

# CD4+CD45RO+CD127-CCR5+ T Cells Define the Expansion of CD127- T Cells in HIV+ Individuals

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## Background

Interleukin-7 (IL-7) is known to be a key cytokine in T cell development and proliferation. In HIV infection, increased IL-7 levels in plasma correlate to high plasma viral load and decreased CD4+ T cell numbers. Similarly, T cells expressing the IL-7 receptor CD127 are reduced in HIV+ individuals suggesting a preferential loss of CD127+ cells. Our data suggest the preferential loss of CD127+ CCR5+ memory CD4+ T cells in HIV infection and/or the increased proliferation of the CD127- CCR5+ subtype.

## Methods

Patients: total of 55 subjects, diagnosed with HIV-1 infection were included in this study.

**Table 1. Patients characteristics.**  
\* IVDU: Intravenous drug user, HMS: homosexual, HH: hemofilic-hemotransfused, HTS: heterosexual, NI: no information.

Patient Characteristics	
Age [Mean Years (SD)]	45 (8)
Sex	N (%)
Female	39 (71)
Male	16 (29)
Risk Factor*	N (%)
IVDU	13 (24)
HMS	18 (33)
HH	1 (2)
HTS	15 (27)
NI	8 (15)
HAART	N (%)
Yes	53 (96.4)
No	2 (3.6)

**Table 2. Virological and immunological parameters.**

Parameters	Mean ± standard deviation
CD4 (cells/ $\mu$ l)	475 ± 278
Viral load Log <sub>10</sub> RNA copies/ml	4.2 ± 1.2
IL-7 (pg/ml)	4.5 ± 3.5

**Blood samples:** whole blood of HIV patients and healthy donors were collected. Plasma was isolated after centrifugation and was immediately cryopreserved and stored at -80° C until use.

**Flow Cytometry:** The monoclonal antibodies (mAbs) used were: CD4-FITC, CD4-PerCP, CD4-APC, CD45RO-FITC, CD3-FITC, CD3-PerCP, CCR5-APC, CXCR4-APC (BD Biosciences) and CD127-PE (R&D Systems). Whole blood samples were stained with mAbs and then analyzed in a FACScalibur flow cytometer (BD).

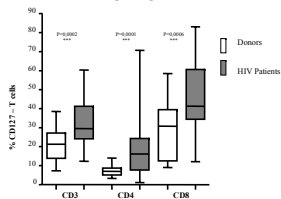
**IL-7 levels:** Plasma IL-7 levels were determined by an ultra sensitive commercial ELISA assay: Quantikine HS Human IL-7 Immunoassay (R&D Systems, Minneapolis, Minnesota) following the manufacturer instructions.

**Plasma viral load (VL):** viral load of HIV patients were measured by the Amplicor Test.

**Statistical analysis:** Non-parametric test (Mann-Whitney) and Spearman's coefficient were used to establish significant differences and correlations among different parameters. Data was analyzed using the GraphPad Prism V4.00 software package.

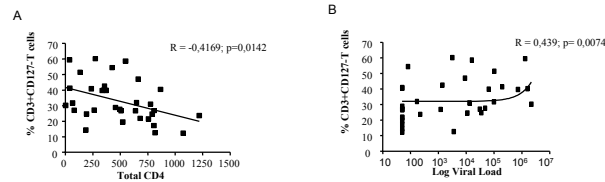
## Results

### Increased proportion of CD127- T cells in HIV+ individuals



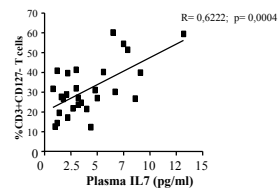
We measured the CD127 expression levels in T cells from 55 HIV patients and 19 healthy donors. We found a significantly higher proportion of CD127- T cells in HIV patients compare to healthy donors. This higher proportion was observed in both CD4 and CD8 T cells.

### CD127 expression on T cells from HIV patients correlates with progression markers



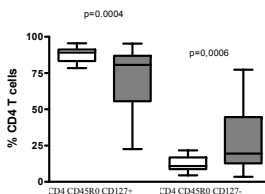
To evaluate if the increase in the proportion of CD127-CD4+ T cells in HIV patients was a consequence of the infection we examined the relationship between this populations and markers of disease progression as CD4 T cell count and viral load. We found a negative correlation between CD127- T cells and the CD4 T cells count (A) and a positive correlation with viral load (B).

### CD127 expression on T cells from HIV patients correlates with IL-7 plasma levels



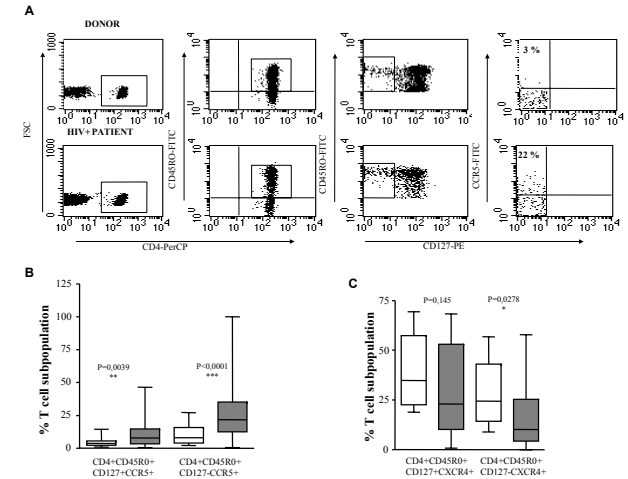
HIV patients have a higher plasma levels of IL-7, so that could explain the lower expression of CD127 in HIV+ individuals. To confirm whether IL-7 was modulating its receptor CD127, we analyzed the correlation between plasmatic IL-7 levels and the proportion of negative CD127 T cells. There was a positive correlation between the cytokine and CD127.

### HIV+ individuals showed an increased proportion of memory (CD45RO+) CD4+ CD127- cells as compared to negative donors



To better understand the expanded CD4+ CD127- T cells, we measured the CD127 expression in different subpopulations of CD4 T cells. We observed that the percentage of CD4+ CD45RO+ CD127- cells was significantly higher in HIV patients than in healthy donors, whereas the percentage of CD4+CD45RO- CD127- cells was just slightly significantly higher (P=0.03)

### CD4+CD45RO+CD127-CCR5+ cells are defining the increased proportion in CD127- T cells



We evaluated the expression of the HIV-coreceptors CCR5 and CXCR4 in the increased subpopulation of CD127- T cells. Representative dot plots of a healthy donor and a HIV+ patient is shown (A). We found an important increase of CD4+CD45RO+CD127-CCR5+ T cells compare to healthy donors (B). That difference was not observed with the other HIV-coreceptor CXCR4 (C). On the contrary, the levels of CD4+CD45RO+CD127-CXCR4+ T cells were lower than in the HIV- individuals. This results suggest that the higher levels of CD127- T cells are defining by the CD4+CD45RO+CD127-CCR5+ T cells.

## Conclusions

- HIV patients showed higher IL-7 plasma levels than healthy donors and a decreased number of CD127+ T cells.
- CD127 expression correlates with progression markers and with plasma IL-7 levels.
- The CD4+CD45RO+CD127-CCR5+ T subpopulation could better define the observed increase in the percentage of CD127- T cells in HIV+ individuals, which suggest the preferential expansion of this subpopulation or a specific depletion of CD4+CD45RO+CD127+CCR5+ T as a consequence of infection.

## Acknowledgments

