Central Memory CD8+ T Cells Appear to Have a Shorter Lifespan and Reduced Abundance As a Function of HIV Disease Progression

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Immunodeficiency in HIV Infection

- Quantitative loss of CD4\(^+\) T cells
- Qualitative changes in cell populations that persist
  McCune JM. *Cell* (1991)

- Loss of recall, or “memory”, responses to antigen

- Loss of polyfunctional CD8\(^+\) “memory” T cells that are able to control HIV viral load

- Such memory responses represent the bedrock upon which the adaptive immune system is based
“Memory” in the T Cell Lineage and Generation and Maintenance of Memory CD8\(^+\) T Cells

- Memory T cells are thought to differentiate from naïve T cells after exposure to antigen
- .. persist for very long periods of time
- .. rapidly proliferate and differentiate into effector T cells after secondary contact with cognate antigen

- Requirements for generation and maintenance of memory CD8\(^+\) T cells include:
  - CD4\(^+\) T cell help
  - a cellular machinery that provides for either *self-renewal* (e.g., through arrested development mediated by transcriptional repressors) or for *limited homeostatic proliferation* (e.g. mediated by IL-7 through the IL-7R\(\alpha\)).

Fearon DT et al. Science (2001)
Luckey CJ et al. PNAS (2006)
Linear Differentiation or Post-thymic CD8+ T Cell Development*

CD45RA+CCR7+  \( \rightarrow \)  CD45RA-CCR7+  \( \rightarrow \)  CD45RA-CCR7-  \( \rightarrow \)  CD45RA+CCR7-

\( T_N \)  \( \rightarrow \)  \( T_{CM} \)  \( \rightarrow \)  \( T_{EM} \)  \( \rightarrow \)  \( T_{EMRA} \)

CD45RA+  \( \rightarrow \)  CD45RA-  \( \rightarrow \)  CD45RA-(+)

CCR7+  \( \rightarrow \)  CCR7-  \( \rightarrow \)  CCR7-(+)

CD28+  \( \rightarrow \)  CD28-  \( \rightarrow \)  CD28-(+)

CD27+  \( \rightarrow \)  CD27-  \( \rightarrow \)  CD27-(+)

CD57-  \( \rightarrow \)  CD57-(+)  \( \rightarrow \)  CD57-(+/-)

CD45RA+/-(+)  \( \rightarrow \)  CD45RA+/-  \( \rightarrow \)  CD45RA+(-)

CCR7-  \( \rightarrow \)  CCR7-(+)  \( \rightarrow \)  CCR7-(+/-)

CD28-  \( \rightarrow \)  CD28-(+)  \( \rightarrow \)  CD28-(+/-)

CD27-  \( \rightarrow \)  CD27-(+)  \( \rightarrow \)  CD27-(+/-)

CD57-(+)  \( \rightarrow \)  CD57-(+/-)  \( \rightarrow \)  CD57-(+/-)

Naive  \( \rightarrow \)  Early 1  \( \rightarrow \)  Early 2  \( \rightarrow \)  Intermediate  \( \rightarrow \)  Late 1  \( \rightarrow \)  Late 2


In vivo $^2$H$_2$O or $^2$H-glucose Labelling showed that Higher Proportions of T Cells Are Short-lived in Advanced HIV Infection Compared to Healthy Controls

- These short-lived cells have a memory/effector phenotype

- Long-lived potential progenitor T cells may be reduced in advanced HIV infection

These Findings Led Us to Ask the Following Questions:

- What is the phenotype and the lifespan of long-lived memory CD8\(^+\) T cells in HIV-negative subjects?

- Does this phenotype and/or its lifespan change in the context of progressive HIV disease?
We also Wanted...

.. to evaluate the applicability of the stable isotope (\(^2\text{H}_2\text{O}\)) / FACS / mass spectrometric method for the analysis of low abundance T cell subpopulations
Stable Isotope *In Vivo* Labelling with $^{2}{H}_{2}O$

$^{2}{H}_{2}O$ Long-term oral administration

Labelling (7 weeks)

Label wash-out (3 w.)

De-labelling (8 weeks)

(FACS)

Cell Isolation (20,000 cells)

DNA

GC/MS

Enrichment of label in DNA from sample cells
Calculations of Decay Constants, Half-lives, and Percentages of CD8$^+$ T Cell Subpopulations Remaining after 7 Weeks of $^2$H$_2$O Labelling

• Loss of label from cellular DNA of each subset was quantified between S2 (week 10) and S4 (week 18)
• The decay constant ($k$) was calculated using the equation for exponential decay:
  \[ k = \frac{-\ln(S2/S4)}{Dt} \]
• The half-life was calculated as:
  \[ t_{1/2} = \frac{\ln(2)}{k} \]
• Some CD8$^+$ T cell subpopulations did not lose label exponentially, which is why we also calculated the percentage of initially incorporated label:
  \[ \frac{S4}{S2} \]
Phenotypes of Sorted CD3⁺CD8⁺ T Cell Subpopulations

**MEMORY & EFFECTOR**

- $T_{CM}$
  - CD45RA⁻CD28⁺CCR7⁺
- $T_{EM1}$
  - CD45RA⁻CCR7⁻ (CD28⁺⁻)
- $T_{EM2}$
  - CD45RA⁻CD28⁻CCR7⁻

**NAÏVE & “RA” EFFECTOR**

- $T_N$
  - CD45RA^{high}CD28⁺CCR7⁺
- $T_{EMRA}$
  - CD45RA^{high}CD28⁻CCR7⁻
## Characteristics of Study Subjects

<table>
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<tr>
<th>Group</th>
<th>Subject ID</th>
<th>Age (Years)</th>
<th>VL (copies/ml)</th>
<th>Years HIV-infected</th>
<th>CD4 count / µl of blood</th>
<th>CD8 count / µl of blood</th>
<th>Weeks of 2H2O labeling</th>
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<td>66</td>
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ND not determined; NA not available

Median CD8 count:

**HIV-negative subjects:**

334 CD8 T cells/µl; range 246 – 563)

versus

**HIV-infected subjects:**

1175 CD8 T cells/µl; range 578-3388)

(p<0.002 HIV-negative versus HIV-infected)
Gating Strategy for Analysis & Sorting

FMO: CD45RA

FMO: CD28

FMO: CCR7

TEM1

TEM2

T CM

TN

TEMRA
$T_{CM}$ Cells Are Lost with Decreasing CD4 Counts

HIV-neg.

CD4 count: 973 431 349 170 66

HIV+

CD28

CCR7

VL: 8,278 30,851 448, 343 15, 534 467,160
Decreased Numbers of CD8$^+$ \( T_{CM} \) cells / \( \mu l \) of Blood Correlate with Decreasing CD4 Counts

\[ r = 0.50 \]
\[ p = 0.02 \]
Label Die-away Curves of CD8+ T Cell Subpopulations

- TN
- TCM
- TEM2
- TEMRA

HIV- (64 yr)
HIV- (59 yr)
HIV- (56 yr)
HIV- (27 yr)
HIV+ (VL 2K)
HIV+ (VL 74K)
HIV+ (VL 2K)
HIV+ (VL 448K)
HIV+ (VL 30K)
$^2$H Enrichment in CD8$^+$ T Cell Subpopulations

- HIV+ (VL 2K)
- HIV+ (VL 74K)
- HIV+ (VL 2K)
- HIV+ (VL 448K)
- HIV+ (VL 30K)
- HIV- (64 yr)
- HIV- (59 yr)
- HIV- (56 yr)
- HIV- (27 yr)

**TN**

**TCM**

**TEM2**

**TEMRA**
Shorter Half-life of CD8⁺ T<sub>CM</sub> Cells in HIV-infected Subjects with High Viral Load

<table>
<thead>
<tr>
<th>CD3⁺CD8⁺ T cell subset</th>
<th>Group</th>
<th>HIV copies/ml (log₁₀)</th>
<th>k (decay constant)</th>
<th>T&lt;sub&gt;1/2&lt;/sub&gt; (half-life in days)</th>
<th>Percentage of cells remaining</th>
<th>Cells/ul</th>
<th>% of CD3⁺CD8⁺ T cells</th>
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<tbody>
<tr>
<td></td>
<td>HIV-</td>
<td>-</td>
<td>0.0119</td>
<td>58.1</td>
<td>51.3</td>
<td>8.3</td>
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<td>HIV-infected</td>
<td>4.3</td>
<td>0.0154</td>
<td>49.3</td>
<td>43.3</td>
<td>241.4</td>
<td>9.9</td>
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<tr>
<td>CD45RA⁻CCR7⁻CD28⁻ (TEM2)</td>
<td>HIV-</td>
<td>-</td>
<td>0.00007</td>
<td>9762</td>
<td>99.6</td>
<td>33.2</td>
<td>8.9</td>
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<tr>
<td></td>
<td>HIV-infected</td>
<td>4.3</td>
<td>0.0069</td>
<td>114.5</td>
<td>68.5</td>
<td>515.9</td>
<td>25.0</td>
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<tr>
<td>CD45RA⁺CCR7⁻CD28⁻ (TEMRA)</td>
<td>HIV-</td>
<td>-</td>
<td>0.0079</td>
<td>97.7</td>
<td>64.9</td>
<td>30.5</td>
<td>9.15</td>
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<td>4.3</td>
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<td>High VL</td>
<td>(± 0.6)</td>
<td>(± 0.0006)</td>
<td>(± 0.7)</td>
<td>(± 0.8)</td>
<td>(± 12.4)</td>
<td>(± 1.1)</td>
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<td>(± 0.6)</td>
<td>(± 0.0006)</td>
<td>(± 0.7)</td>
<td>(± 0.8)</td>
<td>(± 12.4)</td>
<td>(± 1.1)</td>
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</table>

* p < 0.05, ** p < 0.01, *** p < 0.001 HIV-negative versus HIV-infected; ^ A p < 0.05 HIV-negative versus HIV-infected high VL
Lower Percentages of CD8⁺ T_{CM} Cells Remaining Correlate with Higher HIV VL

- **HIV-infected**
- **HIV-negative**

\[ r = -0.90 \]
Expression of IL-7R$\alpha$ or IL-18R1$\alpha$ on T$_{CM}$ or T$_{EMRA}$ CD8$^+$ T Cells

**IL-7R$\alpha$**

![Graph showing expression of IL-7R$\alpha$](image)

**IL-18R1$\alpha$**

![Graph showing expression of IL-18R1$\alpha$](image)

* $p<0.05$, ** $p<0.01$
Summary

• The different kinetic die-away patterns in different CD8⁺ T cell subpopulations demonstrate that the turnover of low-abundance T cell subpopulations can now be studied using the refined stable isotope / FACS / mass spectrometric method.

• Two long-lived CD8⁺ memory/effector T cell subpopulations were found: T_{CM} cells expressing IL-7Rα and T_{EMRA} cells, of which a high fraction expresses CD57.

• T_{CM} cells appear to have a shorter half-life in HIV-infected subjects than HIV-negative subjects and decline numerically with progressive HIV disease.

• T_{EMRA} cells had a long half-life in both HIV-infected and HIV-negative subjects and were significantly increased in all HIV-infected subjects irrespective of their VL.
Summary

• Accepted traits of hematopoietic stem cells (such as higher expression of the transcriptional repressor, bcl6b*, or cell surface expression of IL-18R1α**) could not be ascribed to these human CD8+ memory T cell subpopulations.

• However, a lower fraction of $T_{CM}$ cells in HIV-infected individuals expressed IL-7Rα and the fraction of $T_{CM}$ cells that expressed IL-7Rα trended to decrease with declining CD4 counts.

• The fraction of $T_{EMRA}$ cells expressing IL-7Rα, IL-18Rα, or CD57 was also lower in HIV-infected individuals.

* MandersPM. et al. PNAS (2005)
** Luckey CJ. et al. PNAS (2006)
Conclusions

• These data are consistent with the hypothesis that IL-7Rα+ T_{CM} cells represent “true” memory CD8+ T cells, the loss of which may be responsible in part for the progressive loss of T cell memory function during progressive HIV infection.

• Further exploration of these observations may provide a more complete understanding of the manner in which the CD8+ T cell compartment is eroded, both numerically and functionally, as HIV disease advances.

Ladell K. et al. JI (2008) in press
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