Loss of CD96 Expression as a New Biomarker for T-cell Senescence in HIV-1 Infection

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We declare that we have no conflict of interest
# Canadian Cohort of HIV+ Slow progressors (CCHSP)

<table>
<thead>
<tr>
<th>Slow Progressor subgroups/number of subjects</th>
<th>CD4+ T-cell count at baseline</th>
<th>HIV-RNA viral load (VL) at baseline</th>
<th>Time since infection</th>
<th>ART**</th>
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<tbody>
<tr>
<td>Elite controller (n=45)</td>
<td>&gt;500 cells/mm³</td>
<td>≤50 copies/ml</td>
<td>Any</td>
<td>Naive</td>
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<tr>
<td>Virologic controller (n=68)</td>
<td>&gt;500 cells/mm³</td>
<td>51-3000 copies/ml</td>
<td>&gt;7 years</td>
<td>Naive</td>
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<td>Non-Virologic controller (n=33)</td>
<td>&gt;500 cells/mm³</td>
<td>&gt;3000 copies/ml</td>
<td>&gt;7 years</td>
<td>Naive</td>
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</table>

*Slow progressor: HIV+ study participant meeting any of the above definitions and having no signs of AIDS

**Antiretroviral treatment
CD4 T-cell decline in HIV-infected slow progressors
Loss of immunological and virological control in SPs

CD4 counts/mm³ at baseline (1273)

HIV RNA copies/ml at baseline (1092)

Years since infection (single points represent clinic visits)

CD4 counts/mm³

HIV RNA copies/ml plasma

Number of subjects losing control

EC VC NVC
2% 10% 27%

Total number of subjects

Number of subjects losing control
Transcriptional analysis of PBMCs from subjects losing control

Total PBMCs from n=5
Average loss of CD4 211 cells
Average increase in VL 20 fold

V1 (Before loss of control)
V2 (After loss of control)

Microarray analysis

1.3 fold change p<0.05
207 genes down-regulated
83 genes up-regulated
Why do we need a cure for HIV?

CD96 negatively regulates cytokine production by NK cells

**CD96**

T cell-activated increased late expression (TACTILE) is expressed by both NK and T-cells

Initially thought to enhance NK killing capacity through binding to the nectin receptor CD155 (PVR)


Abundant expression of IFNγ

*Chan et al., 2014 Nat. immunol. 15(5):431-8.*

Potential role of CD96 in negative regulation of cell activation

Down-regulation of CD96 on CD8+ T-cells in HIV infection

Down-regulation of CD96 on SP T-cells upon loss of control

Why do we need a cure for HIV?

CD4 T-cells

CD8 T-cells

Visit 1 (Control)

Visit 2 (Loss of control)

Visit 2 (Loss control)

Visit 1 (Control)
Down-regulation of CD96 on CD4+ and CD8+ T-cells in HIV infection

NK cells

Kruskal-Wallis p=0.0006

p<0.05

p<0.01

MFI of CD96

CD4+ T-cells

Kruskal-Wallis p=0.004

p<0.001

MFI of CD96

CD8+ T-cells

Kruskal-Wallis p<0.0001

p<0.001

p<0.05

MFI of CD96
Down-regulation of CD96 on CD8+ T-cells in HIV infection

**Why do we need a cure for HIV?**

- **Down-regulation of CD96 on CD8+ T-cells in HIV infection**

  - **HIV+**
    - HIV+ (EC)
    - HIV+ (TP)

  - **HIV-**
    - HIV- (EC)
    - HIV- (TP)

- **CD3+CD8+CD45RA^-**
  - HIV+
    - HIV+ (EC)
    - HIV+ (TP)
  - HIV-
    - HIV- (EC)
    - HIV- (TP)

- **CD3+CD8+CD45RA^+**
  - HIV+
    - HIV+ (EC)
    - HIV+ (TP)
  - HIV-
    - HIV- (EC)
    - HIV- (TP)

**Total Memory CD8**

- **Kruskal-Wallis p<0.0001**

  - CD8+CD45RA^+
    - HIV+ (EC)
    - HIV+ (TP)
    - HIV- (EC)
    - HIV- (TP)

**Total Memory CD8**

- **Kruskal-Wallis p<0.0001**

  - CD8+CD45RA^+
    - HIV+ (EC)
    - HIV+ (TP)
    - HIV- (EC)
    - HIV- (TP)

**Kruskal-Wallis p<0.0001**

- Frequencies of CD96^high^ and CD96^dim^ in HIV+ and HIV- individuals.
Low CD96 expression is associated with a more differentiated phenotype

CD8+CD45RA+
- CD96bri CD96dim
- CD8 Naïve (CCR7+CD27+)
- CD8 TEMRA (CCR7negCD27neg)
- Wilcoxon p<0.0001

CD8+CD45RAneg
- CD96bri CD96dim
- CD8 CM (CCR7+CD27+)
- CD8 EM (CCR7negCD27neg)
- Wilcoxon p<0.0001

CD8 Naïve (CCR7+CD27+)
- TEMRA (21.82%)
- Naïve CCR7neg (24.48%)
- Naïve (41.13%)

CD8 TEMRA (CCR7negCD27neg)
- TEMRA (38.38%)
- Naïve CCR7neg (28.35%)
- Naïve (31.95%)

CD8 CM (CCR7+CD27+)
- CM (12.5%)
- EM (9.4%)
- TM (77.75%)

CD8 EM (CCR7negCD27neg)
- EM (29.7%)
- CM (4.7%)
- TM (60.1%)
Low CD96 expression is associated with a senescent phenotype

- CD8+CD45RA+
  - CD96bri
  - CD96dim
  - p<0.0001

- CD8+CD45RA-
  - CD96bri
  - CD96dim
  - p<0.0001

- Total CD45RA+ CD8
  - Frequency of CD38+ cells
  - p=0.001

- Total CD45RA- CD8
  - Frequency of CD38+ cells
  - p=0.0006

- CD8+CD45RA-
  - MFI of CD57+
  - p<0.0001

- Total CD45RA- CD8
  - MFI of CD57+
  - p=0.001

Why do we need a cure for HIV?

Low CD96 expression is associated with a senescent phenotype.
Down-regulation of CD96 is associated with increased activation and increased PD-1 expression.

HIV+ subjects (TP and EC, n=10/group)
Poor replicative capacity of CD96<sup>dim</sup> CD8<sup>+</sup> and CD4<sup>+</sup> T-cells

Why do we need a cure for HIV?

Poor replicative capacity of CD96<sup>dim</sup> CD8<sup>+</sup> and CD4<sup>+</sup> T-cells
Conclusions

- Loss of immunological control (CD4 decline) and virological control (increased VL) is associated with significant down-regulation of CD96 expression on T-cells, mainly CD8.

- Down-regulation of CD96 on CD8 T-cells is associated with a more differentiated phenotype (increased frequencies of effector and terminally differentiated cells).

- Cells down-regulating CD96 expression in HIV infection are enriched with $CD27^{\text{neg}}CD28^{\text{neg}}$ Double negative phenotype.

- These cells express significantly high levels of CD57, typical phenotype of senescent cells.

- These cells were previously shown to express high levels of perforin and IFNγ.

- However, T-cells with the CD96$^{\text{low}}$ senescent phenotype have inferior capacity to proliferate compared to CD96$^{+}$ cells.
Future work

- Validating CD28 and CD27 down-regulation at the transcriptional level (permanent replicative senescence)

- Measuring the telomere and/or assessing the telomerase activity in CD96\textsuperscript{bri} versus CD96\textsuperscript{dim} CD8\textsuperscript{+} memory T-cells

- Characterizing the exhaustion phenotype of CD8\textsuperscript{+} memory T-cells within the CD96\textsuperscript{bri} and CD96\textsuperscript{dim} subpopulations

- Investigating CD96 signaling in T-cells by over-expression of CD96 and triggering with anti-CD96 antibodies using bead systems (in the presence of anti-CD3/anti-CD28 antibodies)

- Investigating the impact of CD96 knockdown with RNA interference on T-cell activation, cytokine production, proliferation and senescence in culture

- Transcriptional profiling of CD96\textsuperscript{bri} and CD96\textsuperscript{dim} ex vivo from memory CD8 T-cells from HIV-infected subjects by microarray to identify a signature associated with T-cell senescence
Backups
Profound loss of metabolic, functional and cytolytic capacities

Recurring exposure and activation/inhibition

Specific up-regulation during HIV infection
Expression correlates with disease progression (phase of infection, viral load, CD4 counts, CD4/CD8 ratio)

- IL-2
- Proliferation
- TNFα
- INFγ
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