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Emergence of Syncytium-Inducing Human Immunodeficiency Virus Type 1 Variants Coincides with a Transient Increase in Viral RNA Level and Is an Independent Predictor for Progression to AIDS

Ingrid Spijkerman, Frank de Wolf, Miranda Langendam, Hanneke Schuitemaker, and Roel Coutinho

To study the dynamics of human immunodeficiency virus (HIV)-1 RNA level around the time of conversion from non-syncytium-inducing (NSI) to syncytium-inducing (SI) phenotype and to study the predictive value of the SI phenotype for progression to AIDS, sequential samples from 123 HIV-infected homosexual men with documented intervals of seroconversion were evaluated. The NSI-to-SI phenotype conversion coincided with a 3-fold increase in median RNA level, which was not observed in matched controls in whom a viral phenotype conversion did not occur. This increase in virus was followed by a decrease to a higher steady-state RNA level than before the switch. After adjusting for RNA level and CD4 T cell count, SI phenotype was an independent marker for progression to AIDS. Hence, phenotype determination will contribute to optimal staging of HIV-infected persons in addition to virus load measurements and CD4 T cell count.

Methods

Study population. The study population consisted of 123 homosexual men enrolled in the Amsterdam cohort study of HIV-1 infection in homosexual men [12], who had a documented seroconversion interval for HIV-1 antibodies during follow-up in the cohort (median seroconversion interval, 3.2 months). Participants were examined every 3 months, and blood samples were obtained. AIDS diagnosis (CDC 1993 AIDS case definition excluding the CD4 T cell criteria [13]) was obtained through follow-up and record linkage with the national AIDS registry. Vital status was determined through hospital admission data and the population register in the town of residency.
HIV-1 RNA level was determined in stored (−70°C) serum samples at seroconversion (defined as the first seropositive sample), at 3 and 6 months thereafter, and subsequently at 1-year intervals until the end of follow-up in the cohort study. In the 123 homosexual men, 1794 RNA measurements were made (median, 14/subject; range, 2−45; interquartile range [IQR], 10−18). Viral phenotype and CD4 cell count were measured every visit.

Fifty-four of the 123 men progressed to AIDS after a median of 4.6 years (range, 0.5−10.3) after seroconversion, and 69 did not develop AIDS (median follow-up, 6.0 years; range, 0.1−10.9). Among those men developing AIDS, 47 died after a median follow-up of 6.9 years (range, 0.8−10.3) from seroconversion. Of the 123 men, 73 (59%) seroconverted between 1985 and 1987 and 50 (41%) between 1988 and the end of 1995. Treatment with three antiretroviral drugs was used by 1 subject at the last visit of the study, while 25 and 23 subjects were treated with two and one antiretroviral drug(s), respectively, for various time periods.

Laboratory methods. Sera were screened for the presence of antibodies to HIV-1 with a commercial EIA (Abbott Laboratories, Abbott Park, IL) and confirmed by Western blot (Genelabs, Herent, Belgium).

HIV-1 RNA was quantified by using a nucleic acid sequence–based amplification assay (NASBA HIV-1 RNA QT; Organon Teknika, Boxtel, The Netherlands) according to the instructions of the manufacturer. The threshold of quantification, with 100 μL of serum, was 10^3 RNA copies/mL. RNA levels were determined in batch purely randomly, thus, without selection by viral phenotype.

Lymphocyte immunophenotyping for peripheral CD4 and CD8 T cells was done by flow cytometry using dual-color immunofluorescence.

To determine HIV-1 phenotype, HIV-1 was isolated from fresh or cryopreserved peripheral blood mononuclear cells by cocultivation with MT-2 lymphoblastoid cells (Medical Research Council-AIDS Reagent Project, Potters Bar, UK). Isolates producing syncytia in MT-2 cells were considered SI (NSI isolates do not replicate in MT-2 cells).

Statistical analysis. The moment of phenotype conversion was estimated as the midpoint between the date of the last NSI phenotype visit and the first SI phenotype visit. For RNA copy numbers below the test threshold of quantification, the amount of RNA was arbitrarily set at 10^6 or 999 RNA copies/mL.

To study the dynamics of RNA level and CD4 T cell count around conversion of viral phenotype, median values of the two markers within fixed time periods (mean ± 3 months) were determined from 3 years before till 4.5 years after NSI-to-SI conversion. For comparison, median values were calculated in a control group who did not convert from NSI to SI phenotype during follow-up and who were matched by length of follow-up (date last NSI visit of control was later than last RNA determination of the case) and CD4 T cell count (±100 × 10^6 cells) at 1 year before phenotype conversion switch. In this analysis, 34 subjects of 42 who showed a phenotype conversion (and 34 controls) were studied, because 8 subjects were excluded for various reasons (3 subjects in whom SI variants were detected from seroconversion onwards, 2 subjects who showed phenotype conversion within 1 year from HIV-1 seroconversion, 1 subject who had no CD4 T cell counts around 1 year before the phenotype conversion, and 2 subjects who could not be matched). If more than one measurement per person was available within one time period, only one measurement (closest to the midpoint of the time period) was taken into account for the median value. If <10 subjects with a measurement were available within a specific time period, this time period was omitted. Differences between groups at each time point were tested with the Mann-Whitney U test (P < .05 was considered statistically significant). Differences in median RNA level between two time points before and after phenotype conversion were tested by Wilcoxon matched-pairs signed-ranks test. Additional analyses were done: excluding RNA measurements at time points with CD4 cell counts <100, studying a subpopulation of participants who were followed for >3 years after the switch, and excluding RNA measurements from the start of antiretroviral treatment.

Also, median RNA levels during time since seroconversion were calculated for those who remained NSI for >9 years after seroconversion (n = 19) and those who converted from NSI to SI phenotype at some time point during follow-up (n = 39). Median RNA levels for the latter group were divided into the NSI course and the SI course and shown as two curves.

Kaplan-Meier survival analysis and Cox proportional hazards analysis were used to study the cumulative incidence of viral phenotype conversion and AIDS and the predictive value of RNA levels, SI phenotype, and CD4 T cell counts for these outcomes. In the Cox analysis, both uni- and multivariate models were fitted, treating the markers as time-dependent covariates. AIDS-free subjects were censored at 1 year after the last visit with RNA determination, at death, or on 1 January 1996. Categories of CD4 T cell count (>500, 300−500, <300 cells × 10^9/L) were defined by the 33rd and 67th percentile and of RNA level by the 25th, 50th, and 75th percentile (<10^4.0, 10^4.0−10^5.5, 10^5.6−10^6.9, >10^6.9 copies/mL). In the time-dependent analysis, the two lowest quartiles of RNA level were combined because no AIDS occurred in the lowest quartile. The 3 subjects with SI phenotype at seroconversion were excluded in the analysis concerning the cumulative incidence of SI phenotype.

Results

Dynamics of HIV-1 RNA level around viral phenotype conversion. Of the 123 homosexual men with documented intervals of seroconversion, SI variants could be detected in 42 at any time during follow-up. In 3 men, SI variants were present from seroconversion onwards. Viral phenotype conversion before AIDS diagnosis was shown in 38 and after AIDS diagnosis in 1. The median interval between the last visit with the NSI phenotype and the first detection of SI variants (n = 39) was 6 months (IQR, 3.2−9.2; minimum, 1.2; maximum, 20.4). The median time from seroconversion to NSI-to-SI switch was 3.8 years (range, 0.4−9.2).

The first detection of SI variants occurred at all levels of RNA; of the 26 subjects who showed a phenotype conversion and who had an RNA determination available within 6 months before conversion, 6 subjects (23%) had RNA levels >4.9 log copies/mL, 5 (19%) between 4.5 and 4.9 log copies/mL, 11
The dynamics of RNA level and CD4 T cell count from 3 years before till 4 years after the NSI-to-SI conversion is shown in figure 1 for 34 patients and 34 matched controls who did not show a viral phenotype conversion. Median RNA levels before the NSI-to-SI conversion were characterized by relatively stable values of ~30,000 copies/mL (3 months before conversion IQR, 13,750–92,000) or 4.5 log copies/mL (figure 1A). Coinciding with the first detection of SI variants, a rapid 3-fold increase in median RNA level was observed to 90,000 copies/mL (IQR, 26,000–260,000) or 5.0 log copies/mL at 15 months after conversion. Subsequently, median RNA level declined to an equilibrium level before conversion. This equilibrium was reached at 33 months after seroconversion at ~50,000 copies/mL (IQR, 17,750–272,500) or 4.7 log copies/mL. In the comparison group consisting of persons who did not develop SI virus, median RNA levels were observed of ~20,000 copies/mL (at the −3 months time point, median, 16,000; IQR, <1000–33,000). Levels remained at about the same level in the NSI group, although fluctuations in median RNA level increased with time because of the small number of measurements at the extreme time points. The difference in RNA level between the group that experienced SI conversion and the NSI group was statistically significant from 9 months before till 33 months after conversion. The median RNA level at 15 months after conversion was significantly higher than the median RNA level at 3 months before conversion ($P = .001$).

The decline in RNA level from 15 months after phenotype conversion might be caused by exhaustion of infectible CD4 T cells. However, no indications were found that very low CD4 T cell counts were associated with low RNA levels (data not shown), and exclusion of RNA measurements at time points with CD4 T cell counts <100 revealed a similar pattern of an increase followed by a decrease in RNA after the conversion. Another explanation for the decline from 15 months after the conversion might be the RNA determinations that are missed because of attrition (participants who were lost to follow-up because of disease progression or death). However, studying participants who were followed for >3 years after the emergence of SI variants revealed a similar pattern.

The potential impact of antiretroviral treatment was studied by excluding RNA measurements from the start of antiretroviral treatment. A similar pattern could still be observed, with even greater differences in RNA level between the group who experienced SI conversion and the NSI group.

At the individual level, the amount of viral RNA around phenotype conversion varied greatly, but the majority showed the pattern of an increase around conversion and a subsequent decrease. All 39 individual curves showed an increase in RNA level around phenotype conversion, varying from 5000 to 1,300,000 RNA copies/mL (median, 100,000) or from 0.1 to 2.0 log copies/mL (median, 0.7), which started between 1 year before and 1 year after the estimated conversion in 34 (87%) subjects and >1 year after the conversion in 5 (13%) subjects. A consequent decrease was observed in 33 (85%) of those who converted, varying from 5000 to 1,000,000 RNA copies/mL (median, 65,000) or from 0.1 to 1.5 log copies/mL (median, 0.4), which started between 6 months and 2 years after the conversion in 22 (56%) subjects and >2 years after the switch in 11 (28%) subjects.

The emergence of SI variants occurred at a median CD4 T cell count of 380 × 10^6/L (IQR, 250–590) (figure 1B). Thereafter, an accelerated decline in CD4 cells was observed compared with the period before the conversion. Median CD4 cell count at 15 months after the conversion was 200 × 10^6/L (IQR, 100–410).

In figure 2, we show the dynamics of median RNA level from seroconversion onward for those whose virus remained NSI for >9 years of follow-up and for those who converted from NSI phenotype to SI phenotype. The group with NSI virus for >9 years had median RNA levels between 10,000 and 25,000 copies/mL in the 6 years after HIV-1 seroconversion. Comparable median RNA levels were found for the NSI course of those who eventually converted in viral phenotype. From the first emergence of SI variants, median RNA levels fluctuated between 55,000 and 112,000 copies/mL in the 6 years after seroconversion, which were higher than RNA levels at the same time point in the other groups.

**Cumulative incidence of SI switch and predictors for switch.** The cumulative incidence of the NSI-to-SI conversion at 3, 6, and 9 years after seroconversion is 12%, 32%, and 37%, respectively. Subjects with RNA level >4.9 log (relative hazard [RH], 1.6; 95% confidence interval [CI], 0.8–3.3, compared with those with RNA <4.5 log) or CD4 T cell count <500 (RH, 1.9; 95% CI, 0.9–3.9, compared with those with counts >500) were at increased risk for phenotype conversion. However, the RHs were not statistically significant.

**Predictive value for progression to AIDS.** In univariate analysis, high RNA level is associated with a faster progression to AIDS (table 1). Subjects with RNA levels >4.5 log had a 4- to 7-fold-increased risk for progression to AIDS. Also, viral phenotype was a strong predictor for progression to AIDS, with a 5-fold-increased risk for those with NSI-to-SI phenotype conversion compared with those whose virus remained NSI. The strongest univariate predictor was CD4 T cell count; subjects with <300 CD4 T cells were 19-fold more likely to develop AIDS than were subjects with higher CD4 T cell counts. In the multivariate analysis, SI phenotype was an inde-
Figure 1. Median RNA level (A) and CD4 T cell count (B) around the time of conversion from non-syncytium-inducing (NSI) to syncytium-inducing (SI) phenotype for 34 patients who converted and for comparison group of 34 patients whose virus remained NSI (matched by duration of follow-up and CD4 T cell count at 1 year before conversion).
dependent predictor for progression to AIDS in addition to RNA level and CD4 T cell count. The RHs of all three predictors decreased, but all remained significantly associated with progression to AIDS. No statistical interaction was found between the markers.

### Table 1

<table>
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<tr>
<th>Covariate</th>
<th>Univariate</th>
<th>Multivariate</th>
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<tr>
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<tr>
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<tr>
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<tr>
<td>&gt;4.9</td>
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<td>3.0 (1.3–6.7)</td>
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<td>Phenotype</td>
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<td>1.0</td>
</tr>
<tr>
<td>SI</td>
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<td>2.0 (1.0–3.7)</td>
</tr>
<tr>
<td>CD4 cell count/L</td>
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<td>&lt;300 x 10^6</td>
<td>8.8 (2.4–32.1)</td>
<td>8.8 (2.4–32.1)</td>
</tr>
</tbody>
</table>

NOTE: NSI, non-syncytium-inducing; SI, syncytium-inducing.

**Discussion**

In this large group of HIV-infected homosexual men with documented interval of seroconversion, we observed a 3-fold increase in the median serum HIV-1 RNA level coinciding with the NSI-to-SI phenotype conversion, which was not observed in matched controls in whom a phenotype conversion did not occur. Since samples from both groups were tested simultaneously, this difference cannot be explained by technical factors. At the individual level, an increase in viral RNA around the first detection of SI variants was observed in all subjects, but the magnitude of the increase varied greatly, which might be explained by intra-assay variation. Also, the temporal relation between the increase in virus and viral phenotype conversion varied between individuals; while in most cases the increase seemed to follow the conversion, in others it seemed to precede the conversion. However, because of uncertainty related to the exact time point of the emergence of SI variants and the frequency of RNA determinations around the switch, firm conclusions cannot be drawn regarding the temporal relation between the RNA increase and emergence of SI variants. The observations of an increase in virus could be biologically explained by a broader T cell host range for SI variants [8], which can...
probably be explained from the fact that SI variants can infect CXCR4-expressing T cells in addition to CCR5-expressing cells [14]. A 3-fold increase in RNA level is important with regard to prognosis, as reported by Mellors et al. [15], who showed a 50%–60% higher risk for progression to AIDS associated with a 3-fold increase in RNA. As antiretroviral treatment is mostly started in subjects with low CD4 T cell counts and high virus loads, it biases the results towards unity. Hence, if treatment were taken into account, the RNA increase might even be greater.

Following the increase, the RNA level is observed to decrease to a new steady state at a higher RNA level than before the switch. No indications were found that this decline was due to exhaustion of infectible CD4 T cells, although no differentiation was made between CXCR4- and CCR5-positive cells, nor due to missing RNA determinations caused by attrition. The RNA decline might be biologically explained by the humoral or cellular immune response (or both) evoked by the SI variants and/or the increase in virus. Another explanation for the RNA decline might be the depletion of activated target cells due to extensive HIV-induced cell loss, which restricts virus production until a new equilibrium is instituted (the so-called predator-prey theory) [16, 17].

Although viral phenotype conversion was more prevalent in persons with RNA levels >10^3 copies/mL and CD4 T cell counts <500 × 10^6/L, the first detection of SI phenotype was observed at all levels of RNA and CD4 T cell count. Moreover, we found similar median RNA levels for subjects with the NSI phenotype regardless of a subsequent SI conversion. These findings indicate that a high replication rate or immunosuppression of the host appeared to be related to the emergence of SI variants but were not obligatory for the emergence of SI variants. Other important factors must be involved, such as the number of mutations required for the evolution from NSI to SI [18]. Further study is needed on the immunologic and virologic factors linked to the emergence of SI variants and the amount of HIV in blood.

Viral phenotype was an independent marker for progression to AIDS after adjusting for RNA level and CD4 T cell count. Our findings indicate that the effect of the SI phenotype on disease progression was partially mediated by its effect on RNA level and CD4 T cell decline. Viral phenotype certainly adds distinct and important information, in addition to RNA level and CD4 T cell count, regarding individual prognosis. One other study reported that SI phenotype at the start of antiretroviral drugs was associated with both a faster CD4 T cell decline and disease progression [19]. Only with respect to CD4 T cell decline, SI phenotype was an independent marker besides RNA level and CD4 T cell count [19].

A clinical implication of these findings is that HIV-infected patients should preferably be treated before the emergence of SI variants to prevent or postpone the conversion of viral phenotype and the associated fast deterioration [3, 5]. By the current criteria for antiretroviral treatment based on RNA level (>10,000 copies/mL) and CD4 T cell count (<500 × 10^6/L), the vast majority of our study population whose viral phenotype converted (37 of the 39) were eligible for treatment before phenotype conversion. The few patients who experience phenotype conversion and do not meet the criteria for therapy are at high risk for disease progression and should be considered for treatment to prevent the associated CD4 T cell decline and/or RNA increase.

In conclusion, we found that the NSI-to-SI phenotype conversion coincided with an increase in RNA level followed by a decrease to a higher steady-state level than before the switch. After adjusting for RNA level and CD4 T cell count, SI phenotype was an independent marker for progression to AIDS. Hence, phenotype determination will contribute to optimal staging of HIV-infected persons in addition to virus load measurements and CD4 T cell count.

References


