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Pre-seroconversion immune status predicts the rate of CD4 T cell decline following HIV infection

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Pre-seroconversion immune status predicts the rate of CD4 T cell decline following HIV infection

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Objective: To study whether immune status prior to HIV seroconversion predicts CD4 T cell decline during HIV infection.

Design: Prospective cohort study including 51 injecting drug users (IDU) who were HIV negative at study entry and seroconverted for HIV during follow-up.

Methods: Cryopreserved peripheral blood mononuclear cells obtained before HIV seroconversion were used to measure naive (CD45RO\textsuperscript{−}CD27\textsuperscript{+}), memory (CD45RO\textsuperscript{+}CD27\textsuperscript{+}), and total CD4 T cell numbers, the fraction of dividing Ki67\textsuperscript{+}CD4\textsuperscript{+} T cells, and CD4 T cell receptor excision circles (TREC). The effect of pre-seroconversion immune status, as defined by these markers, on the rate of CD4 T cell decline during HIV infection was assessed using linear regression for repeated measurements.

Results: IDU with low pre-seroconversion CD4 T cell TREC contents lost CD4 T cells at a significantly faster rate during HIV infection than those with a high CD4 T cell TREC content. IDU with higher pre-seroconversion CD4 T cell numbers had a significantly steeper CD4 T cell decline in the first 3 months of HIV infection, but their CD4 T cell counts remained higher throughout HIV infection. Intermediate levels of pre-seroconversion dividing Ki67\textsuperscript{+}CD4\textsuperscript{+} T cells were associated with a significantly steeper CD4 cell decline than high levels. IDU with the highest pre-seroconversion drug-injecting frequencies showed slower CD4 T cell decline than those who injected less. No correlation was present between pre-seroconversion immune markers and the pre-seroconversion duration or intensity of drug use.

Conclusion: Among IDU, immune status prior to HIV infection as measured by TREC content affects the disease course after HIV seroconversion.

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Keywords: HIV, injecting drug users, TREC, CD4 T cell decline, HIV disease progression, pre-seroconversion immune status

Introduction

It has been proposed that increased levels of immune activation in healthy individuals prior to HIV infection may be associated with faster HIV disease progression \cite{1,2}. However, because of the paucity of patient material obtained prior to infection with HIV, little is known about the extent to which the immune status before HIV seroconversion determines HIV disease course. To our knowledge, only one recent study among homosexual men has investigated this issue \cite{3} and this showed that both low CD4 T cell count and high immune activation (CD4+CD70+ expression) before seroconversion were associated with faster pro-
gression to AIDS. Compared with heterosexual individuals, HIV-negative homosexual men may have increased levels of immune activation owing to increased prevalence of sexually transmitted diseases [4,5]. However, the median incubation time to AIDS and death in risk groups with perhaps even higher immune stimulation, such as injecting drug users (IDU) and people living in Sub-Saharan Africa, does not differ from that among homosexual men in industrialized countries in the era without highly active antiretroviral therapy (HAART) [6-8]. Nevertheless, pre-seroconversion immune status could be a factor explaining the great interindividual variation in HIV disease progression within risk groups, as demonstrated by our previous study among homosexual men [3].

IDU are an interesting risk group in which to study the association between pre-seroconversion immune activation and subsequent HIV disease progression because large interindividual differences in pre-seroconversion immune status may be present as a result of the immune-modulating effects of drug use [9], exposure to injected impurities and microbes, and differences in lifestyle [10,11]. Whether and how drug use affects the immune system (suppression or activation) may depend on the type and frequency of drug use [12,13]. Access to stored serum samples from IDU that were obtained at least 3 months prior to the last HIV-seronegative test result have provided a unique opportunity to study how the rate of CD4 T cell decline during HIV infection depends on the immune status of an individual prior to HIV infection. In addition to a T cell activation marker characterizing the level of T cell proliferation before HIV seroconversion, pre-seroconversion CD4 signal joint T cell receptor excision circles (TREC) were measured as a cumulative marker of an individual’s history of T cell activation.

Methods

Study population
In the Amsterdam cohort study among IDU, which started in 1985, participants visit every 4 months for standard interviews and blood sampling for laboratory testing and storage. Cryopreserved peripheral blood mononuclear cells (PBMC) collected prior to HIV infection (at least 3 months before the last antibody-negative HIV test) were available from 51 IDU out of a total of 66 individuals who seroconverted after April 1989, at which point the collection and storage of PBMC became part of the study routine.

Immune markers
TREC have been used as a marker for thymic output of newly synthesized T cells, but the TREC content (i.e., the average number of TREC/T cell) is affected not only by thymic output but also by T cell proliferation and apoptosis. TREC content, therefore, also reflects the replicative history of a cell population [14,15]. Ki67 is a nuclear antigen that is expressed by cells that are in cell cycle; it can, therefore, be used as a surrogate marker for T cell proliferation ex vivo at the time of blood sample collection [16]. Pre-seroconversion CD4 T cell numbers, CD4 naïve and memory subsets, TREC and CD4 Ki67 expression were measured as previously described [17]. Briefly, PBMC were stained to identify CD4 naïve (CD27+CD45RO−) and memory (CD27+CD45RO+) T cell subsets. After fixation and permeabilization, lymphocytes were stained intracellularly with monoclonal antibody for Ki67, fixed and analyzed on a FACSCalibur (Becton Dickinson, San Jose, California, USA). For TREC analysis, CD4 T cells were isolated from PBMC by positive selection over MiniMACS separation columns (Miltenyi Biotec, Sunnyvale, California, USA); DNA was purified, and the signal joint TREC frequency of this fraction was quantified using real-time polymerase chain reaction [14]. To normalize for input DNA, the C6 constant region, which remains on the genes for the T cell receptor despite receptor rearrangement processes, was amplified in every sample tested. TREC content was defined as the average number of TREC/CD4 T cell, assuming 150 000 cells/μg DNA. Total TREC numbers were calculated as TREC content multiplied by the CD4 cell count. For all immune markers, three categories were defined by the 33rd and 67th percentile.

Drug-use behaviour
Pre-seroconversion duration of IDU was calculated as the number of years from initiation of injecting to HIV seroconversion, subtracting temporary periods in which injecting drug use was suspended (divided into three groups defined by the 33rd and 67th percentile: < 5.3, 5.3-13.9 and > 13.9 years). Pre-seroconversion injecting frequency was calculated as the mean of the daily injecting frequencies reported at all study visits in the 2 years prior to HIV infection (categorized as < 1.5, 1.5-3.0 and > 3.0 times daily).

Statistical analysis
The effect of several markers on the mean CD4 T cell trajectory during HIV infection was assessed with regression analysis for repeated measurements using the proc mixed statement in SAS [18,19]. To correct for the dependence between consecutive CD4 measurements within each individual, a linear mixed effects model was used in which a first-order autoregressive moving-average correlation structure was applied (i.e., each CD4 T cell count value depends on the value of and the variation around the preceding count; this correlation decreases as the time between measurements increased). To satisfy the normality assumption, CD4 T cell numbers were modelled on a square-root
Prior immune status predicts CD4 T cell decline

Prior immune status predicts CD4 T cell decline [20,21]. The CD4 trajectory was modelled from the date of HIV seroconversion until 7 years after seroconversion. The onset of HIV infection is associated with a rapid fall in CD4 T cell numbers followed by a long-term slower decline. Therefore, the CD4 T cell trajectory was modelled in a piecewise manner, allowing the initial slope of the regression line to differ from the slope later in infection [22]. The best fit was achieved when the slope of the modelled mean CD4 T cell number was allowed to change at 3 months after HIV seroconversion. The date of HIV seroconversion was calculated for each individual conditional upon the last negative HIV test and first positive HIV test, using a cohort-specific estimate of the cumulative HIV seroincidence over calendar time [23].

Measurements from the date of HIV seroconversion onwards were entered into the analyses up to 1 October 2002 or until an individual initiated HAART, which was diagnosed with AIDS, died or was lost to follow-up, whichever occurred first. Correlations were calculated using Spearman’s rank correlation coefficients.

Results

Population characteristics

The median age of the 51 HIV-positive IDU was 32 years [interquartile range (IQR), 28–37] and 33% of the study population was female. The median follow-up time since HIV seroconversion was 6.9 years (IQR, 4.0–8.5). By 1 October 2002, 17 IDU had initiated HAART at a median of 3.9 years (IQR, 2.1–6.7) after HIV seroconversion, after which they were excluded from the analyses. Pre-seroconversion markers of immune status were measured using samples taken at a median time of 6.5 months (IQR, 4.0–9.1) prior to the last date on which the IDU tested negative for HIV antibodies, i.e., a median time of 9.7 months (IQR, 6.4–14.1) prior to the estimated date of HIV seroconversion. The distribution of marker values and drug use prior to HIV seroconversion are given in Table 1.

Pre-seroconversion correlations

As reported by others [24], older individuals had lower TREC contents (r = -0.39; P = 0.01). Lower naive CD4 T cell numbers were also associated with older age (r = -0.28; P = 0.05), while Ki67 expression on CD4 T cells (total, naive or memory) was not dependent on age.

Drug-use variables showed no correlation with pre-seroconversion TREC content, whereas higher naive CD4 T cell numbers were associated with higher TREC content (r = 0.33; P = 0.02). The percentage of Ki67+CD4+ T cells did not correlate with TREC content (r = -0.01). Total TREC numbers were highly associated with TREC content (r = 0.89; P < 0.001), with CD4 T cell count (r = 0.41; P = 0.003) and with the numbers of naive CD4 T cells (r = 0.57; P < 0.001).

Pre-seroconversion CD4 T cell numbers showed no correlation with pre-seroconversion TREC content, whereas higher naive CD4 T cell numbers were associated with higher TREC content (r = 0.33; P = 0.02). The percentage of Ki67+CD4+ T cells did not correlate with TREC content (r = -0.01). Total TREC numbers were highly associated with TREC content (r = 0.89; P < 0.001), with CD4 T cell count (r = 0.41; P = 0.003) and with the numbers of naive CD4 T cells (r = 0.57; P < 0.001).

Drug-use variables showed no correlation with immune markers with one exception: a higher daily injecting frequency (during 2 years prior to seroconversion) correlated significantly with increased numbers of naive CD4 T cells (r = 0.31; P = 0.03).

Table 1. Drug use and marker values prior to HIV seroconversion of 51 HIV-infected drug users.

<table>
<thead>
<tr>
<th>Pre-HIV seroconversion characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of drug mainly injected prior to HIV seroconversion</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Heroin + cocaine</td>
<td>28 (55)</td>
</tr>
<tr>
<td>Heroin</td>
<td>9 (18)</td>
</tr>
<tr>
<td>Cocaine</td>
<td>9 (18)</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Methadone</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Median duration of injecting prior to HIV seroconversion (years [IQR])</td>
<td>9.6 (3.9–15.9)</td>
</tr>
<tr>
<td>Median injecting frequency prior to HIV seroconversion (per day [IQR])</td>
<td>2.2 (0.7–3.9)</td>
</tr>
<tr>
<td>Immune markers prior to HIV seroconversion</td>
<td></td>
</tr>
<tr>
<td>Median TREC content per CD4 T cell (IQR)</td>
<td>0.023 (0.012–0.035)</td>
</tr>
<tr>
<td>Median total CD4 TREC × 10^6 (IQR)</td>
<td>25.7 (13.4–37.8)</td>
</tr>
<tr>
<td>Median CD4 T cell count × 10^6 cells/l (IQR)</td>
<td>1070 (850–1310)</td>
</tr>
<tr>
<td>Median naive CD45RO−CD27+CD4 T cells × 10^6 cells/l (IQR)</td>
<td>278 (199–442)</td>
</tr>
<tr>
<td>Median memory (CD45RO−CD27+) CD4 T cells × 10^6 cells/l (IQR)</td>
<td>530 (418–682)</td>
</tr>
<tr>
<td>Median Ki67+CD4+ (% [IQR])</td>
<td>2.1 (1.6–3.1)</td>
</tr>
<tr>
<td>Median Ki67-positive naive CD4 T cells (% [IQR])</td>
<td>0.5 (0.3–0.9)</td>
</tr>
<tr>
<td>Median Ki67-positive memory CD4 T cells (% [IQR])</td>
<td>2.6 (1.9–4.1)</td>
</tr>
</tbody>
</table>

IQR, interquartile range; TREC, T cell receptor excision circles.

a Number of years between initiation of injecting drug use and HIV seroconversion, corrected for periods of abstinence.

b Average number of daily injections in the 2 years prior to HIV seroconversion.

c Figures from 45 individuals not 51.
Modelling of the decline of CD4 T cell numbers
The mean CD4 trajectory during HIV infection was modelled starting from HIV seroconversion (Fig. 1). The mean CD4 T cell decline was significantly more rapid in the first 3 months after HIV seroconversion than at later time points (P = 0.02). The values of the intercepts and slopes for the mean CD4 T cell decline, stratified by pre-seroconversion drug use and immune marker values, are shown in Table 2. For each marker, the highest group was defined as the reference group.

Pre-seroconversion CD4 T cell counts
The cohort was divided into three equal groups based on their CD4 T cell count prior to HIV seroconversion: > 1240, 896–1240 and < 896 × 10^6 cells/l. The lowest category was significantly associated with a slower rate of CD4 T cell decline in the first 3 months of HIV infection compared with the highest category (P = 0.05). After 3 months, the rate of CD4 T cell decline did not differ significantly among the three categories. Despite the steeper initial decline of CD4 T cells in IDU with the highest pre-seroconversion CD4 T cell counts, their CD4 T cell counts remained higher throughout HIV infection compared with IDU who started off with lower CD4 T cell counts; however, the difference with the middle group became smaller with time (Fig. 2a). IDU with the lowest numbers of naive CD4 T cells prior to HIV infection had significantly lower total CD4 T cell numbers at HIV infection and showed no decrease in total CD4 T cell counts in the first 3 months of HIV infection. Thereafter, rates of CD4 T cell decline did not differ significantly with variation in the level of pre-seroconversion naive CD4 T cell numbers, although IDU with the highest pre-seroconversion naive numbers did exhibit higher total CD4 T cell numbers throughout HIV infection. Those with the highest memory CD4 T cell numbers also had the highest total CD4 T cell numbers throughout infection, but the total CD4 T cell count at HIV infection and the rates of CD4 T cell loss did not differ significantly (Table 2). When stratifying by pre-seroconversion naive and memory CD4 T cell percentages instead of absolute numbers, no significant differences in CD4 T cell trajectories during HIV infection were found. IDU with the lowest proportion of memory CD4 T cells prior to HIV infection had slightly higher total CD4 T cell counts during infection but differences were small and all trajectories converged (data not shown).

TREC and age
Compared with IDU with high pre-seroconversion CD4 TREC content (> 0.029/cell), there was a significantly faster decline of CD4 T cell numbers from month 3 to 80 of HIV infection in IDU with low (< 0.016/cell; P = 0.04) and intermediate (0.016–0.029/cell; P < 0.001) TREC contents (Fig. 2b). The intermediate and lowest TREC categories showed similar (i.e., almost overlapping) CD4 trajectories and rates of decline. As TREC content is known to decline with age, a possible confounding effect by age was assessed. In univariate analysis, the oldest age category (> 37 years) showed a faster CD4 T cell decline in months 3 to 80 compared with the two younger age categories (< 30 years (P = 0.02) and 30–37 years (P = 0.001); Fig. 2c). After adjusting pre-seroconversion TREC contents for age and vice versa, the effect of both on CD4 T cell decline during HIV infection remained (Fig. 3 for the effect of TREC stratified by age). Analyzing total CD4 TREC numbers instead of TREC content showed that IDU with the lowest total TREC numbers had significantly lower CD4 T cell numbers at seroconversion compared with IDU with higher pre-seroconversion CD4 TREC numbers. Pre-seroconversion total TREC numbers were not significantly associated with the rate of CD4 T cell decline, although IDU with the lowest total CD4 TREC numbers showed a slightly faster CD4 T cell decline after month 3 than those with the highest TREC numbers, which was borderline significant (P = 0.06; Fig. 2d). In the figure, however, this slope is not clearly steeper, because of back-transformation of the modelled square root CD4 T cell numbers, which decreases visual effects at lower CD4 T cell counts. Stratifying by age did not change the results for total TREC numbers.

Ki67 expression
Patients with the highest fraction of Ki67+CD4+ T cells before seroconversion had the lowest numbers of

![Fig. 1. Mean CD4 trajectory during HIV infection.](image-url)
CD4 T cells until month 70 after seroconversion. Trajectories eventually converged owing to a steeper CD4 T cell decline in IDU with smaller fractions of dividing Ki67+CD4+ T cells, but this was significant only for the intermediate group. A steeper CD4 T cell decline was also observed for the group with intermediate fractions of dividing naive CD4 T cells pre-seroconversion, albeit only during the first three months after infection. Both intermediate groups had significantly higher CD4 T cell numbers at the time of HIV seroconversion (Fig. 2e).

**Drug-use variables**

There was no statistically significant association between the duration of drug injecting before seroconversion and the CD4 T cell trajectory during HIV
infection. However, the average injecting frequency before seroconversion did seem to play a role. For the majority of the time during HIV infection, IDU with the highest injecting frequencies (> 3 times daily) had the highest CD4 T cell numbers (Fig. 2f). Compared with these frequent injectors, intermediate injectors (those who injected 1.5–3.0 times daily) exhibited a significantly steeper CD4 T cell decline after month 3, and their trajectory eventually converged with those who injected < 1.5 times per day.
Prior immune status predicts CD4 T cell decline

Fig. 3. Mean CD4 T cell trajectory by pre-seroconversion CD4 T cell receptor excision circles (TREC) content stratified by age.

Discussion

This study assessed the predictive value of pre-seroconversion CD4 T cell counts, TREC levels and Ki67 expression for subsequent HIV disease progression. The data suggest that immune status prior to HIV infection affects the course of HIV infection in a well-defined cohort of IDU seroconverters and confirmed findings in homosexual men [3]. The latter study showed that individuals with increased levels of T cell activation pre- or post-seroconversion progressed more rapidly to AIDS. We have extended this line of research by determining the CD4 T cell trajectory post-seroconversion and by adding pre-seroconversion CD4 TREC analysis. Because TREC content in CD4 T cells declines with cell division, this marker can be taken as a cumulative measure of previous immune activation.

IDU with high CD4 T cell counts prior to HIV infection had a significantly higher rate of CD4 T cell decline in the first 3 months of infection than IDU with lower CD4 T cell numbers. After this initial phase, the rate of CD4 T cell loss became similar for all three groups. However, the IDU with high pre-seroconversion CD4 T cell counts maintained the highest CD4 T cell counts throughout infection, a finding that supports the previous observation that high pre-seroconversion CD4 T cell counts are associated with slower progression to AIDS in homosexual men [3]. The faster initial decline in IDU with higher pre-seroconversion CD4 T cell counts may be caused by regression to the mean. However, the steeper fall is more likely related to increased HIV replication in those with higher CD4 T cell numbers, because HIV RNA levels shortly after seroconversion have also been shown to be higher in such individuals [25]. Higher levels of virus may induce higher levels of immune activation and consequently more redistribution of activated CD4 T cells to lymph nodes. In our study, with data on viral load available for 27 IDU in the first 3 months of HIV infection, median HIV RNA viral load was higher among those with higher pre-seroconversion CD4 T cell numbers but differences were not significant (3.9, 5.0 and 5.1 log_{10} copies/ml for < 896, 896-1240 and > 1240 X 10^6 cells/l, respectively; P = 0.25, Kruskal-Wallis test). After this initial phase, the median viral load was similar for the three groups between months 6 and 12 of HIV infection [4,61, 4.65 and 4.86 log_{10} copies/ml, respectively (n = 40); P = 0.50, Kruskal-Wallis test]. Our data are also in line with a study in which CD4 T cell numbers at seroconversion were estimated to be higher and subject to a steeper decline after seroconversion among haemophiliacs compared with homosexual men [26].

Our study also shows that IDU with high pre-seroconversion CD4 TREC content lost their CD4 T cells at a significantly slower rate during HIV infection than IDU with low pre-seroconversion CD4 TREC content. Older individuals reportedly lose their CD4 T cells more rapidly than younger individuals [26], but when we stratified our population by age, the advantageous effect of high pre-seroconversion CD4 TREC content remained. Low CD4 TREC contents during HIV infection have been shown to predict faster progression to AIDS [27-29], most likely reflecting HIV-induced T cell activation, which leads to dilution of TREC [14]. In contrast, differences in pre-seroconversion CD4 TREC contents may reflect interindivid-udial differences in the number of infections experienced throughout life and differences in genetic predisposition to immune stimulation in response to infections. Since the extent of HIV-induced activation is associated with disease progression [3], high responders – who are expected to have lower CD4 TREC contents even before HIV seroconversion – would have a more rapid loss of CD4 T cells during HIV infection. Indeed, HIV-infected people with the high immune-responder phenotype HLA-A1+HLA-B8+HLA-DR3+ progress more rapidly to AIDS [30]. The observation that groups with intermediate and low TREC content did not differ in their rate of CD4 T
cell decline after HIV seroconversion suggests that there is a critical level of immune activation that is associated with a higher risk for progression: a level of immune activation that is not reached by the low-responder group with high pre-seroconversion TREC contents. When pre-seroconversion T cell activation was measured using Ki67 (a marker of T cell activation at the time of blood draw), neither this study nor the previous one [3] found a clear-cut predictive value on the CD4 T cell trajectory or on disease progression. However, CD70 did have predictive value in the latter study. Importantly, while Ki67 expression and CD70 expression merely reflect an individual's immune status at a specific moment in time, the average CD4 TREC content is a cumulative and, therefore, better marker of an individual's immune status history.

The association between pre-seroconversion CD4 TREC contents and the post-seroconversion rate of CD4 T cell decline could also be a reflection of interindividual differences in residual thymic output. Individuals with more residual thymic output are expected to have relatively high pre-seroconversion CD4 TREC content and a slower decline of CD4 T cells upon infection. Since TREC content is strongly influenced by T cell proliferation, differences in thymic output, as measured by TREC content, may be obscured by differences in T cell division. We, therefore, also studied the predictive value of the total number of TREC, which is the net result of past and ongoing thymic function and loss of naive T cells. Total TREC numbers are not affected by cell division and may, therefore, be better reflective of thymic function. The association with the rate of CD4 T cell loss during HIV infection was not significant, suggesting that pre-seroconversion thymic function is not predictive of CD4 T cell decline. However, IDU with the lowest numbers of total TREC did have a nearly significantly \( (P = 0.06) \) faster loss of CD4 T cells from month 3 onward compared with IDU having higher pre-seroconversion total TREC numbers. Also age (which correlates with thymic volume) was significantly associated with the rate of CD4 T cell loss upon infection. Therefore, the loss of CD4 T cells induced by the immune-activating effect of HIV may be slowed by the input of new T cells from the thymus in individuals with more thymic output, although the results for total TREC numbers did not clearly confirm this (lacking statistical significance). Since direct markers to analyze thymic output are lacking, and because thymic output in healthy adults is thought to be quite low, it remains to be determined whether differences in thymic output can affect the rate of CD4 T cell decline during HIV infection.

We found no correlation between pre-seroconversion duration or frequency of drug use and almost all pre-seroconversion immune activation parameters, confirming the results of a study comparing HIV-positive and HIV-negative female IDU [31]. Remarkably, we found that IDU with the highest injecting frequency had a slower CD4 T cell decline. Although the mean CD4 T cell count at seroconversion has been shown to be higher in IDU and haemophiliacs compared with homosexual men, the rate of CD4 T cell decline has not previously been found to differ among these three groups [26].

In conclusion, our results show that pre-seroconversion CD4 TREC content is a determinant of the rate of CD4 T cell loss in HIV infection among IDU. Since TREC content is a cumulative marker for an individual's immune history, immune status prior to HIV infection thus affects the disease course after HIV seroconversion. Because the number of patients analyzed was small, these results warrant further research in larger groups of patients and other risk groups.

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