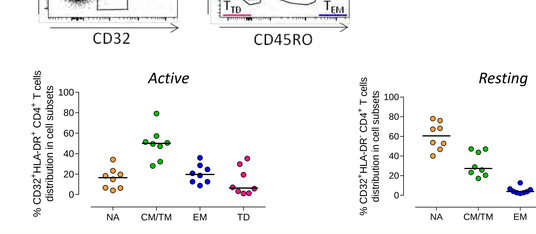




Infected cells from cervical tissue express more CD32 than uninfected cells. CD32+ infected cells are more active than CD32- uninfected and uninfected cells.

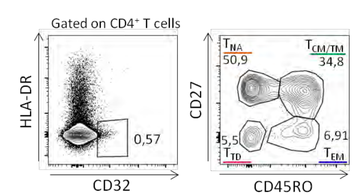
## ✓ Frequency of CD32+ cells in T cell subsets:




Terminal differentiated CD4 T cell subset presents a slight enrichment in active CD32+ cells.

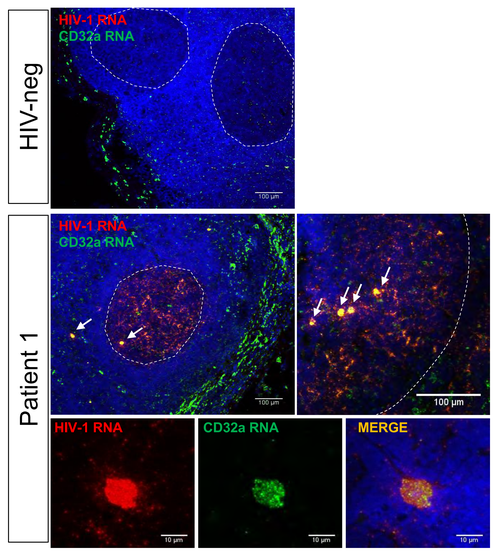
## ✓ Active CD32+ cells preferentially belong to CM/TM CD4 T cell subset

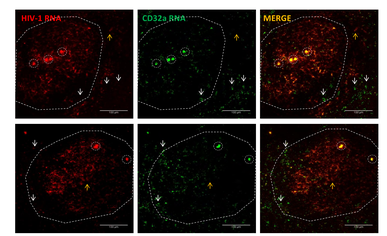


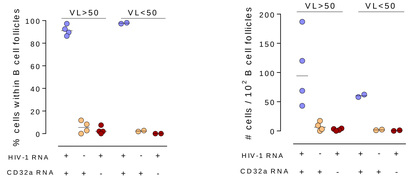

The majority of active CD32+ cells are central memory and transitional memory CD4 T cells. However, the majority of inactive CD32+ cells are naïve CD4 T cells.



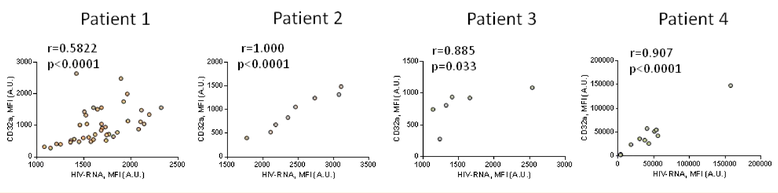

## ✓ CD32a is expressed in productively HIV-1-infected cells in B cell follicles




Most of the HIV-1-infected cells within B cell follicles express CD32a-RNA, both in detectable (VL>50) and in undetectable (VL<50) HIV-infected patients.




Significant correlation between the expression of HIV-1-RNA and CD32-RNA in infected cells in lymph nodes

- ✓ HIV-1 infection induces the upregulation of CD32 in PBMCs and cervical tissue. However, CD32+ cells may be also preferentially infected by HIV-1.
- ✓ Distribution of CD32 among CD4 T cell subsets depends on the expression of HLA-DR. More activated CD32+ cells are preferentially memory cells, while HLA-DR- are naïve cells.
- ✓ In human lymph nodes, CD32a RNA is coexpressed in transcriptionally active HIV-1 infected cells.

*These results suggest that **CD32 might represent a potential marker for transcriptionally active HIV-1 infected cells***

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