Interplay between IL-32 and CD96 expression: potential role in cell senescence and persistent inflammation in HIV infection

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Context

Introduction: Persistent inflammation in HIV infection is associated with disease progression by impacting cellular functions, differentiation and survival. This inflammation also underlies the premature aging of immune cells as reflected by their limited replicative capacity and inflammatory nature which predicts morbidity and mortality in HIV-1 infected individuals. We have previously shown that persistent upregulation of the proinflammatory cytokine IL-32 is associated with loss of immunological and virological control in HIV^{pos} individuals with a history of slow progression. In this study, we aim to further investigate the impact of IL-32 on CD96, a surface immunoglobulin that restricts T-cells activation and that is also downmodulated upon loss of control.

Hypothesis: CD96 loss is associated with increased T cell activation ultimately leading to T CD8 and TCD4 senescence in the context of HIV. IL-32 is upregulated in HIV infected individuals and may impact the CD96 expression, furthermore enhancing premature immuno-senescence.

Objectives: To investigate the loss of expression of CD96 in T cells in the context of HIV and describe their phenotypes. Study the impact of IL-32 on the CD96 expression.

Materials and Methods: CD96 expression together with activation and senescence markers were tested on PBMCs from HIV^{neg} and HIV^{pos} individuals from the Canadian Cohort of HIV-infected slow progressors (SP) and Montreal Primary Infection (PRIMO cohort), by flow cytometry. IL-32 recombinant isoforms were used to stimulate T-cells at 500ng/ml. Mitogenic stimulation was used to measure proliferation of T-cells with high and low CD96 expression.

Results: Our *ex vivo* phenotypic analysis on T-cells (CD4 and CD8) from HIV^{pos} individuals (both typical progressors and SP) compared to non-infected controls expressed showed significantly lower levels of CD96. Importantly, $CD8+CD96^{low}$ compared to $CD8+CD96^{high}$ T-cells exhibited a CD28-CD27-CD57+ phenotype, indicative of cell senescence. In addition, $CD96^{low}$ *versus* $CD96^{high}$ cells showed inferior proliferation capacity in response to mitogenic stimulation. *In vitro* activation of T-cells with IL-32 mediated a significant downregulation of CD96 expression on both CD4 and CD8 cells.

Conclusions: Our results suggest that IL-32 is involved in mechanisms underlying down-regulation of CD96 in HIV infection. This phenotype is likely associated with high level of T-cell activation and potentially cell senescence, which may sustain persistent inflammation. Work is in progress to validate the senescence phenotype at the functional level.

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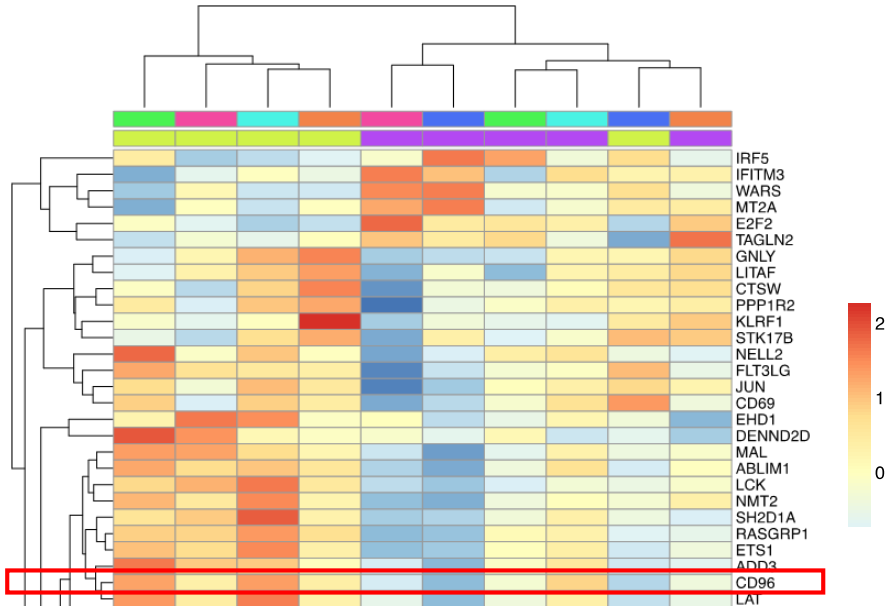
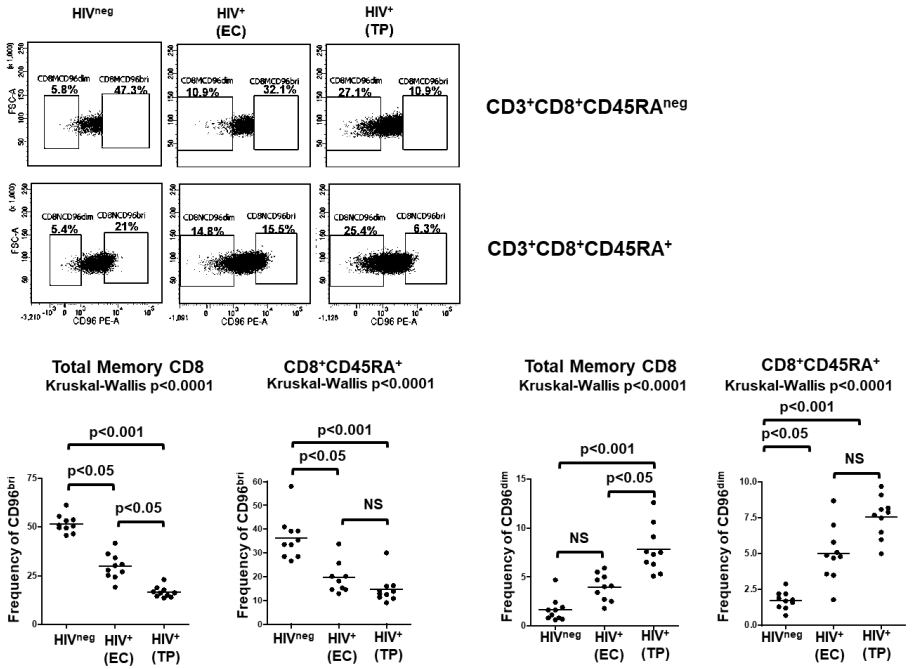
Results

Figure 1 : Down-Regulation of CD96 in HIV+ slow progressors failing immunological and virological control. Heat map showing Transcriptional analysis by microarrays of Total PBMCs from subject losing control (increased viral load and decreased CD4 counts, n=5) between Visit 1 (before loss of control) and Visit 2 (after loss of control). Selection of modulated genes (p <0.05).



Among other factors, we observed a significant loss of CD96 in T cells.

Figure 2 : Change in CD96bright and CD96dim on CD45RA+ and CD45RA- in Typical Progressors (TP), Elite Controllers (EC) and HIV^{neg} individuals. *Ex vivo* Flow cytometry analysis of TP viremic infected individuals (n=10), EC (n=10) (viral load < 50 copy/ml) and HIV neg (n=10).



Both Memory and Naïve CD8 of EC and TP had a lower frequency of CD96bright cells compared to HIV^{neg}, but in contrast had a significantly increased frequency of CD96dim.

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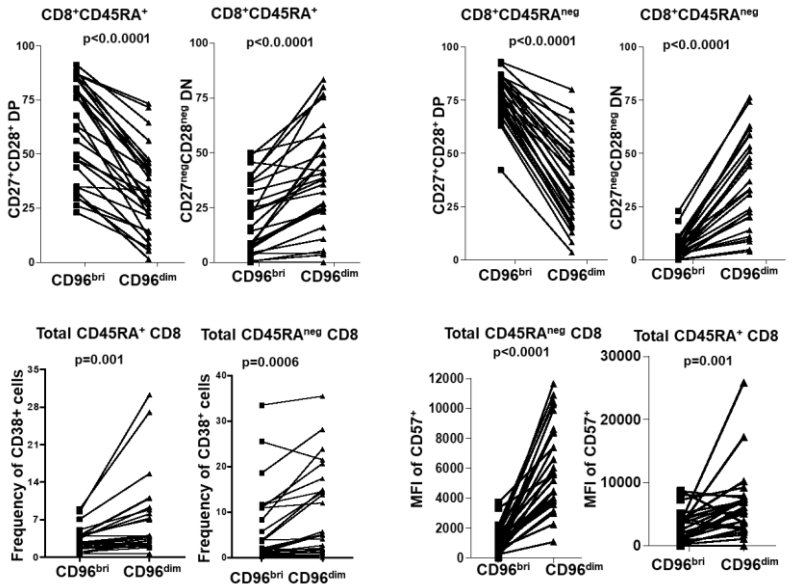
Results

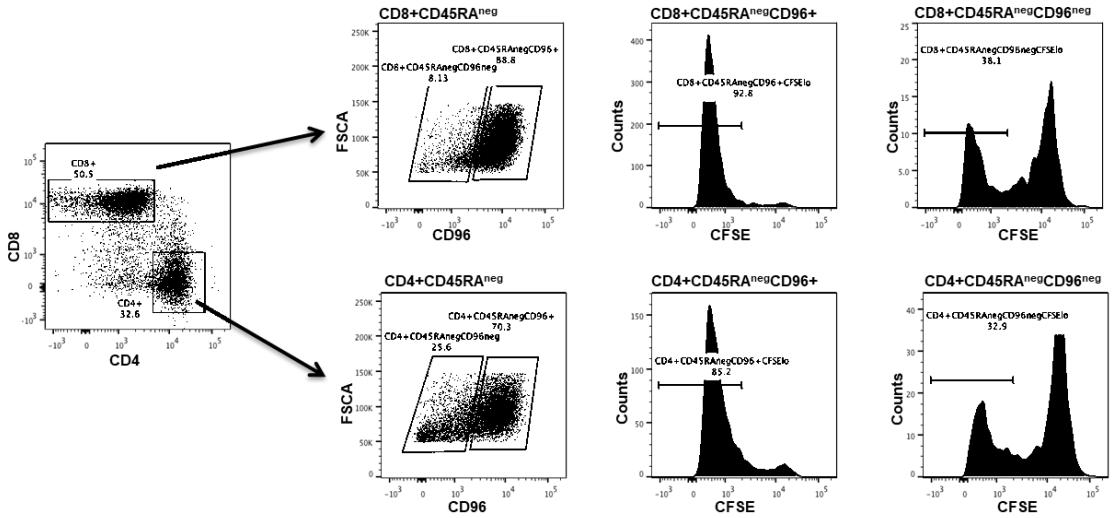
Figure 3 : Low CD96 expression is associated with senescence phenotype. **(Upper left)** CD27 and CD28 expression on CD8+CD45RA+CD96bri and dim and **(Upper right)** CD8+CD45RA-CD96bri and dim (n=30). **(Down left)** expression of activation marker CD38 and **(Down right)** senescence marker CD57 on CD8+CD45RA+CD96bri and dim and CD8+CD45RAneg CD96bri and dim (n = 30).



CD96dim CD8 T cells are more activated than CD96bri CD8 T cells.

CD27-CD28-CD57+ cells are strongly enriched in the CD96dim population indicating a senescent CD8 T cell profile.

Figure 4 : Down-Regulation of CD96 is associated with poor T cell proliferation in response to PHA stimulation. Proliferation assay of memory T CD8 CD96+ and CD96- **(Up)** and memory T CD4 CD96+ and CD96- **(Down)**. Data shown for 72h HA stimulation (10ug/ml) and CFSE staining.



Both CD4 and CD8 CD45RA-CD96- had poor proliferation capacity following PHA stimulation compared to CD4 and CD8 CD45RA-CD96+ .

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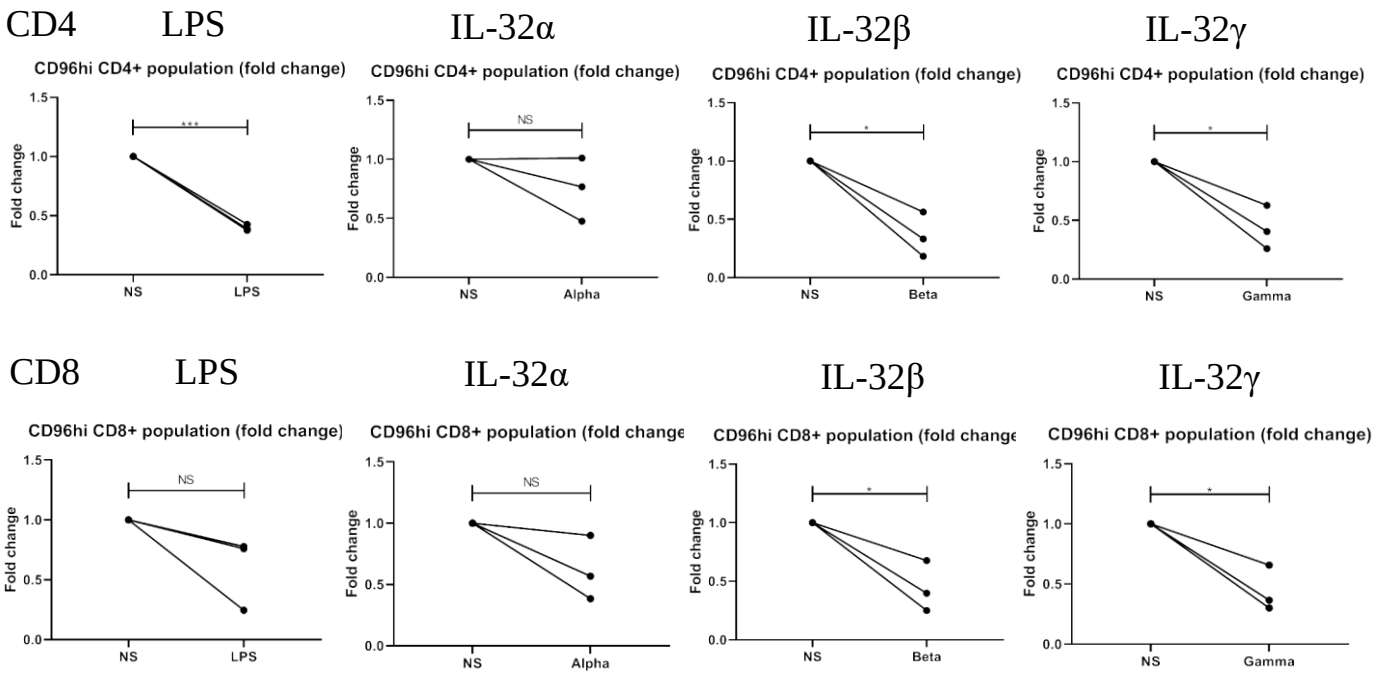
Results and discussion

Figure 5 : Down-regulation of CD96 by IL-32 stimulation. Fold change in CD96 expression following IL-32 isoforms stimulation (Alpha, Beta and Gamma) and LPS.



IL-32 plays a role in CD96 modulation on T CD4 and T CD8 cells

Discussion : Impact of IL-32 on the loss of CD96 expression might contribute to T CD4 and T CD8 cells premature immuno-senescence. Accumulation of these senescent cells with poor proliferation capacity may bias the host defense against HIV infection. CD96dim T cells may also secrete pro-inflammatory cytokines and thus contribute to the chronic inflammation and development of comorbidities in the context of HIV infection. However, this last hypothesis of secretion of proinflammatory cytokines by CD96dim cells remains an open question and is currently under investigation.

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Thank you, Merci.