HIV-TRANSCRIPTION INDUCES CD20 EXPRESSION AND RENDERS CELLS SUSCEPTIBLE TO RITUXIMAB

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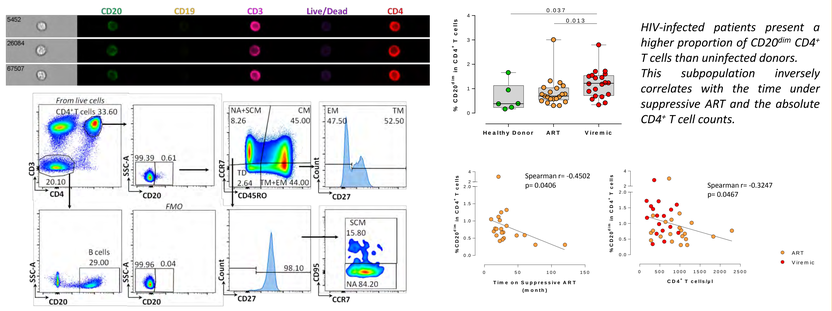
Background. One of the main obstacles to cure HIV is the existence of persistent HIV reservoirs in ART-suppressed patients. New markers with which to identify and target these reservoirs will definitely advance the search for an HIV cure. Here, we studied the expression of CD20 in HIV-infected cells and the use of this molecule to target the persistent viral reservoir.

Methods. Phenotypic characterization of CD20 expression in different CD4⁺T cell subsets was measured by flow cytometry in n=21 ART-suppressed, n=20 viremic and n=6 healthy controls. Intracellular levels of HIV-RNA were measured by the novel FISH/RNA flow cytometry assay, and quantification of HIV-DNA in sorted CD20+ T cells by qPCR. Expression of CD20 and p24 in lymph nodes of HIV+ patients was measured by immunohistochemistry. Moreover, the dynamics of CD20 expression upon HIV infection was examined by ex vivo infection. Finally, the susceptibility of CD20⁺ T cells to therapy with rituximab (anti-CD20 monoclonal antibody) was examined after ex vivo viral reactivation of CD4⁺T cells from ART-suppressed patients.

Results. We systematically found CD4⁺T lymphocytes in blood with dim expression of the CD20 receptor, in both healthy and HIV-infected individuals (0.38% in healthy, 0.70% in ART-suppressed and 1.22% in viremics). These cells had a memory phenotype (mainly central memory) and an increased activation state (values of 26.70 and 30.45% for CD20^{dim} CD4⁺T cells, and 21.30 and 19.20% for CD20^{-} CD4⁺ T cells in ART and viremics, respectively). In patients on ART, CD4⁺T cells expressing CD20 contained viral DNA and were enriched in HIV-RNA compared to cells not expressing this receptor (p=0.002). This population represented the 20% of the total cells expressing HIV transcripts. In lymph node tissues, we observed that a proportion of p24-expressing cells were also positive for the CD20 marker. Moreover, ex vivo infection of unstimulated PBMCs showed that viral replication induced the expression of CD20. Notably, rituximab was able to target CD20^{dim} CD4⁺T cells of ART-suppressed patients by the induction antibody-mediated cell cytotoxicity, and the combination of rituximab and latency reversal agents decreased HIV-RNA+ cells up to 64%.

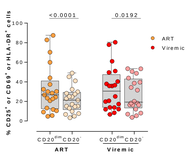
Conclusions. CD20^{dim} CD4⁺T cells harbored transcriptionally active HIV. Anti-CD20 treatment after induction of viral reactivation might represent a novel tool to target the active HIV-reservoir.

✓ A subpopulation of CD4⁺ T cells expresses CD20^{dim}, which associates with HIV infection



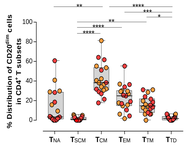
HIV-infected patients present a higher proportion of CD20^{dim} CD4⁺ T cells than uninfected donors. This subpopulation inversely correlates with the time under suppressive ART and the absolute CD4⁺ T cell counts.

✓ CD20^{dim} CD4⁺ T population is more activated than CD20^{-} cells



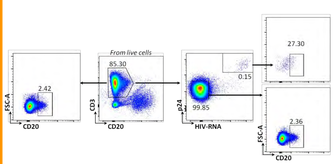
CD4⁺ T cells expressing CD20 show a higher activation state than CD20^{-} CD4⁺ T cells.

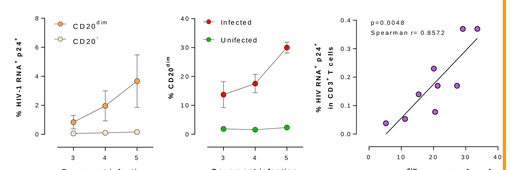
✓ CD20^{dim} CD4⁺ T cells are mainly ✓memory cells



CD20^{dim} CD4⁺ T cells are mainly distributed in memory CD4⁺ T cell subsets.

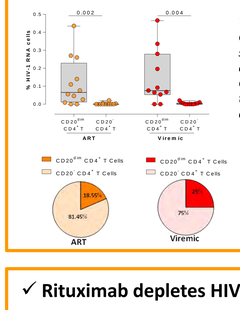
✓ HIV upregulates CD20 in T cells during viral production





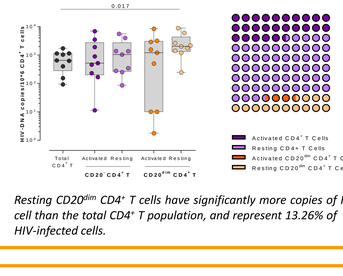
Unstimulated PBMCs from healthy donors were infected with the viral strain NL4.3 for 5 days. The CD20^{dim} CD4⁺ T subpopulation supported HIV infection more efficiently than CD20^{-} T cells. Moreover the contribution of infected CD20^{dim} T cells to the total pool of HIV-infected cells increased over time. Notably, a significant positive correlation was found between the percentage of productive HIV-infected cells and the percentage of CD20⁺ infected cells.

✓ CD20^{dim} CD4⁺ T cells are enriched in cell-associated HIV RNA



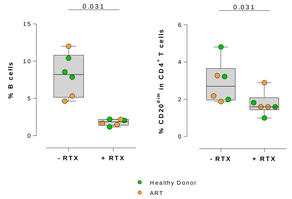
We observed that CD20^{dim} CD4⁺ T cells contained significantly higher levels of HIV RNA than CD20^{-} CD4⁺ T cells, contributing to the total pool of HIV-expressing cells

✓Sorted CD20^{dim} CD4⁺ T cells have similar levels of viral DNA compared with other CD4⁺ T cells

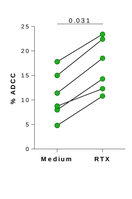


Resting CD20^{dim} CD4⁺ T cells have significantly more copies of HIV-DNA per cell than the total CD4⁺ T population, and represent 13.26% of total pool of HIV-infected cells.

✓ Rituximab depletes HIV-infected CD20^{dim} CD4⁺ T cells by inducing ADCC mediated by NK cells

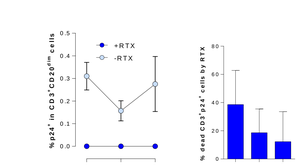


Rituximab reduce B cells and CD20^{dim} CD4⁺ T cells from both healthy donors and ART-suppressed HIV infected patients.

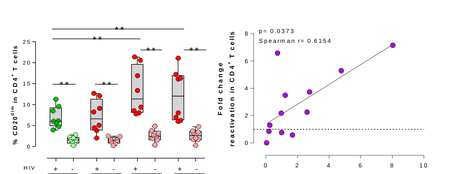


NK cells are responsible of inducing a potent ADCC response that significantly reduce the proportion of CD20^{dim} CD4⁺ T cells

✓ Rituximab impacts ex vivo HIV infected cells and transcriptionally active LRA-reactivated cells

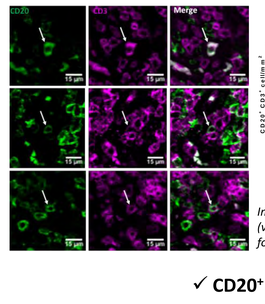


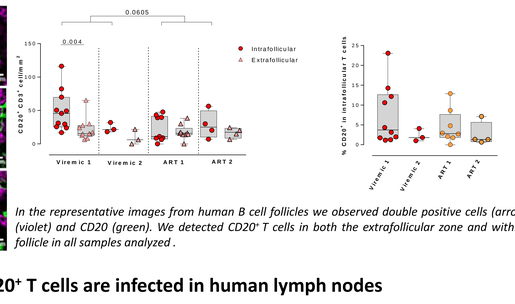
Rituximab eliminates ex vivo infected CD20^{dim} CD4⁺ T cells.



Rituximab targets transcriptionally active HIV-infected cells from ART-suppressed patients after viral reactivation .

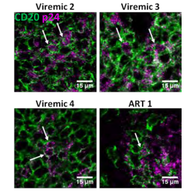
✓ T cells present in B cell follicles also express CD20



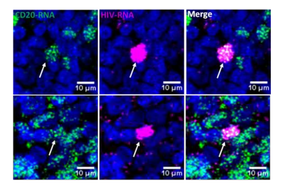


In the representative images from human B cell follicles we observed double positive cells (arrows) for CD3 (violet) and CD20 (green). We detected CD20⁺ T cells in both the extrafollicular zone and within the B cell follicle in all samples analyzed .

✓ CD20⁺ T cells are infected in human lymph nodes

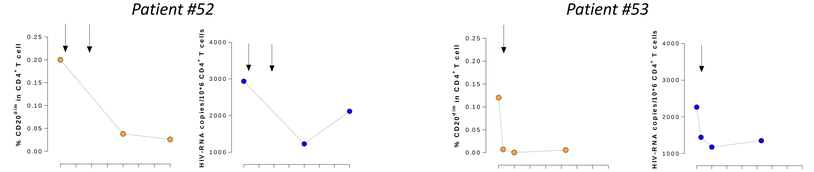


Arrows indicates double positive cells for CD20 (green) and p24 (violet) inside the B cell follicle.



In situ hybridization RNA detection assay was used to visualize HIV RNA and CD20 RNA in lymph node sections from an HIV⁺ viremic patient. Productively HIV infected cells are often associated with the expression of CD20, inside and outside of the B cell follicle

✓ HIV infected ART suppressed patients treated in vivo with Rituximab eliminate CD20^{dim} CD4⁺ T cells and reduce intracellular HIV RNA



Two ART-suppressed patients receiving Rituximab for non-malignant reasons. Frequency of CD20^{dim} cells and copies of intracellular HIV-RNA in CD4⁺ T cells population were measured before and after treatment.

- ❖ In HIV infected patients, higher frequencies of CD20^{dim} CD4⁺ T cells are found, and this population contains HIV-DNA and is enriched in HIV-RNA.
- ❖ In lymph nodes from HIV-infected patients CD4⁺ T cells and productively HIV-infected cells might express CD20.
- ❖ HIV transcription induces upregulation of CD20 in CD4⁺ T cells in vitro.
- ❖ Rituximab targets CD20^{dim} CD4⁺ T cells and impacts transcriptionally-active HIV infected cells.

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