Is There a General Tendency for CD4 Lymphocyte Decline to Speed Up during Human Immunodeficiency Virus Infection? Evidence from the Italian Seroconversion Study

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It has been suggested that the rate of CD4 cell decline accelerates in parallel with decreasing numbers of cells; however, the statistical literature suggests the opposite. CD4 cells were counted about every 6 months in a cohort of 1264 human immunodeficiency virus–infected subjects (the Italian Seroconversion Study cohort). Kaplan-Meier analysis was used to estimate the time for CD4 cells to decline by 100 cells/mm³, conditional on reaching predefined levels. In addition, CD4 cell counts were modeled as a function of time since seroconversion in individuals with ≥5 counts. Kaplan-Meier survival times for a 100 cell/mm³ decrease in CD4 cells increased as lower counts were reached (log rank test, \( P < .001 \)). The shape of the overall fitted curve of the CD4 cell counts does not suggest an increasing rate of decline. Data from the Italian Seroconversion Study cohort do not show a general tendency for accelerating CD4 cell decline in association with lower counts.

Methods

Data source. We derived data from 16 outpatient facilities in Italy for a cohort of HIV-positive subjects who seroconverted between 1980 and 1993. The seroconversion date was estimated as the midpoint between the last negative and the first positive HIV serologic test. Clinical information and data on laboratory parameters (including CD4 cells) were collected about every 6 months. CD4 subset studies were done by flow cytometry, using OKT4 monoclonal antibodies (Ortho Diagnostics, Raritan, NJ). More detailed information on data collection is reported elsewhere [14]. For this analysis, we considered only subjects with at least 2 CD4 cell measurements.

We considered the absolute CD4 cell count, rather than the percentage of lymphocytes that are CD4 cells, since most studies containing results from several analyses of longitudinal CD4 cell data, which indicate that the best fitting curve is linear in the square root (or even logarithm) of the CD4 cell count; that is, the rate of CD4 cell decline is slower in persons with fewer CD4 cells [6–11]. This has been supported by recent studies in hemophiliac men [12, 13]. Here we present results from the Italian Seroconversion Study cohort and attempt to directly assess whether there is a general tendency for the rate of CD4 cell decline to become faster as lower counts are reached.

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mm$^3$ by time from reaching CD4 cell count $x$, if the subject’s count was initially above $x$ cells/mm$^3$. Subjects could be included in more than one Kaplan-Meier curve. A subanalysis censored all patients at 1 January 1989 and at the time of their first treatment to protect results from possible bias due to the effects of antiretroviral therapy. We also did the analysis excluding all subjects for whom the first CD4 cell count was $\geq 6$ months after the estimated date of seroconversion. This was done because if, for example, a subject’s first count was $\geq 500$ cells/mm$^3$ and subsequently fell, it was assumed for the Kaplan-Meier analysis of time for decline from 500 to 400 cells/mm$^3$ that this was the first time the subject’s CD4 cell count had been $<500$ cells/mm$^3$. If the time from seroconversion to the first measured count was long, this assumption is less likely to hold.

To account for possible laboratory error and random daily variation that could have affected some measurements, the analysis was done with the expectation that the subject reached a specific cutoff as soon as he experienced the first of two consecutive counts below the cutoff, rather than only one count below the cutoff.

To assess whether persons excluded from the analysis had a different course of HIV infection than those included, we compared progression to AIDS in the 2 groups. The AIDS-free survival time was measured from the estimated date of seroconversion to the date of AIDS diagnosis, the date of non-AIDS death, or to July 1994. AIDS diagnoses were cross-checked with the Italian National AIDS Registry as described elsewhere [20].

As a further means of assessing the pattern of CD4 cell decline over time, a multilevel approach [21] was used to model the relationship between the CD4 cell count and the time since seroconversion in subjects with $\geq 5$ CD4 cell measurements. This method was previously used to model the pattern of CD4 cell decline [11, 22] and provides efficient estimates of the pattern of the decline while taking into account the fact that measurements within a person are correlated. A two-level model is fitted. Level 1 is the variation in CD4 cell counts that can be explained within an individual at any point in time. This may include variation due to laboratory methods, time of day, or other natural biologic variation. Level 2 is the variation in CD4 cell counts that describes how CD4 cell decline patterns vary among individuals. The method used is similar to fitting separate regression models for each person, although unlike simple regression, the estimated parameters are constrained to come from some parametric distribution, usually a normal distribution. Parameters may be “fixed” in that each subject has the same value as each other, that is, a common intercept or slope, or “random” in that each subject’s parameter can vary with a given distribution (i.e., different slopes and intercepts).

The simple multilevel model in the untransformed scale of CD4 cell counts can be described as: $CD4_i = [(a + u_i) + (b + v_i)t_i] + e_i$, where $CD4_i$ is the CD4 cell count for patient $i$ at time $t_i$, $a$ and $b$ are fixed quantities, the average CD4 cell count at seroconversion (the intercept) and the average rate of decline (the slope), $u_i$ is the extent to which the $i$th individual departs from the average intercept, $v_i$ is the extent to which the $i$th individual departs from the average slope, and $e_i$ is the residual error for patient $i$ at time $t_i$. $u_i$ is assumed to be normally distributed with mean zero and variance $s_u^2$, $v_i$ is assumed to be normally distributed with mean zero and variance $s_v^2$, and $e_i$ is normally distributed with zero mean and variance $s^2$. Thus, to describe this model, it is only necessary to estimate the mean parameters ($a, b$), the variances ($s_u^2, s_v^2, s^2$), and the covariance between $u_i$ and $v_i$ ($s_{uv}$). In the special case where parameters are fixed, $u_i = 0$ and $v_i = 0$ for all $i$, and $s_u^2 = s_v^2 = 0$. Thus, only the overall mean values need to be estimated along with the within-individual variation, $s^2$. The variances describe the average deviation of each individual’s slope and intercept from the mean value and can be used to estimate the range in which most subject’s values are expected to lie (mean $\pm 1.96$ SD).

Higher order terms (e.g., quadratic or cubic) can be added either as fixed or random parameters. Terms were included in the model if they resulted in a significant improvement in fit to the model. Improvement in fit is assessed by log-likelihood ratio tests by comparing the change in $2x \log$-likelihood to a $\chi^2$ distribution with degrees of freedom equal to the number of extra parameters fitted. These analyses were done using the statistical package ML3 [23].

There is evidence that it is appropriate to model changes in the CD4 cell count on a square root scale [11, 24]. This transformation ensures the fitted values of CD4 cells are not negative. Further, while variation in CD4 cell count at the within-individual level can be explicitly built into the multilevel model if desired, previous studies have shown that the square root transformation essentially stabilizes the variation in the CD4 cell count over time [11]. However, the modeling was repeated using untransformed CD4 cell and log-transformed counts for comparison. Because the conclusions were essentially similar, we present results only after taking the square root transformation.

The existence of a distinct group of long-term nonprogressors who maintain high CD4 cell counts for substantial periods has been suggested [25]. These individuals, if they exist [26], may have an effect on the estimates of the overall pattern of decline. To avoid possible biases due to such an effect, we repeated the analysis, fitting the final model to exclude all subjects whose CD4 cell counts never fell below 500 cells/mm$^3$ and who were likely to be long-term nonprogressors in our cohort. To assess whether a further different pattern of decline was evident in those who progressed, the analysis was also repeated including only subjects whose CD4 cell counts fell below 100 cells/mm$^3$.

### Results

Among 1264 subjects enrolled up to November 1993, 1021 (81%) had at least 2 CD4 cell counts. For 75% of the subjects, the seroconversion interval (time between the last HIV-negative and the first HIV-positive test) was $<1$ year and for 95% it was $<2$ years. Among the subjects with $\geq 5$ CD4 cell counts, this interval was even narrower (95% seroconverted within 21 months). Progression to AIDS did not differ between this group of patients and those with $<2$ counts; an AIDS cumulative incidence of 20% was observed $\sim 6$ years after the estimated seroconversion date in both groups.

Table 1 shows estimated times (in months) for 25%, 50%, and 75% of patients to decline by 100 CD4 cells/mm$^3$ from starting CD4 cell levels, numbers of patients contributing ($n$), and the number of patients in whom a 100 cell/mm$^3$ decline was observed (events). The time to decline by 100 cells/mm$^3$ increased at lower CD4 cell counts, ranging from a median of
of longitudinal CD4 cell data [6–13] and have fundamental implications for understanding the cause of CD4 cell decline during HIV infection. Models of AIDS pathogenesis must reconcile the decreasing rate of CD4 cell decline with the concomitant increase in virus burden [13, 29–33].
We realized that in percentage terms, a change from say 600 to 500 cells/mm$^3$ is less of a change than from 200 to 100 cells/mm$^3$, which represents a halving in absolute count. Nonetheless, even in these terms, the average time for the decline of 200 to 100 CD4 cells/mm$^3$ (16.7 months, see table 1) was only about half that needed for a decline from 600 to 300 cells/mm$^3$ (37.2 months, data not shown), a small difference compared with the 10- to 100-fold difference in virus burden between those with 600 and those with 200 CD4 cells/mm$^3$ [29–33]. We primarily concentrated on the absolute decline in CD4 cells (i.e., on a linear scale) rather than percentage terms (i.e., on a logarithmic scale), because CD4 cell changes are usually considered linear [15–19]. We believe models of HIV pathogenesis must be able to explain the dissociation between virus burden and decline in CD4 cells if they are to be considered realistic.

Before drawing firm conclusions, possible biases and limitations of our study should be discussed. First, subjects were excluded from our analysis if they had <2 CD4 cell counts. If there was a tendency for CD4 cells in these subjects to decline more or less rapidly than the subjects included, a bias may exist. However, we found that progression to AIDS was similar in the 2 groups. Furthermore, the proportion of persons excluded was similar at each starting cell count (≥800 cells/mm$^3$, 700 to 800, etc.).

Second, a bias could arise due to the inclusion of subjects in more than one Kaplan-Meier analysis. Since most persons contributed to several such analyses, those with rapidly declining cells might be overrepresented, resulting in an underestimate of time for CD4 cells to drop by 100 cells/mm$^3$ at lower numbers. However, if anything, this is likely to bias the results towards underestimating the time to decline by 100 cells/mm$^3$ at low CD4 cell counts. For subjects whose CD4 cells declined rapidly, the first count might be low, so they would only be included in the lower count analyses. Again, this should result in bias towards underestimating the time to decline by 100 cells/mm$^3$ at low cell numbers.

Another issue is that we assumed that a subject’s CD4 cell levels had not previously fallen below the value at study entry. This is less likely to be true for subjects with higher starting levels, thus resulting in greater underestimation of the median time for a 100 cell/mm$^3$ decline at higher initial CD4 cell levels. In an attempt to address this potential bias, we reanalyzed the data after excluding patients whose first CD4 cell count was obtained >6 months after the estimated date of seroconversion. The results were similar. Also, the increasing trend in time for a 100 cell/mm$^3$ decline was not affected by random errors in CD4 cell measurements, since the results did not change when a subject was deemed to have reached the cutoff after the first of 2 consecutive counts below the cutoff. A group of subjects in the cohort with low CD4 cells was given antiretroviral drugs. This would produce a bias towards slower decline in CD4 cell counts at low levels, since treatment is likely to prolong survival. We addressed this possible source of bias by performing
both an analysis that censored patients during January 1989 (only 9% of the subjects were treated before this date) and by censoring individuals at the point they received their first treatment. Again, these analyses showed a similar trend in CD4 cell decline (data not shown). 

A recent report suggested the possibility of a distinct group of long-term nonprogressors [25] who may experience patterns of decline different from those of other individuals. Others have suggested rapid CD4 cell decline at late stages of disease [1–5]. Multilevel modeling methods, while allowing individual patterns of decline to vary, assume there is an underlying common pattern of decline shared by all persons. Thus, such methods may not be the most appropriate for establishing the existence of subgroups with very different patterns of cell decline. Although we found in general that after square root transformation, CD4 cells declined linearly over time, we cannot rule out the possibility that some persons maintain high CD4 cell levels for long periods and that others may experience rapid cell decline at very late stages of disease. The significance of the random quadratic coefficient confirms that individual patterns vary. However, as the main aim of our study was to describe the general pattern of CD4 cell decline over time in a group of HIV-infected persons rather than to focus on change patterns in small subgroups, these subgroups are of only limited interest. Our results suggest that any group of long-term nonprogressors [25, 26] is likely to be small and to have little impact on the overall pattern of CD4 cell decline. Further, while we cannot rule out the possible subsequent rapid loss of CD4 cells in persons who previously maintained relatively high cell counts, when the analysis was repeated to include only subjects whose CD4 cell counts dropped to low levels, the conclusions remained unchanged. Thus, while some patients may experience rapid CD4 cell decline at late stages of disease, in the average patient, CD4 cells decline more slowly late in infection.

The consistency of conclusions reached by using the analytical approach involving comparison of median times to lose 100 cells/mm$^3$ with those from fitting the multilevel regression model, which does not suffer from most of the potential biases discussed earlier, suggests that any bias is probably not of practical importance. In conclusion, we found no evidence that the rate of CD4 cell decline in HIV infection speeds up as lower CD4 cell levels are reached. To be considered plausible, models of HIV pathogenesis should reflect this feature.

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### References