Markers of HIV-1 infection and its pathogenesis
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Chapter 3

Strong association between failure of T cell homeostasis and the syncytium-inducing phenotype among HIV-1 infected men in the Amsterdam Cohort Study.

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Abstract

Objective: We investigated T cell homeostasis in the Amsterdam Cohort Study, and whether its failure, associated with downward inflection in the total T cell counts, was related to the switch from the non-syncytium-inducing (NSI) to the syncytium (SI) virus phenotype. Methods: For each of 325 homosexual men, the slope of the CD3+ T cell count before and after the estimated T cell inflection point (IP) was determined and correlated with the time of the NSI/SI switch. Results: Median T cell slopes before the IP (pre-IP) were nearly zero regardless of whether AIDS occurred, but the slopes after the IP (post-IP) were associated with clinical outcomes, with a median annual decline of 17.6% among AIDS cases and 4.6% in those remaining AIDS-free. Among subjects considered to have a true IP (≥8.2%/year post-IP), the times of the SI switch and the IP slope were highly correlated (r=0.65); among AIDS cases the SI switch preceded the IP by a median of 0.63 years. Conclusion: These results support the concept of blind T cell homeostasis, and also suggest that HIV-1 SI variants play an important role in the failure of T cell homeostasis.
Introduction

Immunologic and virologic markers such as CD4⁺ T cell count, HIV RNA concentration, and HIV-1 phenotype (non-syncytium-inducing (NSI) or syncytium-inducing (SI) virus variant), play an important role in assessing the stage of HIV disease in HIV-1 infected subjects. Although the relations between the various markers and the natural history of HIV-1 infection have been the subject of many studies, biologic mechanisms that may explain these relations are still far from clear. In order to study those mechanisms, various attempts have been made to model associations between marker trajectories and clinical endpoints such as AIDS and death.

Recently, the hypothesis of blind T cell homeostasis has been proposed, based on the observation that in various experimental and clinical conditions a constant level of circulating T cells is maintained without regard to the subset phenotype (e.g. CD4⁺ or CD8⁺ cells) ¹⁻⁴. For example, MHC Class I knockout mice, which cannot produce CD4⁺ T cells, generate a CD8⁺ T cell lymphocytosis and maintain normal T cell counts ⁵. In HIV-1 infection, progressive loss of CD4⁺ T cells is balanced by an increase in CD8⁺T cells for many years after HIV-1 seroconversion ⁴. Furthermore, it has been found that T cell homeostasis failed in subjects who developed AIDS, as manifested by an abrupt decrease in the CD3⁺ T cell counts a median of 1.5 – 2.5 years before the onset of AIDS ³⁶. The onset of this decline was denoted as the T cell inflection point (IP).

Using methods which were developed to identify T cell IPs on an individual level ⁷, Gange et al. found that more than 75% of participants in the Multicenter AIDS Cohort Study (MACS) who developed AIDS had T cell IPs, with a median time of 1.7 years from the T cell IP to AIDS ⁷. While these methods are not appropriate for clinical diagnosis, they allow for investigation of the pathogenesis of T cell IPs. Several recent studies have investigated HIV RNA and CTL patterns ⁸, HIV genetic diversity, divergence and predicted chemokine co-receptor usage ⁹, and T cell subset composition ¹⁰ in relation to T cell IPs.
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Early in HIV-1 infection, slow-replicating, macrophage-tropic, non-syncytium-inducing (NSI) viruses are the predominant isolates from the peripheral blood. However, in 50% of AIDS cases, there is a shift of the virus population towards the more rapidly replicating, T cell tropic, syncytium-inducing (SI) phenotype virus. The median time interval between the switch from the NSI to the SI virus phenotype and the onset of AIDS has been estimated as 6-18 months and 23 months. The similarity of these intervals to the time between the T cell IP and AIDS suggests that these events may be related. However, no studies have directly investigated whether this is true. Therefore, the present study was undertaken to investigate this relation in the Amsterdam Cohort Study (ACS), in which HIV-1 phenotype has been assessed on cohort members every three months since the beginning of the study in 1984. In the present study, we first evaluated the validity of the blind T cell homeostasis hypothesis in the ACS, determining individual T cell IPs and CD3+ cell slopes before and after this point. Subsequently, the association between the calculated IP parameters and the appearance of the SI virus phenotype was studied.

Materials and Methods

Study group

Since October, 1984, 1294 homosexual men have been enrolled in the Amsterdam Cohort Study (ACS) according to procedures that have been previously described. Briefly, participants have been seen every three months, at which time a medical history was taken, a physical examination was performed, and blood was drawn for immunological and virological laboratory evaluations. Sera were tested for the presence of HIV-1 antibodies with two commercially available enzyme-linked immunosorbent assays (ELISA: Abbott Laboratories, North Chicago, IL, USA; Vironostika Teknika, Organon, Oss, The Netherlands) and results confirmed with Western blot. All serological tests were carried out using fresh sera. T cell subsets were measured as described using fresh cells, using directly conjugated monoclonal antibodies and flow cytometry.
Participants in the present study were selected from the 679 HIV-1 infected individuals (543 seroprevalents and 136 seroconverters) who had been enrolled in the ACS through February 1998, according to three criteria: 1) individuals had to have at least six T cell measurements; 2) they had to have at least three years follow-up; and 3) the first two criteria had to be met before 15 June 1996, the date on which Highly Active AntiRetroviral Therapy (HAART) was introduced in the ACS. No distinction was made between seroprevalent cases and seroconverters. The median age of the participants was 33.7 years (interquartile range (IQR): 29-58). Those who met these criteria (n=338) were divided into three groups; (1) those who developed AIDS, according to the 1987 Center for Disease Control and Prevention case definition, by 15 June 1996 (the AIDS group, n=178); (2) those who developed AIDS between 15 June 1996 and February 1998 (n=13); and (3) those who had not developed AIDS as of February 1998 (the AIDS-free group, n=147). Members of the second group were excluded from analysis in this study.

T cell counts from the third group were truncated at 1 January 1995, to assure that T cell counts in this group were not affected by any imminent development of AIDS. Finally, persons who started HAART within an interval of two years after the IP or NSI/SI switch were excluded from the analysis.

Analysis of T cell inflections and trajectories.
The statistical methods used to estimate T cell inflection points (IP), and the CD3⁺ T cell slopes before and after the IP for a given individual, have been described elsewhere (Figure 1).

Briefly, a segmented regression model of the form $y_{ij} = \beta_0 + \beta_{i1}(t_{ij} - IP_1) + \beta_{i2}(t_{ij} - IP_1)^2 + \epsilon_{ij}$ was fit, where $y_{ij}$ represents the log₁₀ CD3⁺ cell count for the measurement of the $i$th individual at the time $t_{ij}$, $IP_1$ is the estimated time of loss of T cell homeostasis for the $i$th individual, and $\epsilon_{ij}$ is a random normal error term. The parameter $\beta_0$ represents the log₁₀ CD3⁺ T cell count at the time T cell homeostasis fails, and the parameters $\beta_{i1}$ and $\beta_{i2}$ represent the slope of the log₁₀ CD3⁺ cell
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Figure 1 Illustration of the algorithm used for estimating the time of inflection point (IP) and determines the \( CD^3^+ \) T cell slopes before and after IP.

\[
Y_{ij} = \beta_{10} + \beta_{11}(t_{ij} - IP_i)
\]

Measurements of \( \log_{10} CD^3^+ \) cell counts are represented by \(*\). \( CD^3^+ \) measurements determined in the first year after entry study (seroconverters as well as seroprevalent cases) and after mid 1996 (AIDS cases*) or January 1995 (AIDS free cases**) were excluded. The IP for individual \( i \) (IP\(_i\)) is the time for which the segmented regression model (solid lines) gives the smallest residual variability. Furthermore, the \( CD^3^+ \) trajectories before (\( \beta_1 = \) pre-IP slope) and after (\( \beta_2 = \) post-IP slope) the IP are key parameters for evaluating the methods relative to the T cell homeostasis hypothesis (adapted from PNAS 1998,95:10848-10853).
count before and after the IP, respectively. We evaluated the parameters $\hat{\beta}_{11}$ (pre-IP slope), $\hat{\beta}_{12}$ (post-IP slope), and $\beta_{11} - \beta_{12}$ (difference between the pre- and post-IP slopes) for distinguishing individuals in the AIDS and AIDS-free groups. To determine the slope value that best discriminated these two groups, we calculated the value that maximised the sensitivity and specificity for the development of AIDS using receiver-operator characteristic (ROC) curves. Subjects with post-IP slopes steeper than the cut-off were defined as "true inflectors" while those with a slope less steep than the cut-off were defined as "noninflectors".

**Determination of NSI to SI switch and relation of the switch to T cell inflections.**

To detect the presence of syncytium-inducing (SI) HIV-1 variants, peripheral blood mononuclear cells were co-cultivated with MT-2 cells. Briefly, HIV-1 was isolated from $0.5 \times 10^6$ fresh or $1 \times 10^6$ cryopreserved PBMC from infected men by cocultivation with $1.0 \times 10^6$ MT-2 cells (MRC AIDS Reagent Project, Hertfordshire, United Kingdom), as previously described. Cryopreserved cells were used until October 1992, and fresh cells thereafter. Cultures were kept 3 weeks. Virus replication was detected by observation of syncytium formation and detection of HIV-1 p24 in the culture supernatant. Cocultivation of patient PBMC with MT-2 is a sensitive and specific method for detection of SI isolates. From subjects with at least two virus phenotype measurements, the time of the NSI to SI switch was taken as the midpoint between the time of the last NSI measurement and the first SI measurement, with the restriction that the two measurements had to be within 12 months of each other. Furthermore, persons who started HAART within two years after the IP or NSI/SI switch were also excluded (n=9). With this restriction, there were 267 persons with phenotypic measurements. The median number of phenotypic measurements per person was 15 (IQR: 7-23), and the median interval between measurements was 3.6 months (IQR: 3.0-6.3)). The association between the NSI to SI switch and T cell IP was analysed by non-parametric tests (Mann-Whitney and Wilcoxon signed rank test or sign test, depending on the
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symmetry of the distribution) and by least-squares linear regression.

Results

T cell homeostasis and its failure in relation to AIDS.

Of the 325 subjects who met the selection criteria for this study, 178 developed clinically-defined AIDS by June 1996, and 147 remained AIDS-free through February 1998. Three subjects were excluded because they had started with HAART therapy within two years after IP.

Figure 2 illustrates the slopes of the CD3\(^+\) T cell counts before and after the IP for the AIDS (n=176) and AIDS-free (n=146) groups. For both groups, the distribution of the pre-IP slopes was essentially centered around zero (-0.02%/year for the AIDS group and -3.5%/year for the AIDS-free group. In contrast, the post-IP slopes differed significantly (p<0.001) between the two groups: the median post-IP slope was -17.6%/year (IQR: -46.0 to -0.09%) for the AIDS group and +4.6%/year (IQR: -6.1 to 19.0%) for the AIDS-free group. These data are in complete agreement with the T cell homeostasis hypothesis, which predicts CD3\(^+\) cell stability at all time periods in the AIDS-free group and prior to the T cell IP in the AIDS group.

The ROC analysis demonstrated that the post-IP slope (\(\beta_{12}\)) was the strongest predictor for AIDS, followed by the difference in pre- and
Inflection points

post-IP slopes ($\beta_{11} - \beta_{12}$) (data not shown). The optimal cut-off for distinguishing true inflectors from noninflectors based on the post-IP slope was a slope of -8.2%/year. This cut-off, which resulted in sensitivity and specificity for prediction of AIDS onset that were both 66%, was used to define the individuals included in the subsequent analyses of the relations between the T cell IP and AIDS, and the T cell IP to the SI switch, as described below. 118 of the 176 AIDS cases (67%) and 32 of the 146 AIDS free cases (22%) were classified as true inflectors. Among the 118 true inflectors who developed AIDS, the median time between the T cell IP and AIDS was 1.58 years (IQR: 0.83 to 2.6 years). Again, these estimates were not influenced by HAART, because all individuals with an interval of 2 years or less between IP and therapy were excluded.

The median CD3⁺, CD4⁺, and CD8⁺ T cell counts at the visit just prior to the estimated T cell IP were 1490, 420, and 1000 cells/µl, respectively; and 1110, 330, and 760 cells/µl, respectively, at the first visit after the T cell IP.

Relation of the SI switch to the T cell IP and to AIDS

NSI/SI phenotype data were available for 267 of the 325 individuals eligible for this study 133/176 (75.6 %) of the AIDS group, and 134/146 (91.7%) of the AIDS-free group. In addition, to the three subjects described above, an additional six subjects were excluded because the they had a SI-HAART interval shorter than two years. Overall, there was a strong association of T cell IP and SI switch (odds ratio = 3.9; P<0.001), with 52.1% (61 out of 119) of those with a true IP showing an SI switch, but only 21.0%(31 out of 148) of those without a true IP. There were 92 switches from the NSI to the SI phenotype which occurred within a 12-month interval, and subsequent analyses were based on these, with the exclusion of nine cases as described below. The incidence of a switch from the NSI to the SI phenotype was 62/133 (46.6%) among the AIDS group but only 30/134 (22.4%) in the AIDS-free group. For those who had both a SI switch and AIDS (n=62), the median time between these two events was 1.89 years (IQR: 1.3 to 3.4 years). Because applying
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data censoring can cause a selection bias we also performed the same analysis with the total number of SI switches, without any restrictions. An incidence of 47.3% (80 out of 169) among AIDS cases was found compared to 22.7% (32/141) among persons who remained AIDS-free, with a median interval between the switch and AIDS of 2.0 years (IQR: 1.4-3.6).

Subsequently, we analysed the proportion of men who developed AIDS, as a function of the presence or absence of SI switch and T cell IP, as shown in Table 1. There were 119 men who had both eligible NSI/SI phenotype assessments and a true T cell IP (i.e., post-IP slope <-8.2%/yr). Among these true inflectors, the proportion who developed AIDS was high, regardless of whether an SI switch was present (80%) or not present (72%). Among non-inflectors, the proportion who developed AIDS was much lower, although it was somewhat higher in those who had an SI switch (42%) than in those who did not (25%). These results are consistent with the hypothesis that some of the association between the SI switch and the development of AIDS is mediated by the failure of T cell homeostasis. However, since a higher risk of AIDS persisted among the non-inflectors with a SI switch than those without a switch, there appears to be an association of SI with AIDS via a pathway independent of T cell homeostasis failure.

The distribution of the time intervals between the true IP and the SI switch is shown in Fig.3. Among true inflectors who developed AIDS (n=49), the SI switch generally preceded the IP (median interval = -0.63 years, IQR = -1.35 to -0.06 years, p<0.001). However, for true inflectors without AIDS (n=12), these events

Table 1 Occurrence of AIDS stratified by T cell infection and SI phenotype

<table>
<thead>
<tr>
<th></th>
<th>SI switch</th>
<th>No SI switch</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>True inflectors</td>
<td>49/61 = 80%</td>
<td>42/58 = 72%</td>
<td>91/119 = 76%</td>
</tr>
<tr>
<td>Noninflectors</td>
<td>13/31 = 42%</td>
<td>29/117 = 25%</td>
<td>42/148 = 28%</td>
</tr>
<tr>
<td>Overall</td>
<td>62/92 = 67%</td>
<td>71/175 = 41%</td>
<td></td>
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occurred more contemporaneously (median: 0.0 year (IQR:-1.3 – 0.8)). Overall, there was a very strong linear relation between the time of the SI switch and the time of the true IP, as shown in Fig. 4.

Finally, we compared the influence of viral phenotype on T cell slopes before and after the IP. Using only data from the 119 true infectors, the T cell decline after the T cell IP was substantially steeper for men with an SI virus (median slope = -40.2%/year; IQR = -54.8 to -17.3) than for men with NSI viruses (median slope = -25.2%/year; IQR= -44.8 to -15.9). This difference was of borderline statistical significance (p=0.07). As expected there was also no difference regarding the pre-IP slopes (4.7%/year versus 6.3%/year, p=0.16). If the analysis was extended to all 267 subjects (i.e., without regard to whether they were true infectors), there was no difference in the pre-IP slopes (medians -0.06%/year versus -2.7%/year, p=0.3), but subjects who had an SI switch had significantly steeper post-IP T cell declines than those who had an IP without an SI switch (medians = -17.3%/year and -1.1%/year, respectively; p<0.001).
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Figure 3 Time between the first appearance of the SI virus phenotype and the T cell inflection point (IP) of Individuals who developed AIDS or remained AIDS free.

Figure 4 Time between the first appearance of the SI virus phenotype and the T cell inflection point (IP) of individuals who developed AIDS or remained AIDS-free.

Linear regression model
Discussion

This study has provided further support for the blind T cell homeostasis concept, in that a biphasic trajectory was observed, with nearly a stable number of peripheral CD3+ T cells in the first phase followed by a steep decline in CD3+ T cells in the second phase. This started a median of 1.58 years before the onset of AIDS. This is in close accordance with the results found in the MACS, where a median of 1.72 years was found.

In the second part of this study, we examined the association between the appearance of the SI phenotype in peripheral blood and the time and magnitude of the T cell IP. In subjects who developed AIDS, there was a strong temporal association between the T cell IP and the first appearance of SI virus phenotype. Specifically, the SI switch occurred a median of 0.63 years before the T cell IP, which agrees closely with the finding by Shankaprappa et al. that X4 viruses were maximally represented 0.6 years before the T cell IP in 8 homosexual men in the MACS who progressed to advanced HIV-1 disease. While NSI/SI phenotype did not affect the pre-IP slopes, post-IP CD3+ T cell slopes were significantly steeper among SI carriers than NSI carriers.

There are several possible mechanisms by which SI HIV-1 variants may be associated with failure of T cell homeostasis. One of the mechanisms may be an expanded host range of SI variants as compared to NSI variants. CCR5, the co-receptor for NSI variants, is expressed primary on memory T cells whereas CXCR4, the SI co-receptor, is expressed both memory and naive cells. In agreement with this expression pattern, NSI variants were mainly isolated from memory cells whereas SI variants were equally distributed among naive and memory cell populations. Not only the greater target cell population for SI HIV-1 variants but also the infection and destruction of naive cells that normally contribute to the T cell population may both have large impact in T cell homeostasis.

Herbein et al. found that the SI phenotype can be associated with apoptosis of non-infected T cells. Another possible explanation is that in some cases with rapid progression to AIDS, dual tropic SI variants (i.e., capable of replicating in both T
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cells and macrophages) were isolated. It has been postulated that the observed T cell turnover in HIV-1 infection can be viewed as the reverse of the process by which immune reconstitution occurs after stem cell transplantation, and Hellerstein et al. found that T cells from HIV-1 infected individuals had reduced half-lives as compared to those from healthy, HIV negative individuals. Finally, Glushakova et al. found that specific immune responses were enhanced by productive infection of the tissue with NSI isolates, but were blocked by SI isolates. In other words, broadening of the HIV target cell population - including the naive T cell population - together with the SI induced impairment of the immune responses and a reduced T cell half-life, may be the driving forces behind loss of T cell homeostasis and an accelerated progression of infection.

Acknowledgements

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Inflection points

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