Longitudinal analysis of CD4 T cell counts, T cell reactivity and human immunodeficiency virus type 1 RNA levels in persons remaining AIDS-free despite CD4 cell counts <200 for >5 years

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CD4 cell count, T cell reactivity, and human immunodeficiency virus type 1 (HIV-1) RNA load were examined in 10 persons remaining free of AIDS while CD4 cell counts were <200/mm$^3$ for >5 years, so-called low CD4 cell count long-term survivors (low CD4 LTS), and 10 matched rapid and 10 matched intermediate progressors (AIDS within 1 and 1–3 years). Longitudinal analysis of CD4 cell count and T cell reactivity revealed slower declines in low CD4 LTS compared with rapid progressors, with a similar linear increase over time in low CD4 LTS and progressors of 0.1 log/year. There was considerable overlap between groups. The constant difference between low CD4 LTS progressors probably reflects HIV RNA load early after infection, but low CD4 LTS and progressors show similar slopes of HIV-1 RNA load rise.

Several recent studies suggest that human immunodeficiency virus (HIV) load is a very early predictor of disease progression and that virus load in blood remains remarkably stable over time during asymptomatic HIV-1 infection, the so-called set point hypothesis [1–7]. In addition, the use of virus load has been recommended as a surrogate marker to assess the need for antiretroviral treatment or for changes therein [8–10]. Whether the hypothesis of stability of virus load, described as a steady state [1], holds in prolonged asymptomatic HIV-1 infection remains to be determined. We performed a longitudinal analysis of virus load in low CD4 cell count long-term survivors (low CD4 LTS), persons who remain AIDS-free in spite of low CD4 cell counts. These low CD4 LTS were compared with matched progressor controls. To relate longitudinal patterns of virus load with markers of immune deficiency, we first analyzed patterns of CD4 cell decline and loss of T cell reactivity over time in the same groups.

Many different and sometimes mutually exclusive case definitions have been used for LTS [11]. These definitions have in common that they identify persons with an exceptionally benign course of HIV-1 infection in various stages of HIV-1 infection. Three categories of definitions can be distinguished: lack of clinical and immunologic progression [12, 13], long-term survival after clinical progression [14], and lack of clinical progression in spite of a low CD4 cell count. The present study analyzes the third category and uses the case definition for low CD4 LTS proposed by Schrager et al. [11]: persons who have remained free of clinical AIDS for ≥5 years after a decrease in CD4 cell count to <200 × 10$^6$/L.

The analysis of this subgroup may help to identify factors that sustain immune function in spite of low CD4 cell counts and help to identify correlates of arrested disease progression. In a previous study, we reported that independent predictors of AIDS-free survival following a CD4 cell decline below 200 × 10$^6$/L are absence of syncytium-inducing (SI) variants, absence of HIV p24 antigen, and relatively preserved T cell reactivity after stimulation with phytohemagglutinin [15].

**Subjects and Methods**

**Study population.** The study subjects were all homosexual men who participated in the Amsterdam cohort study on HIV infection among homosexual men, which was started in October 1984 [16]. Participants were seen every 3 months. For the present study, we identified a subset of HIV-infected participants who had had at least one CD4 cell count >200/mm$^3$ after entry or seroconversion and who subsequently had a CD4 cell count <200/mm$^3$ at least two times within 12 months. CD4 cell counts >200/mm$^3$ between these two visits were allowed. The time of the first CD4 cell count <200/mm$^3$ has been designated as time zero in the analysis. In a nested case-control analysis, we compared men with an AIDS-free survival according to the 1987 Centers for Disease Control AIDS case definition of ≥5 years (low CD4 LTS) with men who developed AIDS within 1–3 years (intermediate progressors, IP) and within 1 year after time zero (rapid progressors, RP). Low CD4
LTS, RP, and IP were matched for early treatment, defined as antiretroviral treatment that was started before a clinical AIDS diagnosis. Early treatment was started in the Amsterdam cohort study in 1991 in persons with CD4 cell counts <200/mm³. Because of the differences in duration of follow-up, matching for early treatment was performed as follows: low CD4 LTS who never used early antiretroviral treatment could be matched only with IP and RP who likewise never received early antiretroviral treatment. Low CD4 LTS who started early treatment were preferably matched with IP and RP who started treatment at the same moment before or after time zero within a maximum interval of 6 months. If such controls were not available, low CD4 LTS were matched with IP and RP who developed AIDS before the low CD4 LTS started early treatment. The same procedure was used to match for primary prophylaxis against Pneumocystis carinii pneumonia with either cotrimoxazole or pentamidine, which was started in 1990. In addition, low CD4 LTS and controls were matched for age at time zero and calendar year of first CD4 cell count <200 × 10⁶/L.

**Laboratory methods.** Stored serum samples frozen at −70°C were used for measurements of HIV-1 RNA load with a quantitative RNA assay (NASBA; Organon, Oss, Netherlands) [17–19]. Results are expressed as number of HIV RNA copies per milliliter and were log-transformed for analysis. The quantitation limit of this assay is 1000 RNA copies/mL, and sera with undetectable levels were assumed to have 1000 copies/mL in the analysis. Sera were tested for the presence of antibodies to HIV-1 with a commercially available EIA (Abbott, Abbott Park, IL) and confirmed with Western blotting.

T cell subsets were tested with fresh cells. CD4 and CD8 T cells were enumerated by direct immunofluorescence using monoclonal antibodies and a flow cytomtery system. Cocultivation of cryopreserved peripheral blood mononuclear cells with MT-2 cells was used to detect the presence of SI HIV-1 variants [20, 21]. To measure T cell reactivity, fresh cells were stimulated in a whole blood culture system with CD3 monoclonal antibodies [22–24]. Reactivity is expressed in counts per minute per well. HIV p24 antigen was detected in fresh sera with a solid-phase, sandwich type immunoassay (Abbott).

**Statistics.** We studied CD4 cell count, T cell function, and HIV load over time by use of the proc Mixed module of SAS (SAS Institute, Cary, NC) in three separate analyses. To meet the criteria of multivariate normality, these data were log-transformed. Independent variables were group (low CD4 LTS, IP, or RP), years since time zero, and matching indicator.

Two potential covariance structures between the observations were explored: compound symmetry, which is characterized by a constant correlation between observations from the same person over time, and an autoregressive structure assuming a decrease in the correlation with longer time periods. The covariance structure resulting in the highest likelihood was selected. Second, we analyzed the presence of an interaction between time and group. In the absence of an interaction, we examined whether there was an overall time effect. In case a time effect was detected, we analyzed with the likelihood ratio test whether this was best described by a linear, second-order, or third-order model. All data were used to define the model. Subsequently, outlying observations were identified by visual inspection of the data, by use of two criteria. First, observations that were outliers from the model were selected. Second, we explored whether these observations were also outliers within the individual. If the latter was the case, the observations were removed; if not, all observations of the individual remained in the model.

Individual virus load trajectories of the low CD4 LTS were analyzed by use of linear regression. Finally, the Pearson correlation coefficient ($r$) was calculated. The square of the correlation coefficient ($r^2$) represents the proportion of variation of a dependent variable that is explained by an independent variable, the so-called explained proportion. Here, we use it to analyze which proportion of virus load variation is explained by variation in CD4 cell count and T cell function.

**Results**

**Characteristics at Baseline and Follow-Up**

In 186 men (mean age, 38 years), CD4 cell counts dropped below 200/mm³ during follow-up. The probability of remaining AIDS-free >5 years was 16% (95% confidence interval, 10%–23%), and 10 men were identified as low CD4 LTS. Subsequently, each low CD4 LTS was successfully matched with 1 IP and 1 RP. The mean time between the first HIV-positive visit and the first CD4 cell count <200/mm³ was 3.1 years in the low CD4 LTS, 3.0 years in the IP, and 3.0 years in the RP. SI variants were present at time zero in 0 of 10 low CD4 LTS, 4 of 10 IP, and 5 of 9 RP. Two low CD4 LTS, 6 IP, and 4 RP were positive for HIV p24 antigen. Five years after time zero, SI variants were present in 6 low CD4 LTS. Early antiretroviral treatment was performed as follows: low CD4 LTS who never received early antiretroviral treatment could be matched only with IP and RP who likewise never received early antiretroviral treatment.

**Immunologic Parameters**

**CD4 cell count.** For modeling of the CD4 cell count in low CD4 LTS, IP, and RP, 728 values could be used. Table 1 shows the group medians and interquartile ranges of the CD4 cell counts around time zero in low CD4 LTS and progressors and at year +5 in the low CD4 LTS. Note that the median CD4 cell count at a given time point is the mean CD4 cell count in the 6-month interval around that time point (3 months before until 3 months after the time point). This explains why the median CD4 cell count at time zero can be >200.

Figure 1 is a graphic presentation of the course of the crude log-transformed CD4 cell counts over the entire follow-up period in the low CD4 LTS, IP, and RP. These data were used for modeling the CD4 cell counts in each group. Fifteen outlying observations (2%) were removed before the final analysis was
Table 1. Median values and interquartile ranges of CD4 cell count, T cell reactivity, and HIV-1 RNA load at different time points in long-term survivors with CD4 cell counts <200/mm³ (low CD4 LTS), intermediate progressors (IP), and rapid progressors (RP).

<table>
<thead>
<tr>
<th>Year</th>
<th>CD4 cell count</th>
<th>T cell reactivity</th>
<th>HIV RNA load</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LTS</td>
<td>IP</td>
<td>RP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>220 (171–240)</td>
<td>188 (173–206)</td>
<td>177 (148–215)</td>
</tr>
<tr>
<td>+0.5</td>
<td>247 (196–273)</td>
<td>162 (106–203)</td>
<td>80 (50–138)</td>
</tr>
<tr>
<td>+1</td>
<td>210 (158–260)</td>
<td>116 (85–158)</td>
<td>80 (50–138)</td>
</tr>
<tr>
<td>+5</td>
<td>140 (45–210)</td>
<td>1407 (305–2250)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Year: no. of years before or after initial CD4 cell decline below 200/mm³. T cell reactivity was measured after stimulation with anti-CD3 antibodies. Individual CD4 cell count at given time point is mean CD4 cell count in 6-month interval around that time point (3 months before until 3 months after); thus, median CD4 cell count at time zero can be >200.

* Only 2 observations were available for T cell reactivity in LTS at year −1.

done. The autoregressive covariance structure was found to be most appropriate for these data. CD4 cell decline in the low CD4 LTS can best be described by a linear function with a mean decline of 0.08 log/year (figure 2). The CD4 cell decline in progressors is accelerated and can best be described by a second-order function for IP and a third-order function for RP.

The correlation coefficient (r) between CD4 cell counts and HIV-1 RNA load was .1760 (P < .003), resulting in an explained proportion (r²) of .03.

T cell reactivity. The raw data on T cell reactivity, of which 450 counts were available, show a decline in T cell reactivity in the groups (table 1). Six extreme observations were removed (1.3%). The autoregressive covariance structure was most appropriate for modeling the T cell reactivity data. The low CD4 LTS had a decline of 0.16, IP of 0.46, and RP of 0.64 log cpm/year (figure 3).

The correlation between T cell reactivity and HIV-1 RNA load was −0.5387 (P < .001), resulting in an explained proportion of .29.

HIV-1 RNA Load

HIV-1 RNA load was measured at 6-monthly intervals in the time interval between −2 and +2 years and at 12-monthly
figure 2. Predicted CD4 cell count: modeling of CD4 cell count as dependent variable of time in years since CD4 cell count decline below \(200 \times 10^6/L\). Years \(-2\) to \(-6\) are years preceding; years \(2\)–\(8\) are years following CD4 cell count decline below \(200 \times 10^6/L\).

Figure 3. Modeling of T cell reactivity after stimulation with monoclonal antibodies against CD3 as dependent variable of time in years since CD4 cell count decline below \(200 \times 10^6/L\). Years \(-1\) through \(-3\) are years preceding; years \(2\)–\(8\) are years following CD4 cell count decline below \(200 \times 10^6/L\).

intervals in the interval \(-7\) to \(-2\) and \(+2\) to \(+5\) years, provided sera were available. In total, 277 samples were tested for this study, of which the group medians around time zero and at year 5 are presented in table 1 and figure 4. The correlation between the individual observations over time was best described by the compound symmetry structure. Six outlying observations (2%) were removed before the final analysis was done. There was a significant effect of time \((P < .001)\) and group \((P < .001)\) but no interaction effect between group and time \((P = .77)\), implying that the group effect is constant over time. Hierarchic testing of the three time functions showed that the fit of a linear model was better than that of a second- or third-order function. Therefore, we used linear modeling (figure 5). The constant difference between RP and low CD4 LTS in log load was 0.96. The intercept (the estimated load at time zero) was 4.12 in the low CD4 LTS, 4.79 in the IP, and 5.08...
Figure 4. Group profiles of observed virus load: mean log-transformed HIV-1 RNA levels as observed in years preceding and following CD4 cell count decline below 200 × 10^6/L.

Figure 5. Predicted virus load: modeling of HIV-1 RNA levels as dependent variable of time in years since CD4 cell count decline below 200 × 10^6/L. Years −2 to −6 are years preceding; years 2–6 are years following CD4 cell count decline below 200 × 10^6/L.

Discussion

We explored longitudinal differences between HIV-infected low CD4 LTS and matched progressors regarding HIV-1 RNA load, CD4 cell count, and T cell reactivity. We analyzed quantitative and qualitative differences, the first referring to different rates of increase or decline and the latter to different trends: linear or higher-order functions. Only quantitative differences were found for HIV-1 RNA load, which was low but not stable in the low CD4 LTS. Moreover, no indication for a differential...
increase between low CD4 LTS and progressors was found. The group medians and interquartile ranges showed considerable overlap between low CD4 LTS and progressors regarding the level of HIV-1 RNA load. However, the real difference between low CD4 LTS and progressors may have been underestimated in this study because of the detection limit of the assay. Differences in HIV-1 RNA load between low CD4 LTS and progressors already appear early after infection, but these data clearly show that load does not remain stable throughout the asymptomatic stage of HIV-1 infection, as has been suggested in the set point hypothesis [1–7, 25]. The increase in load over time was similar in the 3 groups, resulting in a delay of 6–10 years before the low CD4 LTS reached the baseline levels of load of the progressors. The increase in load over time reported in this study may have been preceded by a longer interval of a stable increasing or even decreasing load following seroconversion, which needs further exploration.

With respect to the CD4 cell profiles, both quantitative and qualitative differences were found between groups. In the low CD4 LTS, the CD4 cell decline following and preceding the CD4 cell drop below 200 is best described by a linear function. However, in the RP and IP, the CD4 cell decrease shows a marked acceleration as the CD4 cell count comes close to 200.

Regarding T cell reactivity, there was a clear difference in the rate of decline in the 3 groups, with the RP and IP showing a much faster drop in T cell reactivity when the CD4 cell counts fell below 200/mm³. A better discrimination can be expected in a longitudinal analysis of T cell reactivity after stimulation with phytohemagglutinin [15]. However, because of a change in the method to measure T cell reactivity after phytohemagglutinin stimulation in 1992, this analysis was not possible. The removal of outlying observations had no impact on the estimator of the virus load rise or CD4 cell count and T cell function decrease (data not shown).

Comparison of the correlation coefficients shows that the association between HIV-1 RNA load and T cell reactivity is stronger than between load and CD4 cell count in this population with low CD4 cell counts. The lower correlation with CD4 cell count may be explained by the fact that fluctuation in HIV-1 RNA load is not associated with short-term changes in CD4 cell count [26]. Apparently, HIV-1 RNA load is a better predictor of T cell reactivity than of CD4 count at a given time point.

There were several arguments to use repeated-measurements modeling as opposed to more conventional methods in the present study. First, a crude analysis of the data at different time points would have provided much less information. Statistical inference would have been hampered by multiple testing in a small data set. In contrast, the modeling in the present study markedly raised the power by examining longitudinal patterns for each marker in a single analysis. Also, these analyses provide insight into qualitative differences of temporal changes. Use of the conventional least-square regression analysis in this respect would have been inappropriate, since persons differ with respect to the number of observations. Here, the benefits of the application of repeated measurements were shown. Other markers and determinants can be studied as well with this analysis, such as the role of cytotoxic lymphocyte response [27].

Nevertheless, the results have to be interpreted with caution because of the small numbers, and confirmation in other and larger studies is necessary.

The results of the analysis show that the 3 groups are strongly discriminated by different trends of CD4 cell decline. In contrast, analysis of HIV-1 RNA load reveals a similar trend of linear increase of load over time in the 3 groups. Presumably, HIV-1 RNA load is discriminatory throughout HIV-1 infection, with a constant difference between low CD4 LTS and progressors.

In summary, we showed that HIV load in low CD4 LTS is low in comparison with that in progressors. However, virus load goes up over time in this group, and there is an ongoing decrease in CD4 cell count and T cell reactivity. In addition, SI variants appeared in 6 of 10 low CD4 LTS. Progression in this group appears to be delayed but not arrested. Therefore, as in all persons with low CD4 cell counts, appropriate antiretroviral and prophylactic treatment is necessary for these persons.

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References


