Virus and host determinants of HIV-1 infection and AIDS pathogenesis
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Chapter 7

"In vitro replication kinetics of HIV-1 variants in relation to virus load in long-term survivors of HIV-1 infection"

H. Blaak, M. Brouwer, L.J. Ran, F. de Wolf, H. Schuitemaker

In vitro replication kinetics of human immunodeficiency virus type 1 (HIV-1) variants in relation to virus load in long-term survivors of HIV-1 infection

In 7 long-term survivors (LTS) and 8 progressors, all carrying solely non-syncytium-inducing (NSI) variants, a possible correlation between in vitro virus replicative capacity, virus load, and clinical course of human immunodeficiency virus type 1 (HIV-1) infection was analyzed. Late in infection, 3 LTS and 7 progressors had a high virus load, which coincided with the presence of rapid-replicating viruses. In contrast to progressors, LTS maintained relatively high and stable CD4\(^+\) T cell counts. Four LTS persistently had relatively slow-replicating viruses and a low viral load, even after 6.6 to 9 years of seropositive follow-up. All virus isolates from 1 of these LTS had a 4-aa deletion in nef. These results suggest a correlation between the in vitro replicative capacity of NSI HIV-1 variants and virus load. The presence of HIV-1 variants with relatively low replicative capacity throughout infection may have contributed to the beneficial clinical course in half of the LTS in this study.
progressed to AIDS, maintain a reduced replicative capacity throughout the infection, thereby enabling LTS to maintain low virus load and high CD4+ T cell counts. Therefore, we analyzed virus load in relation to the in vitro replicative capacity of biological virus clones obtained early and late in infection from 7 LTS and 8 persons who progressed to AIDS in the presence of solely NSI variants. In addition, since defective nef genes have been implicated as being instrumental for low replicative capacity and long-term survival,[470-473] the nef sequences of the biological variants isolated from LTS and progressors in this study were analyzed.

MATERIALS AND METHODS

Subjects. By October 1994, 12 participants of the Amsterdam Cohort studies on AIDS had an asymptomatic follow-up of at least 9 years with a relatively normal mean CD4+ T cell count (>400/mm3). Additionally, these persons harbored solely NSI variants and did not receive any anti-HIV drug treatment during the course of infection. These individuals were termed LTS.[436] Seven of these 12 LTS were studied here (ACH78 [L1], ACH441 [L2], ACH583 [L3], ACH709 [L4], ACH68 [L5], ACH337 [L6], ACH750 [L7]). L5 underwent splenectomy 72 months after entry in the cohort studies.

For comparison, 8 progressors were selected on the basis of the presence of solely NSI variants during their clinical course. Three of them were rapid progressors (AIDS diagnosis after 43 to 76 months of follow-up: ACH424 [P1], ACH537 [P4], ACH53 [P5]), 4 were typical progressors (AIDS diagnosis after 99 to 109 months of follow-up: ACH19 [P2], ACH38 [P6], ACH1081 [P7], ACH142 [P8]), and 1 was termed a slow progressor (AIDS diagnosis after 136 months of follow-up, after a 10 year period of high and stable CD4+ T cell counts: ACH517 [P3]).

From L1 - L7 and P1 - P3, at least 2 peripheral blood mononuclear cell (PBMC) samples were used for analysis. The first sample was obtained as early as possible after seroconversion or entry in the cohort studies. The other sample was obtained as late as possible, which was -9 years after seroconversion or entry for most LTS, 6.6 years for L4, and about the time of AIDS diagnosis for the progressors. From P4-P8, a sample derived from a time point close to AIDS diagnosis was analyzed (Table 1).

Analysis of CD4+ T cell counts. T lymphocyte immunophenotyping for the CD4+ T cells was carried out at 3-month intervals by flow cytometry. PBMC were stained with CD4 monoclonal antibody according to standard procedures for cell cytometric analysis.

Virus isolation and determination of infectious cellular virus load. Virus was isolated under limiting diluting conditions as previously described.[594,220] Briefly, participant PBMC (0.5 to 4 x 10^4 cells/well in 48 or 96 wells) were cocultivated with phytohemagglutinin (PHA)-stimulated healthy donor PBMC (10^5/well) in 96-well microtiter plates. Every week, culture supernatants were tested for p24 antigen by an in-house p24 antigen-capture ELISA.[394] At the same time, one-third of the culture volume was transferred to new 96-well plates, and 10^5 fresh PHA-stimulated healthy donor PBMC were added to propagate the culture. The proportion of productively infected CD4+ T cells was calculated with the formula for Poisson distribution: F = 1 -e^{-m(F0)}, in which F0 is the fraction of negative cultures.

PBMC from wells tested positive were transferred to 25-ml culture flasks containing 5 x 10^8 fresh PHA-stimulated PBMC in 5 ml of medium to grow virus stocks. From these cultures the cell-free supernatant was stored at -70°C until use. One million of the infected PBMC were used for DNA analysis. To the remaining PBMC, MT2 cells (1 x 10^6) were added to analyze syncytium-inducing capacity of the virus clones.[89]

Quantification of RNA in serum. HIV-1 RNA was quantified in serum by using a nucleic acid sequence-based amplification assay (HIV-1 RNA QT. Organon Teknika, Boxtel, The Netherlands). One hundred microliters of serum was added to 900 ul of lysis buffer containing guanidinium thiocyanate, Triton X-100, and Tris-HCl. Three synthetic RNAs (QA, QB, QC) of known high, medium, and low concentration, respectively, were added to the lysis buffer containing the released nucleic acid. These Q-RNAs served as internal calibrators, each differing from the HIV-1 wild-type RNA by only a small sequence.[233] Subsequently, RNA was isolated as previously described.[239] Amplification of wild-type HIV-1 RNA and Q-RNAs is based on primer extension of primer 1 (nt 682 - 711 in relation to the HX2B gag sequence, 5'-ACTCTCTTGGTTCCCCTTCACTGTATCGAAA-3') RNA, and Q-RNAs is based on primer extension of primer 2 (nt 569 - 590: 5'-AGTGGGGGGGACATCAAGCAGCCATGCAGCATAATCTTAA-3') RNA. Subsequently, RNA was added to propagate the culture. The proportion of productively infected CD4+ T cells was calculated with the formula for Poisson distribution: F = 1 -e^{-m(F0)}, in which F0 is the fraction of negative cultures.

PBMC from wells tested positive were transferred to 25-ml culture flasks containing 5 x 10^8 fresh PHA-stimulated PBMC in 5 ml of medium to grow virus stocks. From these cultures the cell-free supernatant was stored at -70°C until use. One million of the infected PBMC were used for DNA analysis. To the remaining PBMC, MT2 cells (1 x 10^6) were added to analyze syncytium-inducing capacity of the virus clones.[89]
by the hybridized ruthenium-labeled probes is proportional to the amount of amplificate.

On the basis of the relative amounts of the four amplificates, the original amount of wild-type RNA in the sample was calculated.

**Testing of replicative capacity of virus clones.** From each subject 1 to 5 biological virus clones per time point were analyzed for their replicative capacity. From most persons, <5 clones from the early time point were analyzed (Table 1), because <5 virus clones could be recovered as a result of a low virus load. The selected viruses had different time points of first detection during clonal isolation. The titer of the virus stocks was quantified by determination of the TCID$_{50}$ in PHA-stimulated PBMC derived from the same donor and cultured for 16 days.

To determine the kinetics of virus production, 75 μl of the culture supernatant was harvested every 2nd day and stored at 4°C until analysis. At day 8 of culture, 4 x 10$^6$ freshly PHA-stimulated PBMC in 2 ml of medium were added to the cultures. Supernatants harvested at all different time points were analyzed for the presence of viruses in an in-house p24 antigen-capture ELISA.$^{[19]}$ p24 production per ml of supernatant was determined and corrected for the differences in volume of culture supernatants between the moments of sampling.

**Nef sequence analysis.** From 10$^6$ infected PBMC, obtained after propagation of the clonal virus stocks, proviral DNA was isolated. PBMC were lysed in L5 lysis buffer (0.08 M GuSCN [Life technologies Gibco BRL, Gaithersburg, MD], 0.08 M Tris-Cl, pH 6.4, 0.035 M EDTA, 2% (wt/vol) Triton X100)$^{[21]}$ and stored at -70°C until use. DNA was precipitated by addition of isopropanol in a 1:1 ratio (vol/vol) and centrifugation for 15 min at 13,000 g. DNA pellets were washed twice with 70% ethanol and resuspended in water.

Nef DNA was amplified by a nested polymerase chain reaction (PCR) with primers Nef A (nt 2408 - 2428) in relation to the SF2 env sequence: 5'-GTCTAGAATCCTAAGAAATG-TTG-3' (sense orientation) and LTR BCAT (nt 519 - 545 of the LTR sequence: 5'-CAGTCCTAAAGGCAAAGCAT-3' (antisense) in the first reaction and primers Nef B (nt 2526 - 2546 of the env sequence: 5'-ATCTAGAAGAATAAAGACG-3' (sense), and Nef C (nt 350 - 370 of the LTR sequence: 5'-AAGTCTGGCAGGAAAGCTC-3' (antisense)) for the second reaction.

For both reactions DNA was denatured for 5 min at 98°C, followed by 30 cycles of 1.5 min of denaturation at 95°C, 1.5 min of annealing at 48°C, and 1.5 min of extension at 72°C, and a subsequent extra 5-min extension at 72°C and soaking at 4°C. Five microliters of DNA was amplified in 50-μl reactions containing 1 x Taq buffer (Promega, Madison, WI), 0.2 mM of each dNTP, 100 μg of each primer, 1.5 mM MgCl$_2$, and 1 U of Taq DNA polymerase (Promega). Five microliters of the first reaction was used as input for the nested PCR reaction.
PCR products were purified by use of a spin PCR purification kit (Quiagen, Chatsworth, CA).

The positive strands were sequenced using Sequenase DNA polymerase (United States Biochemicals, Cleveland) with primers Nef B; LTR 1A (nt 412 - 438 of the nef sequence: 5'-AGATATCCACTGACCTTTGGATGGTGC-3'), and Nef 1C (nt 96 - 126 of the nef sequence: 5'-AGCATCTC6AGACCT66AAAAACATGGAGC-3').

Both DNA purification and sequencing procedures were performed according to the instructions of the manufacturer.

Statistical analysis. Differences between the 3 groups of phenotypically distinct virus variants in the increase in p24 production between days 4 and 8 of culture and the maximal p24 production were analyzed by one-way analysis of variance. The Mann-Whitney U test was used to analyze the relationship between the presence of rapid-replicating virus variants and the infectious cellular load. The correlation between the infectious load and the RNA load in serum was analyzed by use of the Spearman's correlation coefficient ($r_s$).

Fig. 1. Replication kinetics of biological virus clones (continued on next page).
RESULTS

Correlation between HIV-1 replication characteristics and clinical course. Biological virus clones of 7 LTS and 3 progressors, obtained from PBMC that originated from an early and late time point during follow-up (Table 1), were analyzed with respect to their replicative capacity (Fig. 1a and b). The replicative capacity of each virus was reflected by the rate of p24 accumulation early in culture (between days 4 and 8) and the maximum levels of p24 production reached within 14 days of culture.

The mean increase in p24 production between days 4 and 8 of culture (0.25 µg/ml) and the mean maximal p24 production (1.26 µg/ml) of all viruses analyzed were used as reference for determining the replication phenotype of each single virus clone (Fig. 1c). Variants with a p24 increase between days 4 and 8 and a maximal p24 production above average were typed rapid (upper right quadrant in Figure 1c; mean values, 0.1 µg/ml and 0.9 µg/ml). Viruses with only one measure above average were typed as variants with intermediate replicative capacity (upper left and lower right quadrant; mean values, 0.2 µg/ml and 1.2 µg/ml, respectively). The mean values of both measures were significantly different between the three phenotypically distinct groups (P < 0.001).

At the early time point, all participants carried viruses with relatively low replication kinetics. Only LTS L6 and L7 additionally had viruses with high replication kinetics at this time point. In 4 of the 8 LTS (L1 - L4), all late stage viruses that were analyzed also had relatively low replication kinetics; L4 even seemed to have developed less-replication-competent viruses over time.

Three LTS (L5 - L7) and the progressors (P1 - P3) had viruses with high replicative capacity late in infection. In these subjects (with the exception of L7), the proportion of rapid-replicating viruses had increased compared with that at the early time point, suggesting a shift to more-replication-competent variants during the course of infection. In L6 and P2, all late-stage virus variants analyzed had high replication kinetics, while in the other subjects viruses with high replication kinetics and viruses with lower replication kinetics co-existed.

Virus load in relation to replicative capacity of HIV-1. To study whether the replicative capacity of HIV-1 variants correlated with virus load, the infectious cellular load at the time points of virus isolation was calculated. The contribution of HIV-1 variants with different replicative capacity to the virus load was estimated for each participant at each time point. This was achieved by extrapolating the relative contribution of rapid-, intermediate-, and slow-replicating viruses to the total infectious cellular load (Fig. 2).

Early in infection, all participants had a low cellular virus load (1 to 16 TCID/10^6 CD4+ T cells), which consisted of slow- and/or intermediate-replicating viruses in most persons. The 2 LTS (L6 and L7) in whom viruses with high replicative capacity were detected had the highest infectious cellular load (Fig. 2a).

Because of the low virus load, <5 biological clones were obtained at the early time point for most persons (Table 1). From 3 of these (L4, L5...
HIV-1 replication and virus load in LTS

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Fig. 2. Contribution of biological virus clones with different replicative capacity to infectious cellular load expressed as TCID/10^6 CD4^+ T cells. Proportion of each phenotype (a, slow-replicating; •, rapid-replicating; •, intermediate) within analyzed groups of viruses was extrapolated to total cellular load early (a) and late (b) in infection.

and P3), the replication kinetics of virus clones from an additional time point were analyzed, ~1 year after the early time point. Also at this time point, only viruses with relatively low replicative capacity were present (data not shown) and the cellular virus load was still low (see also Figure 3).

The 4 LTS (L1 - L4) who only had slow- and intermediate-replicating viruses late in infection also maintained a low infectious cellular load (1 to 29 TCID/10^6 CD4^+ T cells). Interestingly, all 3 progressors and the 3 LTS (L5 - L7) with rapid-replicating variants late in infection had a high cellular load at this time point (83 to 288 TCID/10^6 CD4^+ T cells) (Fig. 2b).

We additionally analyzed the replicative capacity of biological virus clones derived from 5 persons with a progressive disease course at a time point close to AIDS diagnosis (P4 - P8; Table 1). For each subject, the infectious cellular load at this time point, consisting solely of NSI variants, is given in Table 2. For comparison of replicative capacity, slow- (n = 7) and rapid-replicating viruses (n = 7) from the first experiment were included.

In contrast to the rapid-replicating control viruses, which first showed detectable p24 production at days 2 to 6 of culture, the slow-replicating control viruses did not produce detectable p24 levels until days 10 to 14 of culture. Therefore, the viruses from P4 - P8 were not classified by the combination of their increase in p24 production between days 4 and 8 of culture and the maximum p24 production, as in the first experiment, but by the combination of the first day of detection and maximal p24 production relative to the means of the control viruses (day 8 and 0.2 μg/ml, respectively). In progressors P5 - P8, but not in P4 who had a low infectious cellular load at the time point of analysis, viruses with high replicative capacity were detected (Table 2).

Both early and late in infection, the infectious cellular load was significantly higher in subjects with rapid-replicating viruses than in subjects from whom no rapid-replicating viruses were detected (early median load, 12.5 [n = 2] versus 2.5 [n = 8], p = 0.04; late median load, 171 [n = 10] versus 27 [n = 5]; P< 0.001).

Table 2. Replicative capacity and infectious cellular load around AIDS diagnosis

<table>
<thead>
<tr>
<th>Participant</th>
<th>No. of biological virus clones analyzed</th>
<th>Replicative capacity, no. (%)</th>
<th>Infectious cellular load (TCID/10^6 CD4^+ T cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slow</td>
<td>Intermediate</td>
<td>Rapid</td>
</tr>
<tr>
<td>P4</td>
<td>4</td>
<td>0</td>
<td>4 (100)</td>
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<tr>
<td>P5</td>
<td>6</td>
<td>1 (16.67)</td>
<td>4 (66.67)</td>
</tr>
<tr>
<td>P6</td>
<td>5</td>
<td>1 (20)</td>
<td>3 (60)</td>
</tr>
<tr>
<td>P7</td>
<td>6</td>
<td>0</td>
<td>3 (50)</td>
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<tr>
<td>P8</td>
<td>6</td>
<td>0</td>
<td>1 (16.67)</td>
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</table>
Infectious cellular load and RNA load in serum. It could be argued that a high infectious cellular load, in particular the high load observed in 3 of the LTS, might be an in vitro artifact caused by activation of latently infected cells during culture procedures. Therefore, we analyzed the RNA levels in serum samples obtained at the late time points for all study participants (with the exception of P6). In agreement with our previous observations, the log-transformed values of both measures of virus load were significantly correlated ($r = 0.67, P = 0.006$). The low infectious cellular load in L1 - L4 was accompanied by a low RNA serum load ($<10^4$ RNA copies/ml of serum), while L5 - L7 and the progressors had both a high infectious cellular and a high serum RNA load ($>10^4$ RNA copies/ml of serum) (see also Figure 3).

Virus load in relation to clinical course. The finding of LTS with high virus load raised the question of whether this high load reflected the onset of disease progression in these subjects. However, during the 1- to 2-year follow-up period beyond the late time point, the CD4$^+$ T cell counts in L5 - L7 remained relatively stable (Fig. 3). Analysis of the infectious cellular virus load and viral RNA in serum at several additional time points during follow-up of these LTS revealed a relatively high cellular load (78 and 74 TCID/10$^6$ CD4$^+$ T cells, respectively), even at earlier time points in L6 and L7.

The time span between detection of a high load and the most recent measurement of high CD4$^+$ T cell counts was 43, 70, and 59 months for L5, L6, and L7, respectively. During this period, the number of CD4$^+$ T cells remained relatively stable (41, 13, and 1 CD4$^+$ T cell(s)/µl/year for L5, L6, and L7, respectively). In contrast, the CD4$^+$ T cell decline observed in the progressors (P1 - P3 and P8) appeared to coincide with or even precede the moment of first detection of high infectious cellular load (CD4$^+$ T cell decline between moment of first detection of high infectious cellular load
Fig. 4. Nef consensus sequences of biological virus clones derived early and late in infection from 3 progressors (P) and 5 LTS (L), aligned with consensus sequence (cons) derived from all clones. Locations of putative functional domains (reference 475) are shown above consensus sequence.

PxxP = proline repeat sequence; PKC = protein kinase C phosphorylation site; PPT = polypurine tract. * 2 sequences of late time point are depicted, since both genotypes were representative for 50% of biological virus clones analyzed (n = 4).
and AIDS diagnosis: 117, 82, 97, and 66 CD4 T cells/μl/year for P1, P2, L3, and P8, respectively).

Together, these data show that some LTS maintain high and relatively stable CD4 T cell counts for an extended period of time in the presence of high numbers of infected cells and relatively high levels of viral RNA in serum.

**Nef sequence analysis.** It has previously been demonstrated that some LTS harbor HIV-1 variants with a defective nef gene\(^{[470-473]}\). We analyzed whether the slow replication kinetics of viruses studied here also resulted from the presence of defective nef genes. Nef sequences of the slow-replicating biological virus clones of L1-L4 were analyzed. In addition, nef sequences of both slow- and rapid-replicating virus clones of L6 and of progressors P1 - P3 were analyzed (Fig. 4).

Intersubject variation in nef sequences was observed, yet previously described functional domains\(^{[475]}\) were generally intact. No specific mutations could be related to the viruses from either progressors or LTS nor to slow- or rapid-replicating viruses. In 4 subjects, the majority of viruses had an insertion of 4-aa at position 25. However, this insertion was observed in viruses with different phenotypes. Mutations in the (PxxP)\(_3\) domain (proline repeat sequence; positions 69 to 78) were observed in all viruses of L1 and in all late viruses of L2. The R-to-K substitution at position 71 in this domain has been described previously for variants from LTS\(^{[475]}\), although the nef sequences of these particular variants were shown to be functional in vitro\(^{[476]}\). All viruses of L3 had a cysteine at position 138, recently described to affect replication capacity in H9 cells\(^{[472]}\). However, this same mutation was also present in the nef of the rapid-replicating viruses isolated from the typical progressor P2 at the moment of AIDS diagnosis.

Possible dysfunctional nef genes were observed in the viruses with slow kinetics of L4, which had a 4-aa deletion at positions 156 to 159. This deletion was present in at least one of the viruses isolated from PBMC in 1989 and was still present in all 3 viruses isolated from PBMC in 1992.

**DISCUSSION**

A small number of HIV-infected persons harboring solely NSI variants show no signs of progression to disease. However, 50% of all AIDS patients also harbor solely NSI variants. The absence of SI variants therefore cannot be the sole explanation for absence of disease progression. Next to a role for host factors\(^{[479,480,462,463,465-467,468]}\), viral characteristics other than syncytium-inducing capacity might contribute to the differential clinical course observed between HIV-1-infected persons. Here we tested the hypothesis that NSI variants present in LTS may have relatively attenuated replication capacity and are unable to achieve a high virus load in vivo, thereby explaining at least in part the beneficial clinical course observed in LTS.

Biological virus clones were isolated from 7 LTS and 8 subjects with progressive HIV-1 infection and analyzed for their replicative potential. Early in infection, all participants analyzed had relatively slow-replicating viruses. Two LTS who had the highest infectious cellular load at the early point of analysis additionally had rapid-replicating variants at this time point.

At the time point late in infection, still only relatively slow-replicating viruses could be detected in 4 LTS. Accordingly, these LTS maintained low levels of RNA in serum as well as a low infectious cellular load. This is in agreement with the recently described low levels of viral mRNA transcripts late in infection in LTS\(^{[336]}\). In contrast, from the other 3 LTS and the progressors late in infection, either solely rapid-replicating viruses or co-existing rapid- and relatively slow-replicating viruses were detected. These persons all had an increased virus load, suggestive of a correlation between in vitro HIV-1 replicative capacity and virus load in vivo.

Increasing cellular load as well as RNA load has been associated with CD4 T cell decline and disease progression\(^{[25,28,28,208,261-264,462,463]}\). It is therefore conceivable that the high load in 3 of the LTS is a sign of progression. However, these 3 LTS maintained high and relatively stable CD4 T cell counts for a subsequent 43 to 70 months, suggesting that some LTS remain healthy for prolonged periods of time despite the presence of rapid-replicating viruses and a high virus load. In support of this, Rump et al.\(^{[477]}\) recently reported the existence of LTS with continuously high virus
load as measured by serum p24 antigen(477).

The maintenance of relatively stable CD4+ T cell numbers in the face of increasing infectious load can have several explanations. In agreement with the low frequencies of infected cells in lymph nodes(337), the magnitude of CD4+ T cell turn-over seems to be much smaller(358) than previously suggested(23,24). The loss of CD4+ T cells might than be explained from a combination of HIV-1-mediated killing and a failing homeostasis. The capacity by which new T cells can be generated might be genetically determined, thereby contributing to differences in CD4+ T cell decline between individuals.

Additionally, the low degree of CD4+ T cell depletion might be due to the absence of an effective anti-HIV CTL response(478). In support of this, only low frequencies of CTL precursors directed against epitopes of multiple HIV-1 proteins have been observed in participant L6 (Pontesilli O, personal communication).

Finally, since viral cytopathic capacity may be a mechanism through which CD4+ T cells are depleted(90), it could be envisioned that increased viral replicative capacity, which causes the increase in virus load, is not necessarily associated with increased cytopathicity of these viruses. Indeed, viruses isolated from persons with differing progression rates yet similar virus load were found to be differentially cytopathic in a SCID-hu mouse model(479).

After 6.6 (L4) or even >9 years of infection, 4 LTS still harbored slow-replicating variants. To explain the absence of evolution to variants with a more rapid phenotype, several hypotheses might be envisioned. First, viruses are under selective pressure by host cellular immune responses. The persisting slow-replicating viruses in our study subjects may be best adapted to withstand cellular immunity, whereas changes associated with more rapid replicative capacity may generate epitopes that are well-recognized by CTL, causing immediate elimination of that variant. It has been postulated previously that the presence of CTL directed against conserved epitopes prevents the evolution of virus populations(209). In this view, the slow-replicating variants may have mutations in relatively conserved sequences, which allow them to escape immune surveillance, yet at the same time result in severe attenuation.

Next to specific immune pressure, viral characteristics may play a role in virus evolution. It is conceivable that the virus variants that establish infection in a particular person, compared with the initial variants in another person, are genetically closer to a genotype that is associated with more rapid replication. In this case, it would be just a matter of time to allow accumulation of relevant mutations(207). Finally, slow replication kinetics might be due to aberrations in HIV-1 regulatory genes(480,481).

The best-studied in this respect is the nef gene. The absence of functional nef was associated with a lack of viral pathogenicity in the SCID-hu mouse model(482) and in simian immunodeficiency virus strain mac-infected rhesus monkeys(483). This is in agreement with the observation that nef is required for efficient replication in primary cells(179-181). More recently, nef was associated with HIV-1 pathogenesis in humans by the finding that some LTS have HIV-1 variants with defective nef genes(470-473).

Some of the slow-replicating viruses in our study carried changes in nef. Some of these changes have been observed before in LTS(477) but were not associated with attenuated function in vitro(476). It has been suggested that a cysteine residue at position 138 may attenuate nef function(472). However, the presence of a cysteine at this position in nef of slow- as well as rapid-replicating viruses in our study supports the finding that this mutation is irrelevant for nef function(476). With the exception of L4, who had viruses with a relatively large deletion in the nef gene, it seems unlikely that deviations in nef contribute to the absence of disease progression in the group of LTS studied here.

In conclusion, we have shown that some persons classified as LTS persistently harbored slow-replicating viruses associated with a low virus load, which might explain the absence of progression in these persons. The existence of LTS with rapidly-replicating viruses and a high virus load, however, suggests that the basis for long-term survival of HIV-1 infection differs between persons. In this light, it could be hypothesized that different yet specific combinations of several favorable conditions may result in the absence of disease progression.
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Next to lymphocytic lymphoma/leukemia, viral
characteristics also play a role in tumor evolution
of T-cell neoplasms. The impact of viral infection
on the development of these tumors may vary,
depending on the tropism of the virus in host tissues.
Published studies indicate that the virus type may
influence the tumor progression, with some viruses
appearing to be more closely associated with
lymphoma development than others.

After 6 to 8 (10) years of infection, a
LTS still harbors non-replicating variants. To
explain the absence of evidence in mice with a
more rapid phenotype, several hypotheses might
cannot be dismissed. First, viruses are under selective
pressure by host cellular immune responses. The
non-replicating variants in our study
subject may be less equipped to elude host
immune responses, whereas changes associated
with more rapid replicative capacity may generate
epitopes that are well-recognized by CTLs, causing
dominant immune selection of that variant. It has been
published previously that the presence of CTLs
directed against conserved epitopes prevents the
expression of viral populations. In this view, the
non-replicating variants may have mutations in
a relatively conserved sequence, which allow them
to escape immune surveillance, yet at the same
time result in severe attenuation.

The best-studied T-cell lymphoma is the
malignant T-cell leukemia/lymphoma. The incidence of
T-cell lymphoma in adults with HIV infection in the
United States, Europe, and Asia has increased over
time. The global increase in HIV incidence is
associated with an increasing burden of
malignant T-cell lymphoma. In developed countries
and in high-income countries, the incidence of
malignant T-cell lymphoma is higher than in low-income
countries. In high-income countries, a significant
difference in the incidence of malignant T-cell
lymphoma exists between men and women. In
developed countries, a significant difference in the
incidence of malignant T-cell lymphoma exists between
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