In 7 long-term survivors (LTS) and 8 progressors, all carrying solely non-syncytium-inducing 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 virus load, even after 6.6–9 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 non-syncytium-inducing 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.

Some human immunodeficiency virus type 1 (HIV-1)–infected persons maintain high and stable CD4 T cell counts and normal T cell function and remain free of clinical symptoms for prolonged periods of time [1]. Concomitantly, these persons, classified as long-term survivors of HIV-1 infection (LTS), have lower virus load than do persons with progressive disease [2–4].

The absence of progression in LTS may be determined by an interplay between host factors and viral factors. Both humoral and cellular immune responses have been implicated in long-term survival. In LTS, high titers of antibodies with neutralizing activity have been detected [2, 3], although other studies reported no differences in neutralizing capacity between antibodies derived from LTS and progressors [4, 5]. Strong and persistent cytotoxic T lymphocyte (CTL) responses against several HIV-1 epitopes have been detected in LTS; however, vigorous CTL responses are also readily detected during the asymptomatic phase in progressors [6–8].

Next to the immune response, host genetic factors may influence the length of the asymptomatic period. Several combinations of histocompatibility complex genes [9] are associated with the length of the asymptomatic period. Also, heterozygosity for a 32-bp deletion in the gene of one of the cofactors for HIV-1 entry, the C-C chemokine receptor 5 [10–13], was associated with longer AIDS-free survival [14, 15].

Viral characteristics, such as cytotropism, cytopathicity, and replicative capacity, have been associated with different stages of infection, suggesting an important role for HIV-1 phenotype in the clinical course of HIV infection [16–23]. On sexual, vertical, and parenteral transmission, infection seems to be initiated by macrophage-tropic variants [18, 24], while a shift from preferentially macrophage-tropic to preferentially T cell–tropic HIV-1 populations is correlated with progression to disease [19]. In 50% of persons, this shift is associated with the emergence of syncytium-inducing (SI) variants [25]. Persons developing SI variants show an increased virus load, accelerated CD4 T cell loss, and rapid progression to AIDS compared with persons harboring solely non-syncytium-inducing (NSI) variants [21, 25–27].

LTS in general and 50% of all persons who do progress to AIDS carry solely NSI variants, suggesting that other viral characteristics might contribute to the differences in the clinical course of infection. Indeed, replication capacity of HIV-1 has been shown to be associated with virus load, CD4 T cell decline, and disease progression [16, 17, 21, 23]. In these studies, however, the rapid replication was associated with the SI phenotype or the phenotype was not determined. Whether differences in replicative capacity among NSI variants might account for the differences in the course of infection between persons harboring solely NSI variants had not been studied before.

We hypothesized that NSI variants in LTS, in contrast to NSI variants in persons who 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 biologic 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 [28–31], the nef sequences of the biologic 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/mm^3). Additionally, these persons harbored solely NSI variants and did not receive any anti-HIV drug treatment during the course of infection. These persons were termed LTS [1]. 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 43–76 months of follow-up: ACH424 [P1], ACH537 [P4], ACH53 [P5]), 4 were typical progressors (AIDS diagnosis after 99–109 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: ACH617 [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 described previously [19, 27]. Brieﬂy, participant PBMC (0.5–10^4 cells/well in 48 or 96 wells) were cocultivated with phytohemagglutinin (PHA)-stimulated healthy donor PBMC (10^5/mm^3). Additionally, these persons harbored solely NSI variants and did not receive any anti-HIV drug treatment during the course of infection. These persons were termed LTS [1]. Seven of these 12 LTS were studied here (ACH78 [L1], ACH441 [L2], ACH583 [L3], ACH709 [L4], ACH68 [L5], ACH337 [L6], ACH750 [L7]).

Table 1. Characteristics of subjects in HIV-1 study.

<table>
<thead>
<tr>
<th>Participant</th>
<th>Date of entry or follow-up</th>
<th>Disease stage by end of follow-up (months after seroconversion or entry)</th>
<th>Early sample</th>
<th>Late sample</th>
<th>Interval between samples (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>+ 8 Nov 84</td>
<td>Asymptomatic (124)</td>
<td>17 2</td>
<td>115 5</td>
<td>98</td>
</tr>
<tr>
<td>L2</td>
<td>+ 21 Jan 85</td>
<td>Asymptomatic (149)</td>
<td>16 3</td>
<td>111 6</td>
<td>95</td>
</tr>
<tr>
<td>L3</td>
<td>+ 18 Feb 85</td>
<td>Asymptomatic (138)</td>
<td>24 3</td>
<td>109 5</td>
<td>85</td>
</tr>
<tr>
<td>L5</td>
<td>- 14 Aug 85</td>
<td>Asymptomatic (122)</td>
<td>43 2</td>
<td>80 3</td>
<td>37</td>
</tr>
<tr>
<td>L6</td>
<td>+ 6 Nov 84</td>
<td>Asymptomatic (143)</td>
<td>18 1</td>
<td>100 5</td>
<td>82</td>
</tr>
<tr>
<td>L7</td>
<td>+ 23 Mar 85</td>
<td>Asymptomatic (138)</td>
<td>24 4</td>
<td>113 5</td>
<td>89</td>
</tr>
<tr>
<td>P1</td>
<td>- 14 Mar 88</td>
<td>AIDS (43)</td>
<td>16 5</td>
<td>112 5</td>
<td>96</td>
</tr>
<tr>
<td>P2</td>
<td>+ 25 Oct 84</td>
<td>AIDS (99)</td>
<td>24 5</td>
<td>98 5</td>
<td>74</td>
</tr>
<tr>
<td>P3</td>
<td>+ 27 Feb 85</td>
<td>AIDS (136)</td>
<td>15 2</td>
<td>111 5</td>
<td>96</td>
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<tr>
<td>P4</td>
<td>- 14 Jul 86</td>
<td>AIDS (43)</td>
<td>NA NA</td>
<td>37 4</td>
<td>NA</td>
</tr>
<tr>
<td>P5</td>
<td>+ 5 Nov 84</td>
<td>AIDS (76)</td>
<td>NA NA</td>
<td>77 6</td>
<td>NA</td>
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<tr>
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<td>+ 24 Nov 84</td>
<td>AIDS (100)</td>
<td>NA NA</td>
<td>102 5</td>
<td>NA</td>
</tr>
<tr>
<td>P7</td>
<td>- 22 Sep 85</td>
<td>AIDS (101)</td>
<td>NA NA</td>
<td>94 6</td>
<td>NA</td>
</tr>
<tr>
<td>P8</td>
<td>+ 22 Nov 84</td>
<td>AIDS (109)</td>
<td>NA NA</td>
<td>93 6</td>
<td>NA</td>
</tr>
</tbody>
</table>

NOTE. NA, not applicable.

* Long-term survivors (L) or progressor to AIDS (P).

End of follow-up is defined as moment of AIDS diagnosis for progressors and July 1997/start of therapy for LTS.
PBMC, MT2 cells (1 × 10^6) were added to analyze syncytium-inducing capacity of the virus clones [33].

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, Netherlands). One hundred microliters of serum was added to 900 μL of lysis buffer containing guanidinethiocyanate, 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 [34].
Figure 1. Replication kinetics of biologic virus clones. Healthy donor peripheral blood mononuclear cells that had been stimulated with phytohemagglutinin were infected with 1250 TCID$_{50}$ of biologic virus clones obtained from LTS (A) and disease progressors (B) early (I) and late (II) in infection. Cultures were maintained for 16 days, and new target cells were added on day 8. p24 production in supernatant harvested every second day was measured. Replication phenotype (C) was based on mean values for increase in p24 production during days 4 and 8 of culture and maximal p24 production reached within 14 days of culture. Open symbols, slow-replicating; solid symbols, rapid-replicating; shaded symbols, intermediate.

Subsequently, RNA was isolated as previously described [4]. 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′-ACTTTTTTTGAACCTGATATGTATG-TGCCATATCAGCATAATCTAA-3′; antisense; T7-RNA polymerase recognition site underlined), by avian myeloblastosis virus reverse transcriptase (AMV-RT). Extension is followed by degradation of the template RNAs by RNase H, synthesis of the second DNA strand through extension of primer 2 (nt 569–590: 5′-AGTGAGGGGAGCATCAAGCCATGAAA-3′) by AMV-RT, and cyclic and isothermal (41°C for 90 min) RNA synthesis by T7-RNA polymerase.

Amplificates were hybridized with an HIV-1–specific bead-oligo (i.e., a biotin-oligo bound to streptavidin-coated magnetic beads acting as solid phase) and ruthenium-labeled probes, each specific for the wild type and control amplificates. Magnetic beads carrying the hybridized ampliticate-probe complex are captured on the surface of an electrode by means of a magnet. Voltage applied to this electrode triggers the electrochemoluminescence reaction, and the light emitted 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, 1–5 biologic 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 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 healthy donor PBMC.

From each virus clone, 1250 TCID$_{50}$ was added to 5 × 10$^6$ PHA-stimulated PBMC derived from the same donor on which the TCID$_{50}$ was determined. To keep the volume of the inocula from exceeding 1.25 mL, virus stocks with <10$^3$ TCID$_{50}$/mL were excluded from replication analysis. Virus and PBMC were incubated in a 1.5-mL volume for 2 h at 37°C. PBMC were then washed, resuspended in 5 mL of fresh recombinant interleukin-2 (20 U/mL; PROLEUKIN; Chiron, Amsterdam)–supplemented medium, and cultured for 16 days.

To determine the kinetics of virus production, 75 µL of the culture supernatant was harvested every second day and stored at 4°C until analysis. At day 8 of culture, 4 × 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 virus in an in-house p24 antigen–capture ELISA [32]. p24 production per milliliter of supernatant was determined and corrected for the differences in volume of culture supernatants between the moments of sampling.

 nef sequence analysis. From 10$^5$ infected PBMC, obtained after propagation of the clonal virus stocks, proviral DNA was isolated. PBMC were lysed in L6 lysis buffer (0.08 M GuSCN [Life Technologies Gibco BRL, Gaithersburg, MD], 0.08 M Tris-CI, pH 6.4, 0.035 M EDTA, 2% (wt/vol) Triton X-100 [35]) 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′-GTCTAGACTAAAGAATAGTG-3′, sense orientation) and LTR BCAT (nt 519–545 of the LTR sequence: 5′-GAACCTAAGCAGGATATTTGAGCC-3′, antisense) in the first reaction and primers Nef B (nt 2526–2546 of the env sequence: 5′-ATCTAGAAGATAAGACGAGC-GAAGTCCTCC-3′, sense) and Nef C (nt 350–370 of the LTR sequence: 5′-AAGTCTAGGCG-GAAATCCC-3′, antisense) for the second reaction.

For both reactions, DNA was denatured for 5 min at 95°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 overnight at 4°C. Five microliters of DNA was amplified in 50-µL reactions containing 1× Taq buffer (Promega, Madison, WI), 0.2 mM each dNTP, 100 ng 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 (Qiagen, Chatsworth, CA).

The positive strands were sequenced by use of Sequenase DNA polymerase (United States Biochemicals, Cleveland) with primers Nef B, LTR 1A (nt 412–438 of the nef sequence: 5′-AGATATTCCACTGACCTTTGGATGGTCG-3′), and Nef 1C (nt 96–126 of the nef sequence: 5′-AGCATCTCGAGACCTTGGAATAACATGGAGC-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 Spearman’s correlation coefficient.

**Results**

**Correlation between HIV-1 replication characteristics and clinical course.** Biologic virus clones of 7 LTS and 3 progressors, obtained from PBMC that originated from an early and a late time point during follow-up (table 1), were analyzed with respect to their replicative capacity (figure 1A, 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 (figure 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, 0.5 μg/mL and 1.6 μg/mL, respectively); variants with both measures below average were typed slow (lower left 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 quadrants; mean values, 0.2 μg/mL and 1.2 μg/mL). The mean values of both measures were significantly different between the 3 phenotypically distinct groups (P < .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 coexisted.

**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 (figure 2).

Early in infection, all participants had a low cellular virus load (1–16 TCID/10⁶ 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 capaci-

![Figure 2](https://example.com/figure2.png)
ity were detected had the highest infectious cellular load (figure 2A).

Because of the low virus load, <5 biologic clones were obtained at the early time point for most persons (table 1). From 3 of these (L4, L5, 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 (1–29 TCID/10⁶ 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 (83–288 TCID/10⁶ CD4 T cells) (figure 2B).

We additionally analyzed the replicative capacity of biologic 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 NS1 variants, is given in table 2. For comparison of replicative capacity, slow- (n = 7) and rapidly-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 2–6 of culture, the slow-replicating control viruses did not produce detectable p24 levels until days 10–14 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).

**Figure 3.** Longitudinal analysis of CD4 T cell counts and virus load. Nos. of CD4 T cells (●) were routinely measured every 3 months during follow-up. Infectious cellular load (●) was calculated from outcome of clonal isolation procedure and is expressed as TCID/10⁶ CD4 T cells. HIV-1 RNA copy numbers (○) were determined by nucleic acid sequence–based amplification assay. Follow-up is indicated in months after HIV-1 seroconversion or seropositive entry in cohort studies. Arrowheads on x-axis indicate time points from which biologic virus clones analyzed for replicative capacity were obtained.
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 \( n = 2 \) vs. 2.5 \( n = 8 \); \( P = .04 \); late median load, 171 \( n = 10 \) vs. 27 \( n = 5 \); \( P < .001 \)).

**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 [36], the log-transformed values of both measures of virus load were significantly correlated (Spearman correlation coefficient = .76, \( P = .002 \)). 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 (figure 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 (decline 41, 13, and 1 CD4 T cell/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 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 [28–31]. 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 biologic 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 (figure 4).

Intersubject variation in nef sequences was observed, yet previously described functional domains [37] 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) domain (proline repeat sequence; position 69–78) were observed in 4 subjects, with an insertion of 4 aa at position 25. This same mutation was also present in L3 and L4. 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).

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

<table>
<thead>
<tr>
<th>Participant</th>
<th>No. of biologic 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>P4</td>
<td>4</td>
<td>Slow 0</td>
<td>4 (100)</td>
</tr>
<tr>
<td>P5</td>
<td>6</td>
<td>Intermediate 1 (16.67)</td>
<td>4 (66.67)</td>
</tr>
<tr>
<td>P6</td>
<td>5</td>
<td>Rapid 1 (20)</td>
<td>3 (60)</td>
</tr>
<tr>
<td>P7</td>
<td>6</td>
<td>0</td>
<td>3 (50)</td>
</tr>
<tr>
<td>P8</td>
<td>6</td>
<td>0</td>
<td>1 (16.67)</td>
</tr>
</tbody>
</table>

0.001).

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.

**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
**Figure 4.** *nef* consensus sequences of biologic 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 [37] 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 biologic virus clones analyzed (n = 4).
factors [2, 6–8, 14, 15], viral characteristics other than syncytiun-inducing capacity might contribute to the differential clinical course observed between HIV-1–infected persons. Here we tested the hypothesis that NS1 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.

Biologic 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 harbored slow-replicating virus strains were found to be continuously infected beyond 43–70 months, suggesting that some LTS of functional virus load were found to be differentially cytopathic in a SCID-hu mouse model [51].

After 6.6 (L4) or even >9 years of infection, 4 LTS still harbored slow-replicating variants. To explain the absence of progression 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 [52]. In this view, the slow-replicating variants may have mutations in relatively conserved sequences that 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 [53]. Finally, low replication kinetics might be due to aberrations in HIV-1 regulatory genes [54, 55].

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 [56] and in simian immunodeficiency virus strain mac–infected rhesus monkeys [57]. This is in agreement with the observation that nef is required for efficient replication in primary cells [58–60]. 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 [28–31].

Some of the slow-replicating viruses in our study carried changes in nef. Some of these changes have been observed before in LTS [37] but were not associated with attenuated function in vitro [38]. It has been suggested that a cysteine residue at position 138 may attenuate nef function [30]. 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 [38]. 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
rapid-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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