HIV Reservoir Size Is Correlated to NK-mediated Killing of Effector Memory CD4 Cells

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Background: The identification of immune control mechanisms of the viral reservoir might help to develop new strategies to cure HIV. Antibody-dependent cell-mediated cytotoxicity (ADCC), largely mediated by natural killer (NK) cells, has been reported to be highly relevant to control HIV. However, the role of ADCC-NK at controlling the cells that compose the viral reservoir is currently unknown.
Methods: The intrinsic susceptibility of Naïve (NA), Stem Cell Memory (SCM), Central Memory (CM), Effector Memory (EM) and CD20dim CD4 T cells to ADCC NK-mediated killing was measured by a novel flow cytometry-based assay. n=15 ART-suppressed and 5 elite controllers (EC) patients were included. Isolated CD4 T cells were stained with the markers eF670 and PKH67 and coated with the gp120Bal recombinant protein. Cells were incubated with plasma from an HIV viremic patient and autologous NK cells at ratio 1:1 for 3 hours. Then, cells were stained with anti CD3, CCR7, CD45RO, CD32, CD95, CD20 and HLA-DR antibodies. NK-mediated killing was calculated as the disappearance of cells measured with the addition of flow cytometry particles. Total HIV-DNA and HIV-RNA was quantified by qPCR.
Results: Results from all analyzed patients showed that each CD4 T cell subset had a different susceptibility to killing, being CD20dim>EM>CM>NA>SCM more prone to be killed (ANOVA Friedman test p=0.0015). Moreover, whereas no differences in the killing of the whole CD4 population were observed between cohorts (Mann-Whitney test, p=0.6109), EC showed higher potency to kill CD20dim and EM (median 61.69, 52.04, 48.04, 33.65, 33.44% for CD20dim, EM, CM, NA, SCM, respectively) (ANOVA Friedman test p=0.0162), while ART-suppressed patients were more efficient at killing EM and CM (median 51.36, 49.32, 41.92, 37.17, 30.26% for EM, CM, CD20dim, NA, SCM, respectively) (ANOVA Friedman test p=0.0273). A more efficient killing of CD20dim cells, which has been found to be a marker of transcriptionally active HIV (poster #329), was detected in EC compared to ART-suppressed patients (Mann-Whitney test, p=0.0316). Importantly, an inverse correlation between the capacity of NK cells to kill the EM subset and the viral reservoir was observed (rho=-0.5143 p=0.0203 for viral DNA and rho=-0.6084 p=0.0399 for viral RNA).
Conclusion: The susceptibility of different CD4 T cell subsets to ADCC-NK killing differs. The ADCC activity against EM cells, one of the most HIV-transcriptionally active cell subsets, was highly correlated to the size of the persistent HIV-reservoir. Inducing the specific killing of EM cells might significantly help to diminish the persistent reservoir.

Flow cytometry-based ADCC method

Figure 1. Diagram of the ADCC method

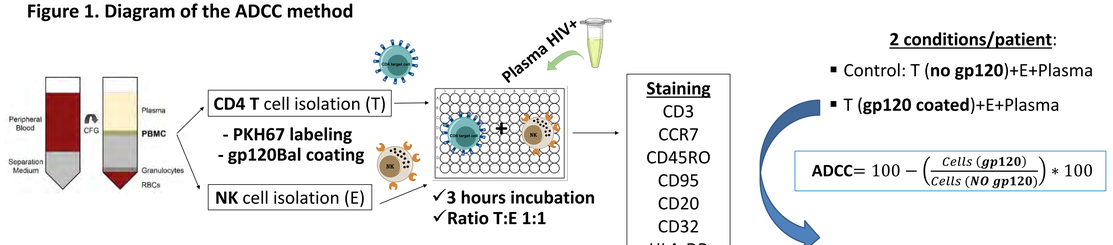


Figure 3. Gating strategy

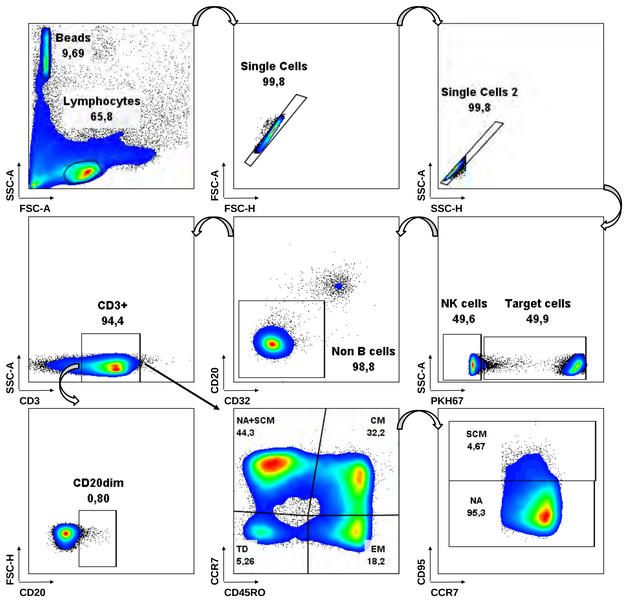


Figure 2. All subsets are efficiently coated with the gp120Bal protein

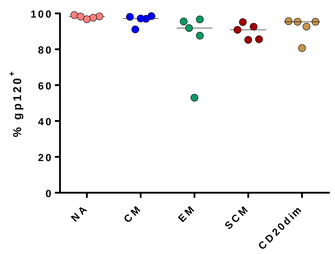


Table 1. Parameters of patients included in the study

Cohort	Number of patients	CD4 cell count (cells/µl) (mean)	Time on ART-suppressed (years) (mean)
Elite controllers	5	1108 [840-1610]	N/A
ART-suppressed	15	917 [630-1830]	7 [4-13]

Assessment of the ADCC response of NK cells from HIV-treated patients and EC against CD4 T cell subsets

Figure 4. Different susceptibility of CD4 T subsets to ADCC-NK killing

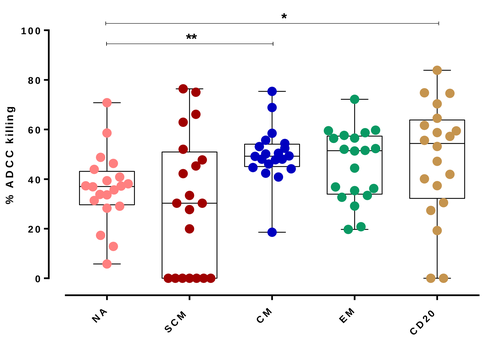


Figure 5. NK cell activity in EC and ART-suppressed patients

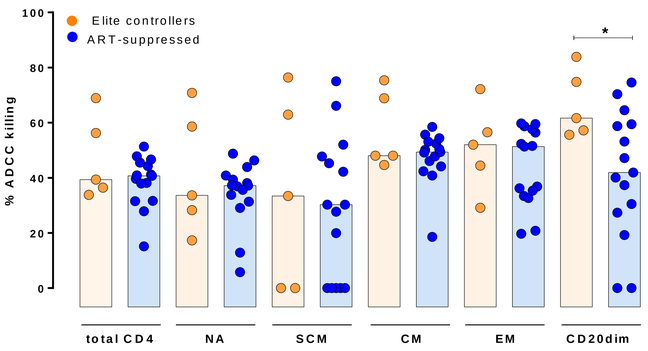
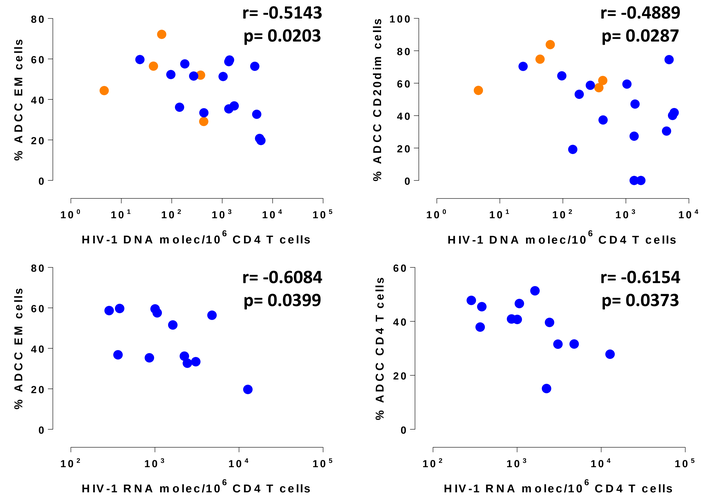


Figure 6. Reservoir size inversely correlates with the killing of cell subsets



- ✓ Not all CD4 T cell subsets are equally susceptible to ADCC-NK killing
- ✓ EC show higher capacity to eliminate the CD20dim cells, a subpopulation described to contain transcriptionally active HIV (Poster #329)
- ✓ ADCC NK-mediated killing of EM cells was highly correlated with the size of the HIV-1 reservoir

These results indicate the importance of studying the individual characteristics of different CD4 T cell subsets that compose the persistent HIV-reservoir. Moreover, harnessing NK-immune responses toward HIV-containing cells might help to impact the size of the HIV-reservoir.

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