Relationship of CD8+ T Cell Non-Cytotoxic Anti-HIV Response to CD4+ T Cell Number in Untreated Asymptomatic HIV-Infected Individuals

JoAnn C. Castelli1, Steven G. Deeks1, Stephen Shiboski2, Jay A. Levy1

1Department of Medicine and 2Department of Biostatistics,
University of California, San Francisco, CA 94143-1270

Running title: CD8+ cell anti-HIV responses in infected subjects

Scientific Heading: Immunobiology

Grant and Training Support: J. Castelli is a trainee under the National Institutes of Health Training Program (T32 AI07395). These studies were supported by a grant from the National Institutes of Health (RO1 AI49926-01).

Reprint Requests:

Jay A. Levy
UCSF
Department of Medicine
San Francisco, CA 94143-1270
jalevy@itsa.ucsf.edu

Word count: Abstract 103, Text 1196
Abstract

During chronic HIV infection, asymptomatic individuals demonstrate a strong CD8+ cell noncytotoxic antiviral response (CNAR). With the onset of symptoms or reduction in CD4+ cell counts, CNAR decreases. Presently, infected individuals are recommended to receive antiretroviral therapy if CD4+ cell counts fall below 350 cells/µl. To determine whether CNAR activity lends support to this time to initiate antiretroviral treatment, we examined CNAR in 20 healthy untreated HIV-infected men exhibiting a range of CD4+ cell numbers. Our results indicate that the asymptomatic untreated HIV-infected individuals with less than 300 CD4+ cell/µl had a significantly lower CNAR than those with higher CD4+ cell counts. These data on CNAR in untreated healthy HIV-infected individuals support the current recommendation for when to initiate antiretroviral therapy.

Corresponding Author: Jay A. Levy, M.D. jalevy@itsa.ucsf.edu
Introduction

In HIV-infected individuals, antiretroviral therapy is recommended when CD4+ cell counts fall below 350 cells/µl, viral load rises above 30,000 copies/ml, or HIV-related symptoms appear \(^1\). Although immunologic responses also decrease during chronic infection, this function has not been used to determine when antiretroviral therapy should be initiated. One of the immunologic responses reduced over time in HIV-infected individuals is the CD8+ cell noncytotoxic antiviral response (CNAR).

CNAR is first observed during the acute stage of HIV infection and then can be maintained for more than 10 years \(^2\). In chronic HIV-infected individuals, the extent of CNAR correlates directly with the clinical state and the CD4+ cell counts \(^2-7\). For example, chronic HIV-infected individuals who are asymptomatic and have more than 500 CD4+ cells/µl have a strong CNAR. Conversely, individuals who are symptomatic and have lower CD4+ cell counts have a significantly weaker CNAR \(^3,5,6,8\). The clinical importance of CNAR is strongly suggested by studies of HIV and SIV infection in primates. Administration of anti-CD8 antibodies caused a return in virus production in these animals \(^9-11\). The rapid rebound in viremia appears to reflect a noncytotoxic mechanism for control of virus replication.

Our goal was to determine whether CNAR in untreated asymptomatic individuals could provide an immunologic measure that supports the time now recommended to initiate antiretroviral therapy. To address this question, we compared the extent of CNAR in CD8+ cells from 20 randomly selected untreated asymptomatic individuals having a broad range of CD4+ cell counts.
Materials and Methods

Study Subjects

After informed consent, peripheral blood was drawn from a cohort of 20 randomly selected men, chronically infected with HIV, who were clinically healthy and not on therapy. Two of the twenty subjects completed antiretroviral treatment three years and five years before enrollment, while all others were therapy-naive. All subjects contracted HIV through sexual contact and with the exception of one individual (infected 2 years ago), seroconverted more than 10 years before enrollment. Plasma viral loads were measured by the bDNA technique. This study received approval of the Committee for Human Research, University of California, San Francisco, CA.

Cell Culture

Blood samples from clinically healthy seronegative and from HIV-1 seropositive subjects were collected by venipuncture. Complete blood counts and T cell subset identification was assessed from EDTA-treated blood. Peripheral blood mononuclear cells (PBMC) were isolated from the heparinized blood by Ficoll gradient separation. Cells were cultured at 3 x 10^6 cells/ml in complete RPMI 1640 medium, containing 10% heat-inactivated (56°, 30 min.) fetal bovine serum (Gibco BRL, Rockville, MD), 1% penicillin/streptomycin, 2 mM glutamine, and 10% purified human IL-2 (Roche Diagnostics, Indianapolis, IN).

Acute Infection Assay

From the PBMC of seronegative individuals, CD4+ cells were obtained by immunomagnetic (IM) bead separation (Miltenyi Biotech, Auburn, CA), cultured at 3 x 10^6
cells/ml in complete medium, supplemented with 10% purified human IL-2. CD4+ cells were then stimulated with PHA-L (3 µg/ml) for 3 days at 37°C before being infected with HIV-1. For recovery of CD8+ cells, PBMC from seropositive subjects were first stimulated with 3 µg/ml PHA-L (Sigma Chemicals, St. Louis, MO) for 3 days.

After 3 days of PHA stimulation, CD8+ cells were isolated from the seropositive PBMC cultures by positive selection with IM beads. The PHA-stimulated CD4+ cells were infected with HIV-1SF33, a β-chemokine-insensitive, CXCR4-dependent viral isolate, at a titer in which peak reverse transcriptase (RT) activities were reached at 7 days in infected CD4+ cells cultured alone. CD8+ cells and CD4+ cells were cocultured in duplicate at input CD8+:CD4+ cell ratios ranging from 0.25:1-2:1, in complete medium, supplemented with 100 U/ml recombinant human IL-2 (generously provided by Glaxo IMB, Geneva, Switzerland). Culture supernatants were monitored for RT activity and percent suppression calculated as described previously.

**Statistical Analysis**

Exact Wilcoxon rank sum tests were used to evaluate differences in the distributions of markers of antiviral response for symmetry and the presence of outliers between individuals with CD4+ cell counts of more than or less than 300. Differences were deemed significant if exact P-values were <0.05. For analysis purposes, a limit of detection (50 HIV RNA copies/ml) was set for undetectable viral load values.
Results and Discussion

Study Group Characteristics A comparison of 20 randomly selected subjects showed a distinct division in the range of CD4+ cell counts at greater than 481 CD4+ cells/µl (>300 CD4+ cells/µl) and at less than 300 CD4+ cells/µl (<300 CD4+ cells/µl) (Table 1).

CD4+ cell count, CD8+ cell count, and plasma viral loads differed significantly between the two groups. The mean CD4+ cell count for those individuals in the group with higher CD4+ cell counts was 629 cells/µl whereas the mean cell number for those individuals in the group with lower CD4+ cell counts was 220 cells/µl (p=0.001). CD8+ cell numbers were higher (1605 CD8+ cells/µl) in individuals with higher CD4+ cell counts than in individuals with lower CD4+ cell counts (677 CD8+ cells/µl) (p=0.01). Plasma viral load values were lower (4173 HIV RNA copies/ml) in individuals with higher CD4+ cell counts than in individuals with lower CD4+ cell counts (26,314 HIV RNA copies/ml) (p=0.03).
Table 1. Characteristics of study subjects with different CD4+ cell numbers

<table>
<thead>
<tr>
<th></th>
<th>&gt;300 CD4+ cells/µl</th>
<th>&lt;300 CD4+ cells/µl</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>46</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(34-59)</td>
<td>(31-55)</td>
<td></td>
</tr>
<tr>
<td>CD4+ cell number/µl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>629 +/- 164</td>
<td>220 +/- 58</td>
<td>0.001</td>
</tr>
<tr>
<td>Range</td>
<td>(481-1011)</td>
<td>(136-298)</td>
<td></td>
</tr>
<tr>
<td>CD8+ cell number/µl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>1605 +/- 1074</td>
<td>677 +/- 331</td>
<td>0.01</td>
</tr>
<tr>
<td>Range</td>
<td>(581-3623)</td>
<td>(204-1371)</td>
<td></td>
</tr>
<tr>
<td>Viral Load (RNA/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>4173 +/- 8988</td>
<td>26,314 +/- 37,189</td>
<td>0.03</td>
</tr>
<tr>
<td>Range</td>
<td>(50-29,000)</td>
<td>(50-117,963)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. T cell subsets and plasma viral load values, obtained at the time CNAR was assessed, are presented as median and range (in parentheses). P values were determined by Wilcoxon rank sum test for paired samples.

**Antiviral Response** By analyzing suppression of HIV replication as a function of CD4+ cell number, a difference in the extent of suppression was observed in individuals with less than 300 CD4+ cells/µl in comparison to those individuals with higher CD4+ cell counts, (Fig 1A).

A statistically lower number of CD8+ cells (average cell ratio of 1.1:1) was required to reach 90% suppression of HIV replication in the group with higher CD4+ cell counts than in the
group with less than 300 CD4+ cells (average cell ratio of 2.35:1) (p=0.01) (Figure 1B). In some subjects with less than 300 CD4+ cells/µl, the CD8+:CD4+ cell input of >2:1 in the coculture (Figure 1B) indicated an inability of CNAR to reach 90% suppression at the ratio of 2:1.

Two of the subjects in the present study represented exceptions. One showed strong CNAR with CD4+ cell counts below 300 cells/µl. This individual did not have a substantially different CD4+ cell count, CD8+ cell count, or plasma viral load from the other individuals in this group. The other individual had poor CNAR but an exceptionally high CD4+ cell count (1033 cells/µl) and an undetectable viral load. This individual had the highest CD4+ cell count of the cohort along with a higher percentage of CD4+ cells than CD8+ cells. Both individuals seroconverted more than ten years ago and were antiretroviral therapy-naïve.

The past and these present findings suggest that the HIV-infected individual with a strong CNAR, despite the low CD4+ cell count, would remain healthy for a longer time than the individual with a low CNAR and high CD4+ cell count. Other subjects who show similar features need to be followed over time to assess this possibility.

Using an exploratory analysis of the extent of CNAR in association with viral load, we observed a trend between low CNAR and high viral load (data not shown). Nevertheless, the number of subjects in each CD4+ cell number group was too low to conclude statistically a relationship of CNAR to viral load.

The present study of untreated HIV-infected individuals, with no HIV-related symptoms, confirms that the extent of CNAR correlates with CD4+ cell counts. Asymptomatic individuals with CD4+ cell counts below 300 cells/µl showed less ability to suppress HIV replication than
those with higher CD4+ cells counts. The findings provide immunologically-based evidence supporting the current recommendation for the time to initiate combination antiretroviral therapy. The results suggest that CNAR could be an additional marker to help determine the optimal time to begin antiviral treatment.

Acknowledgements

The authors thank Sue Fujimura for her technical assistance.
References


asymptomatic compared to those who have progressed to Acquired Immunodeficiency Syndrome. Blood. 1998;92:3105-3114.


Figure 1. CD8+ cell noncytotoxic antiviral response in HIV-infected individuals with different CD4+ cell numbers. CD8+ cells from HIV-seropositive individuals were isolated
from PHA-stimulated PBMC and cocultured at different input cell ratios with PHA-stimulated seronegative CD4+ cells, acutely infected with HIV-1SF33. The ratio of CD8+: CD4+ cells required to reach $\geq 90\%$ suppression of HIV replication is indicated for each infected individual A) within a range of CD4+ cells/$\mu$l (O) and B) after dividing the subjects into two groups either with less than (▲) or greater than (■) 300 CD4+ cells/$\mu$l. Percent suppression is calculated from the amount of reverse transcriptase (RT) activity $^{14}$ in the supernatants of the cocultures of CD8+ and CD4+ cells compared to the RT activity in the supernatants from infected CD4+ cells cultured alone. Solid lines represent the average CD8:CD4+ cell ratio for $\geq 90\%$ suppression.
Relationship of CD8+ T Cell Non-Cytotoxic Anti-HIV Response to CD4+ T Cell Number in Untreated Asymptomatic HIV-Infected Individuals

JoAnn C Castelli, Steven G Deeks, Stephen Shiboski and Jay A Levy