Course and Clinical Significance of CD8+ T-Cell Counts in a Large Cohort of HIV-Infected Individuals

Marie Helleberg,1 Gitte Kronborg,4 Henrik Ullum,2 Lars P. Ryder,3 Niels Obel,1 and Jan Gerstoft1

1Department of Infectious Diseases, 2Department of Clinical Immunology, and 3The Tissue Type Laboratory, Copenhagen University Hospital, Rigshospitalet, Copenhagen, and 4Department of Infectious Diseases, Copenhagen University Hospital, Hvidovre Hospital, Denmark

Objectives. To examine trajectories of CD8+ T-cell counts before and after combination antiretroviral therapy (cART) in human immunodeficiency virus (HIV)-infected individuals and associations with mortality.

Methods. CD8+ T-cell counts were measured in 3882 HIV-infected individuals who received care in Copenhagen during 1995–2012. Reference values were obtained from 1230 persons from the background population. Mortality rate ratios were estimated by Poisson regression.

Results. CD8+ T-cell counts were elevated during untreated HIV infection and remained elevated through 10 years of cART. A slight drop of 130 cells/µL (interquartile range, −160 to 410 cells/µL) in the median CD8+ T-cell count was observed after cART initiation. CD8+ T-cell counts stabilized at approximately 900 cells/µL (95th percentile of the background population, 835 cells/µL). Markedly elevated CD8+ T-cell counts at cART initiation were associated with a poor increase in the CD4+ T-cell count (relative risk, 2.22; 95% confidence interval [CI], 1.42–3.48). Individuals with a CD8+ T-cell count of <500 cells/µL 1 year after cART initiation had an increased mortality rate (mortality rate ratio, 1.73; 95% CI, 1.29–2.32) and a higher proportion of deaths attributable to AIDS-related conditions, compared with individuals with CD8+ T-cell counts of ≥500 cells/µL. After receiving cART for 10 years, a CD8+ T-cell count of >1500 cells/µL was associated with increased non-AIDS-related mortality (mortality rate ratio, 1.82; 95% CI, 1.09–3.22), compared with a CD4+ T-cell count of 500–1500 cells/µL.

Conclusions. CD8+ T-cell counts are elevated during HIV infection and do not normalize despite long-term cART. Low CD8+ T-cell counts are associated with increased AIDS-related mortality. Marked elevations in CD8+ T-cell counts after long-term cART are associated with increased non-AIDS-related mortality.

Keywords. HIV; CD8; immunological recovery; immune activation; mortality.

Human immunodeficiency virus (HIV) infection leads to perturbations of T-cell homeostasis and a skewed distribution of T-cell subsets [1]. In contrast to the gradual decrease in CD4+ T-cell count seen in the majority of untreated HIV-infected individuals, CD8+ T-cell counts are elevated in most cases until HIV infection becomes severe, when CD4+ and CD8+ T-cell counts are depleted. While the majority of HIV-infected individuals achieve normal CD4+ T-cell counts after long-term receipt of combination antiretroviral therapy (cART) [2], there are continuous quantitative, qualitative, and functional defects in the CD8+ T-cell compartment despite successful treatment, although some of these defects may be reversed by early initiation of cART [3–6].

The elevation of CD8+ T-cell counts during untreated HIV infection is presumably caused by increased peripheral CD8+ T-cell proliferation due to antigen stimulation and immune activation, but the mechanisms are not well understood [7]. All CD8+ T-cell subsets are increased in HIV-infected individuals, but the proportion of terminally differentiated CD8+ T cells are disproportionately elevated, and the proportion of naive cells is reduced [8–10].

The clinical implications of abnormal CD8+ T-cell counts during HIV infection have not been fully
elucidated. Recent studies have shown that a low ratio of CD4+ to CD8+ T cells among treated HIV-infected individuals is associated with increased morbidity and mortality [11, 12]. The homeostasis of the CD4+ and CD8+ T-cell compartments are regulated differentially [5, 7]; thus, CD8+ T-cell counts may predict prognosis independently of CD4+ T-cell counts.

The aim of the present study was to describe changes in CD8+ T-cell counts during untreated HIV infection and during long-term cART in a large population-based cohort of HIV-infected individuals and to examine associations with CD4+ T-cell count increases and mortality.

METHODS

Data Sources

The Danish HIV Cohort Study, described in details elsewhere [13], is a population-based nationwide cohort study of all HIV-infected individuals who have received care in Danish HIV centers after 1 January 1995. CD4+ and CD8+ T-cell count and HIV RNA load measurements are extracted electronically from laboratory data files. Data on vital status and migration and causes of death were retrieved from the Danish Civil Registration System [14] and the National Registry of Causes of Death.

References values for CD8+ T-cell counts were obtained from a sample of 1230 persons from the background population. This group consisted of healthy staff, blood donors, and stem-cell donors.

CD4+ and CD8+ T-cell counts were measured by the single platform lyse-no-wash procedure, using Becton-Dickinson TRUcount beads and monoclonal antibodies against CD3, CD4, and CD8.

Study Population

We included all HIV-infected individuals aged ≥16 years at diagnosis, who received care in HIV centers in Copenhagen in the period 1 January 1995 to 31 December 2012. Individuals with no available data on CD8+ T-cell counts were excluded from the study. For specific analyses we included subsets of this population who fulfilled specified criteria as described in detail later.

Statistics

Differences in CD8+ T-cell counts between groups defined by origin, route of infection, age, CD4+ T-cell count, HIV RNA load, cytomegalovirus (CMV) serostatus, hepatitis B virus (HBV) coinfection, hepatitis C virus (HCV) coinfection, and HIV status were analyzed by the Mann–Whitney U test and the Kruskal–Wallis test. We included all variables from univariate analyses in multivariate linear regression analyses. Correlations between CD8+ T-cell counts and HIV RNA loads were assessed by Spearman rank correlation.

Relative risks (RRs) of a poor CD4+ T-cell count increase and of death were analyzed using Poisson regression. In analyses of CD4+ T-cell count increase, time was calculated from date of cART initiation until the date of the CD4+ T-cell count measurement 1 year thereafter (±30 days). We analyzed mortality in 3 separate analyses, defining baseline as the date of CD8+ T-cell count at cART initiation, 1 year after cART initiation, or 10 years after cART initiation. Time was calculated from the date of CD8+ T-cell count measurement until death, emigration, or 31 December 2012, whichever occurred first. Mortality rate ratios for individuals with CD8+ T-cell counts of <500 cells/µL, 500–1500 cells/µL, 1500–2000 cells/µL, or ≥2000 cells/µL versus 500–1500 cells/µL were estimated by Poisson regression, with adjustment for sex, age, route of HIV transmission, year of HIV diagnosis, and CD4+ T-cell count (measured the same date as the CD8+ T-cell count under analysis). The reference CD8+ T-cell count of 500–1500 cells/µL roughly corresponded to the 25th–75th percentile and a CD8+ T-cell count of ≥2000 cells/µL corresponded to the 90th percentile in the HIV-infected population at start of cART.

In sensitivity analyses of mortality, we replaced CD8+ T-cell counts in the analyses with total lymphocyte counts measured at the same date as the CD8+ T-cell count.

Stata 8.0 (StataCorp, College Station, Texas) and Excel 2010 (Microsoft; Redmond, Washington) were used for data analyses.

Ethics

The study was approved by the Danish Data Protection Agency. Ethics approval and individual consent are not required by Danish legislation governing this type of study.

RESULTS

A total of 4191 HIV-infected adults were followed in HIV centers in Copenhagen during the study period. Of these, 309 individuals were excluded because of lack of data on CD8+ T-cell counts. We thus included 3882 individuals, of whom 3060 (79%) were male; the median age at the time of the first CD8+ T-cell count measurement was 39 years (interquartile range [IQR], 32–47 years); 2012 (52%) were men who have sex with men (MSM), and 388 (10%) were infected through injection drug use; 3427 (88%) received cART during follow-up. Median follow-up was 8.6 years (IQR, 4.1–13.7 years). During 29 896 person-years of follow-up 94 344 CD8+ T-cell counts were measured; 14 129 (15%) were measured before cART initiation, and 47 293 (50%) were measured >5 years after cART initiation.

In the group of persons from the background population, 419 (52%) were male, and the median age was 33 years (IQR, 27–43 years). The median CD4+ and CD8+ T-cell counts in this group were 820 cells/µL (IQR, 630–1000 cells/µL) and 450 cells/µL (IQR, 340–570 cells/µL), respectively.
**Distribution of CD8+ T-Cell Counts at the Start of cART**

Table 1 summarizes CD8+ T-cell counts among 2284 HIV-infected individuals who had their CD8+ T-cell count measured at cART initiation. The median CD8+ T-cell count was 900 cells/µL (IQR, 590–1300 cells/µL; Table 1), which was slightly higher than the 95th percentile in the background population (835 cells/µL). Among HIV-infected individuals with a CD4+ T-cell count of <200 cells/µL at the start of cART, the median CD8+ T-cell count was 630 cells/µL (IQR, 380–950 cells/µL), compared with 1016 cells/µL (IQR, 740–1500 cells/µL) among those with a CD4+ T-cell count of ≥200 cells/µL. There were only slight differences between other subgroups: CD8+ T-cell counts were higher among MSM, compared with heterosexual men and women, and among individuals aged ≥50 years, compared with those aged <50 years. CD8+ T-cell counts were significantly higher than in the background population in all subgroups (P < .001 for all comparisons). There was no correlation between CD8+ T-cell count and HIV RNA load (ρ = -0.01; P = .72).

**CD8+ T-Cell Counts and Other Viral Infections**

There were no significant associations between CD8+ T-cell count and HBV or HCV coinfection at the time of cART initiation (Table 1). Individuals who were seropositive for CMV had higher CD8+ T-cell counts than those who were seronegative, but the difference was small and not statistically significant in multivariate analysis. A year after cART initiation, when 71% of the HIV-infected individuals had an HIV RNA load of <40 copies/mL, CMV-seropositive status was associated with a higher median CD8+ T-cell count, compared with the CMV-seronegative group (943 cells/µL [IQR, 680–1300 cells/µL; n = 1976] vs 789 cells/µL [IQR, 499–1100 cells/µL; n = 387]; P < .01).

**Trajectories of CD4+ and CD8+ T-Cell Counts and Ratio of CD4+ to CD8+ T Cells**

In analyses of trajectories of CD8+ T-cell counts, we included 865 individuals for whom CD8+ T-cell counts were available at least 2 years before and 1 year after cART. A total of 28442 CD8+ T-cell counts were measured for these patients, with a median interval of 98 days (IQR, 83–127 days). CD8+ T-cell counts were stable during chronic, untreated HIV infection. Within the first 2 years after cART initiation, the median change in CD8+ T-cell count was −130 cells/µL (IQR, −160 to 410 cells/µL; Figure 1). CD8+ T-cell counts remained high, compared with data for the background population, with no change the following 8 years (median change, 0 cells/µL; 95% confidence interval [CI], −240 to 250 cells/µL). There was a strong correlation between CD8+ T-cell counts 2 years before cART initiation and 5 years after cART initiation (ρ = 0.42; P < .001).

If individuals without serial CD8+ T-cell count measurements 2 years before and 1 year after cART initiation were included in analyses (n = 2284), the median CD8+ T-cell count at cART initiation was lower (Table 1), while CD8+ T-cell counts at other time points were not significantly different (data not shown). The lower CD8+ T-cell count at cART initiation was explained by inclusion of individuals who had advanced HIV disease and severe lymphopenia at the time of HIV diagnosis and cART initiation.

Figure 2 shows the distributions of CD4+ and CD8+ T-cell counts at cART initiation and 10 years thereafter. The variance in CD8+ T-cell counts decreased, but the mean changed only little after cART initiation, which is in contrast to the significant increase in both mean and variance of CD4+ T-cell counts. Even...
after 10 years of cART, CD8⁺ T-cell counts were significantly higher and CD4⁺ T-cell counts lower among HIV-infected individuals, compared with the background population (P < .001 in both analyses).

Changes in the ratio of CD4⁺ to CD8⁺ T-cell counts during the first 2 years of cART had a closer correlation to changes in CD4⁺ T-cell counts than changes in CD8⁺ T-cell counts (ρ = 0.49 [P < .001] and ρ = 0.39 [P < .001], respectively).

We assessed CD8⁺ T-cell counts by viral load 1 year after cART initiation and found no differences in CD8⁺ T-cell counts between different viral load strata (Supplementary Figure 1).

**CD8⁺ T-Cell Counts and Mortality**

The association between CD8⁺ T-cell count and risk of death changed over time of cART receipt (Table 2). Individuals with CD8⁺ T-cell counts of <500 cells/µL at the time of cART initiation had increased mortality, compared with those with CD8⁺ T-cell counts of 500–1500 cells/µL. Both individuals with CD8⁺ T-cell counts of <500 cells/µL and those with CD8⁺ T-cell counts of >2000 cells/µL 1 year after cART initiation had increased mortality. Individuals with CD8⁺ T-cell counts of >1500 cells/µL 10 years after cART initiation had increased mortality, whereas low CD8⁺ T-cell counts were not...
Figure 2. Distributions of CD8⁺ and CD4⁺ T-cell counts (dark grey) at initiation of combination antiretroviral therapy (cART; A and B, respectively) and 10 years after cART initiation (C and D, respectively), compared with the background population (light grey). Abbreviation: HIV, human immunodeficiency virus.
associated with an increased risk of death. All analyses were adjusted for CD4+ T-cell count. Analyses stratified by CD4+ T-cell count (<200, 200–500, and >500 cells/µL) yielded similar results (data not shown).

In analyses restricted to individuals with a fatal outcome, time to death was markedly shorter for individuals with CD8+ T-cell counts of <500 cells/µL, compared with those with higher CD8+ T-cell counts (Table 2), and a higher proportion of deaths were AIDS related. Among individuals with CD8+ T-cell counts of <500 cells/µL at cART initiation, 24 deaths (22% of all deaths) were AIDS related, compared with 36 deaths (13%) among those with CD8+ T-cell counts of ≥500 cells/µL (P = .04). Among individuals with CD8+ T-cell counts of <500 cells/µL 1 year after cART initiation, the corresponding values were 15 (24%) and 20 (8%), respectively (P < .01). There were no AIDS-related deaths >10 years after cART initiation.

To examine whether associations between CD8+ T-cell counts and mortality were explained by virological failure, we reanalyzed the data while censoring individuals at the time of virological failure (defined as an HIV RNA load of >1000 copies/mL >180 days after cART initiation). Results did not differ significantly from results of the original analyses (Supplementary Table 1).

We repeated the analyses by using total lymphocyte counts instead of CD8+ T-cell counts. Findings were quite different from findings of associations between CD8+ T-cell counts and mortality. Low total lymphocyte counts were associated with increased mortality, and the association became stronger after long-term cART receipt, while there was no association between high total lymphocyte count and mortality (Table 3).

**DISCUSSION**

In this study examining trajectories of CD8+ T-cell counts in HIV-infected individuals before and after long-term cART, we found that CD8+ T-cell counts were significantly elevated in untreated HIV-infected individuals and decrease only slightly after cART initiation. CD8+ T-cell counts were elevated in all subgroups examined. Individuals with markedly elevated CD8+ T-cell counts at treatment initiation had an increased risk of having a poor CD4+ T-cell count gain. After cART initiation, CD8+ T-cell counts converged toward levels above the 95th percentile in the background population and remained there despite >10 years of cART. Low CD8+ T-cell counts within the first year after cART initiation were associated with increased mortality, with a high proportion of AIDS-related deaths. After long-term cART, markedly elevated CD8+ T-cell counts were associated with increased non–AIDS-related mortality.

The observed lack of normalization of CD8+ T-cell counts after long-term cART is somewhat surprising, since previous studies have shown that CD8+ T-cell proliferation is correlated with HIV replication and decreases markedly after initiation of cART [15, 16], and is in contrast to the fact that the majority of HIV-infected patients experience CD4+ T-cell count recovery toward levels comparable to those of the background population. CD8+ T-cell counts were slightly higher in MSM and in individuals with CMV infection. These findings are similar to results of previous studies [17, 18] and indicate that ongoing infections other than HIV infection may exacerbate elevations of CD8+ T-cell counts; however, their contribution first becomes evident once HIV replication is controlled.

There was a clear association between CD8+ T-cell counts before and after cART initiation. Previous studies have shown that CD8+ T-cell counts correlate with proviral DNA [19] and that
the increase in CD8+ T-cell counts during primary HIV infection coincides with expansion of the HIV reservoir [20, 21]. Together, these findings could suggest that the CD8+ T-cell count reflects the size of the viral reservoir. In contrast, we found no correlation between CD8+ T-cell counts and HIV RNA loads. Our finding of an association between high CD8+ T-cell counts and a poor CD4+ T-cell count response to cART is in line with previous studies showing that poor CD4+ T-cell count gain is associated with hyperactivation of CD8+ T cells, increased rates of peripheral CD8+ T-cell proliferation, and depletion of naive cells [22].

HIV-infected individuals with relatively low CD8+ T-cell counts during the first year of cART had increased mortality. Low CD8+ T-cell counts were associated with increased mortality, independently of age and CD4+ T-cell count. The association persisted even when individuals were censored at virological failure and is therefore not explained by uncontrolled HIV replication. CD8+ T cells have important functions in the control of infections and for immune surveillance. CD8+ T cells inhibit and kill tumor cells through production of interferon γ and through cytotoxic effects [23]. Thus, depletion of CD8+ T cells may cause an increased risk of cancer. Approximately one

Table 2. Mortality Among Human Immunodeficiency Virus-Infected Individuals, by CD8+ T-Cell Count and Time After Combination Antiretroviral Therapy (cART) Initiation

<table>
<thead>
<tr>
<th>Variable, by Time After cART Initiation</th>
<th>CD8+ T-Cell Count, Cells/µL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;500</td>
</tr>
<tr>
<td>0 y&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Patients, no. (%)</td>
<td>425 (18.6)</td>
</tr>
<tr>
<td>MRR (95% CI)</td>
<td>1.47 (1.14–1.87)</td>
</tr>
<tr>
<td>Time to death, y</td>
<td>3.4 (1.4–7.6)</td>
</tr>
<tr>
<td>Observation time for those censored, y</td>
<td>8.2 (4.6–14.0)</td>
</tr>
<tr>
<td>Patients with fatal outcome who died within 1 y, %</td>
<td>20</td>
</tr>
<tr>
<td>1 y&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Patients, no. (%)</td>
<td>284 (12.4)</td>
</tr>
<tr>
<td>MRR (95% CI)</td>
<td>1.73 (1.29–2.32)</td>
</tr>
<tr>
<td>Time to death, y</td>
<td>2.2 (0.6–6.3)</td>
</tr>
<tr>
<td>Observation time for those censored, y</td>
<td>6.4 (2.9–10.1)</td>
</tr>
<tr>
<td>Patients with fatal outcome who died within 1 y, %</td>
<td>30</td>
</tr>
<tr>
<td>10 y&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Patients, no. (%)</td>
<td>133 (9.3)</td>
</tr>
<tr>
<td>MRR (95% CI)</td>
<td>1.26 (0.67–2.37)</td>
</tr>
<tr>
<td>Time to death, y</td>
<td>1.3 (0.3–2.3)</td>
</tr>
<tr>
<td>Observation time for those censored, y</td>
<td>3.8 (2.2–5.8)</td>
</tr>
<tr>
<td>Patients with fatal outcome who died within 1 y, %</td>
<td>35</td>
</tr>
</tbody>
</table>

Data are median value (interquartile range), unless otherwise indicated. The median CD8+ T-cell count in the background population was 450 cells/µL (5th–95th percentile, 220–835 cells/µL).

Abbreviations: CI, confidence interval; MRR, mortality rate ratio.

<sup>a</sup> Data are for 17 231 person-years of follow-up and 380 deaths.

<sup>b</sup> Data are for 15 526 person-years of follow-up and 325 deaths.

<sup>c</sup> Data are for 5600 person-years of follow-up and 119 deaths.

Table 3. Mortality Rate Ratios Among Human Immunodeficiency Virus (HIV)-Infected Individuals, by Total Lymphocyte Count and Time After Combination Antiretroviral Therapy (cART) Initiation

<table>
<thead>
<tr>
<th>Time After cART Initiation</th>
<th>Total Lymphocyte Count, Cells/µL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1000</td>
</tr>
<tr>
<td>0 y</td>
<td>380</td>
</tr>
<tr>
<td>1 y</td>
<td>325</td>
</tr>
<tr>
<td>10 y</td>
<td>119</td>
</tr>
</tbody>
</table>

The reference value of 1000–2000 cells/µL for total lymphocyte count corresponded to the 25th–75th percentile, and a total lymphocyte count of >2600 cells/µL corresponded to the 90th percentile in the HIV-infected population at start of cART.
fourth of the deaths among HIV-infected individuals with low CD8⁺ T-cell counts during the first year of cART occurred within a year. This reflects the high risk of AIDS-related events among individuals with severe immunodeficiency at cART initiation. It is possible that redistribution of lymphocytes from blood to tissues with ongoing pathological processes is part of the explanation for the observed association between low CD8⁺ T-cell counts and increased short-term mortality.

Markedly elevated CD8⁺ T-cell counts after long-term cART were associated with a moderate increase in non–AIDS-related mortality. An association between high CD8⁺ T-cell counts and increased mortality has previously been observed in HIV-negative populations [24, 25]. Elevated CD8⁺ T-cell counts are associated with immune activation [26]. Whether there is an association between immune activation and an increased risk of non–AIDS-related mortality in the long term (eg, deaths due to cardiovascular disease, cancer, and liver disease) needs to be explored in future studies.

The present study is limited in that we did not have data on the subsets of CD8⁺ T cells or proportions of activated CD8⁺ T cells. Strengths of the study include the large study population with data on CD8⁺ T-cell counts and long-term follow-up.

We conclude that CD8⁺ T-cell counts are continuously elevated in HIV-infected individuals and not normalized despite 10 years of cART. Individuals with CD8⁺ T-cell counts below the median of the background population during the first year of cART have increased mortality, with an elevated risk of AIDS-related death within a year. Marked elevations in CD8⁺ T-cell counts after long-term cART are associated with increased non–AIDS-related mortality.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank the staff of our clinical departments, for their continuous support and enthusiasm. All of the authors contributed to the conception and design of the study and/or the analyses and interpretation of the data. The manuscript was drafted by M. H. and J. G. and was critically reviewed and subsequently approved by all authors.

Potential conflicts of interest. N. O. has received research funding from Roche, Bristol-Myers Squibb, Merck, Sharp, and Dohme, GlaxoSmithKline, Abbott, Boehringer Ingelheim, Janssen-Cilag, and Swedish Orphan. J. G. has received research funding from Abbott, Roche, Bristol-Myers Squibb, Merck, Sharp, and Dohme, ViV, Swedish Orphan, and Gilead. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

21. Ananworanich J, Vandergeeten C, Chomchee N. Early ART intervention restricts the seeding of the HIV reservoir in long-lived central memory


