To clarify the frequency and prognostic significance of a plasma human immunodeficiency virus (HIV) RNA load below the detection threshold during the natural history of infection, an ultrasensitive assay was used to identify persons with low virus loads in a cohort of 111 untreated subjects with a known date of seroconversion. Six persons had HIV RNA loads <40 viral copies (VC)/mL during the first years of HIV infection. The probability of meeting the criteria for long-term nonprogression was higher in these subjects (P = .043). However, a virus load <40 VC/mL was rare during the natural history of infection, even during the first years of symptomless HIV carriage. Such data confirm the general trend of disease progression in the entire population of HIV carriers.

Because human immunodeficiency virus (HIV) replicates actively and progressively in lymphoid tissue during the symptomless HIV carriage period, measurement of plasma viral RNA load plays a prominent role in the management of HIV-infected persons. Because this parameter is considered to be the most accurate predictor of disease progression, a high virus load is the primary indication for the onset of antiviral therapy in symptomless HIV-infected persons.

Another reason for assessing the plasma HIV RNA load is to determine the efficacy of antiretroviral drugs, as the purpose of this therapy is to reduce the virus load below the detection threshold of the quantitative assay for the longest possible period. Until recently, the quantitation limit by commercial assays was 400 or 500 viral copies (VC)/mL of plasma [1–3]. New molecular biology procedures can detect low virus loads, with a quantitation limit of 20 [4], 40 [5], or 50 VC/mL, depending on the assay used [6]. Published studies based on these new technologies have shown that antiviral drugs (highly active antiretroviral therapy or tritherapy) may decrease the HIV RNA load below this detection threshold in some persons [7]. However, the frequency and significance of such a low virus load in untreated subjects is unknown. Present knowledge about the natural history of HIV infection suggests that such a low level of HIV RNA load should be observed during the first years of infection, when the viral RNA concentration is stabilized around a steady-state value under the pressure of antiviral immune response following the primary infection, especially in symptomless persons maintaining a steady CD4 T cell count for ≥10 years without antiviral therapy intervention (termed long-term nonprogressors [LTNPs]) [8–10].

To clarify the frequency and prognosis significance of a plasma HIV RNA load <40 VC/mL during the natural history of infection, we used an ultrasensitive assay to identify persons with such low virus loads in a cohort of untreated subjects with a precisely defined date of seroconversion.

**Patients and Methods**

**Study population.** The 111 subjects in the study have been prospectively followed in our outpatient clinic through regular visits since the discovery of their seropositivity (mainly diagnosed through biologic screening of blood donations). They were recruited from a larger cohort of HIV-infected persons if they had a documented date of seroconversion determined through a negative assay within the 6 months preceding the first positive assay at enrollment. The date of seroconversion was estimated as the midpoint between the most recent negative and first positive test. A clinical primary infection, even well documented, or a known period of exposure to infection was not retained as criteria for inclusion. In all subjects, the diagnosis of HIV infection was made by ELISA (Diagnostics Pasteur, Marnes-La-Coquette, France; Abbott Laboratories, Rungis, France), with confirmation by Western blot (Diagnostics Pasteur). At each visit, each person had a physical examination to determine clinical status (according to Centers for Disease Control [CDC] criteria) and provided blood specimens for laboratory evaluation. This cohort of subjects with a documented date of HIV infection was followed a mean of 8.4 years (range, 3–14).

**Methods.** We determined plasma HIV RNA load by NucliSens HIV type I (HIV-1) quantitative assay, a second-generation assay
for HIV-1 RNA quantitation based on nucleic acid sequence amplification (NASBA; Organon Teknika, Boxtel, The Netherlands) with improved sensibility. The assay was done according to the manufacturer's instructions [5]. Results were expressed as VC per milliliter of plasma. The quantitation limit was 40 VC/mL [5].

The 111 HIV-infected subjects had been screened annually [11] by analysis of blood samples collected throughout the follow-up period and assayed by first-generation NASBA HIV-1 QT for which the quantitation limit was 400 VC/mL [1]. All persons with <400 VC/mL (without any antiviral therapy) at more than one visit were selected for the present study (we did not retain subjects who had <400 VC/mL for only one visit so that we could consider the prognosis value of the phenomena only with a significant duration). In this highly selected group, all samples containing <400 HIV RNA VC/mL were tested by the ultrasensitive quantitation assay on a new plasma sample stored at –80°C. For the same reason, only subjects with <40 VC/mL (without receiving any antiviral therapy) at more than one visit were considered.

Among persons who had plasma HIV RNA loads of <400 VC/mL on at least two visits, the mean time during which the plasma HIV RNA load was <400 VC/mL was compared between two subgroups: persons with <40 VC/mL for at least two visits and those without. The same two subgroups were also compared for a 50% loss in CD4 T cells, a CD4 T cell count of <200/mm³, progression to stage B or C (by CDC classification), start of treatment, and decease. We also looked for long-term nonprogression (a steady CD4 T cell count during ≥10 years of infection without any antiviral therapy) in the two subgroups.

Statistical analysis. Results were expressed as mean or median (range). The relationship between categorical variables was tested by χ²- or Fisher’s exact tests; quantitative data were compared by Mann-Whitney nonparametric test. All time-dependant parameters were estimated by the Kaplan-Meier method, and curves were compared by log rank test.

Results

Among the 111 persons with a precise date of seroconversion, 17 (15.3%) had an HIV RNA load of <400 VC/mL on at least two visits. Of these 17 persons, 6 (35.3%) had an HIV RNA load of <40 VC/mL on at least two visits (5.4% of the whole cohort, table 1). The mean period during which these 6 patients had an HIV RNA load of <40 VC/mL was 3.3 years (range, 2–5). Figure 1 shows the HIV RNA load over time as determined by the 400 VC/mL and 40 VC/mL assays in the 6 subjects with <40 VC/mL. Beyond year 6 of infection, none of the 6 subjects had <40 VC/mL unless they received antiviral therapy. Of the 17 persons with <400 VC/mL, the HIV RNA load was <400 VC/mL in the 6 persons with <40 VC/mL and in the 11 other subjects, for a mean of 7.0 (range, 2–12) and 3.9 years (range, 1–8), respectively (P = .15). During follow-up of the same 17 persons, a loss of 50% of CD4 T cells was observed in 3/6 subjects with <40 VC/mL versus 4/11 other subjects (P = .9). Among the same 17 persons, a CD4 T cell count <200/mm³ was observed in 1/6 persons with <40 VC/mL versus 1/11 other subjects (P = .8).

At year 10 of follow-up, 5/6 subjects with <40 VC/mL versus 3/11 other subjects were considered LTNPs (P = .043). At the end of the follow-up, 1/6 persons with <40 VC/mL versus 1/11 other subjects had progressed to CDC stage B or C (P = .5). At the same time, 3/6 subjects with <40 VC/mL versus 7/11 other subjects received antiretroviral therapy (P = .12). None of the 6 persons with <40 VC/mL had died, versus 1/11 other subjects (P = .65).

Discussion

The major finding of the study is that a virus load <400 VC/mL is rare during the natural history of HIV infection, even during the first years of symptomless HIV carriage. In our series, only a small proportion of persons had a virus load below the limit of the ultrasensitive quantitative assay, and none had such a value over the entire follow-up period, even persons who met criteria for long-term nonprogression. These data are in accordance with the observation that HIV replicates actively and progressively in lymphoid tissue during the whole symptomless period of infection and confirms the general biologic trend of disease progression in the entire population of HIV carriers. Thus, if a plasma HIV RNA load <40 VC/mL were to be included in the criteria for long-term nonprogression, the proportion of true LTNPs would strongly decrease beyond the first years of infection. In our series of 111 subjects with a known date of seroconversion, this proportion would be zero. However, the probability of presenting the classical criteria of long-term nonprogression was higher in subjects with an HIV load of <40 VC/mL.

Table 1. Main characteristics of the 6 human immunodeficiency virus (HIV)-infected persons with plasma HIV RNA loads below the detection limit of 40 viral copies/mL on at least two visits.

<table>
<thead>
<tr>
<th>Patient, sex</th>
<th>Age at HIV seroconversion (years)</th>
<th>Risk factor for HIV infection</th>
<th>No. of years of HIV infection</th>
<th>CD4 cell count at 1st and last year of follow-up (mm³)</th>
<th>Criteria for long-term nonprogression at end of follow-up period</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, M</td>
<td>25</td>
<td>Homosexual</td>
<td>13</td>
<td>213–309</td>
<td>Yes</td>
</tr>
<tr>
<td>2, M</td>
<td>29</td>
<td>Homosexual</td>
<td>12</td>
<td>470–437</td>
<td>Yes</td>
</tr>
<tr>
<td>3, F</td>
<td>21</td>
<td>Heterosexual contact</td>
<td>13</td>
<td>623–700</td>
<td>Yes</td>
</tr>
<tr>
<td>4, F</td>
<td>26</td>
<td>Intravenous drug user</td>
<td>10</td>
<td>627–513</td>
<td>Yes</td>
</tr>
<tr>
<td>5, M</td>
<td>40</td>
<td>Homosexual</td>
<td>12</td>
<td>710–815</td>
<td>Yes</td>
</tr>
<tr>
<td>6, M</td>
<td>28</td>
<td>Homosexual</td>
<td>7</td>
<td>549–209</td>
<td>No</td>
</tr>
</tbody>
</table>

CD4 T cell count >500/mm³ after ≥10-year period of HIV infection without any antiviral therapy.
RNA load <40 VC/mL than in the other subjects. Again, it appears that the LTNPs only represent the tail of a continuous distribution of latency periods of the disease.

By maintaining an HIV RNA load <40 VC/mL over several years in symptomless or asymptomatic patients with a previously high level of viral replication, the drugs induce a situation that is rarely observed during the natural history of infection. Thus, even if the viral replication resists therapy, drug therapy appears to be more efficient than the antiviral immune system in suppression of plasma virus load. Indeed, the more the virus load is lowered under treatment, the more the antiviral effect of drugs on the virus load is durable [12]. Moreover, persons with an HIV RNA load <20 VC/mL during treatment had a better prognosis than persons with virus loads 20–400 VC/mL during therapy [12]. In our study, persons who had <40 VC/mL on at least two visits had longer periods during which their HIV RNA loads were <400 VC/mL. This indicates that there is a correlation between a low virus load and the duration of the virus load at this level.

In conclusion, the subjects who had HIV RNA loads below the limit of the ultrasensitive quantitation assay more frequently met the criteria for long-term nonprogression for the ensuing years. This may constitute an argument for initiation of antiretroviral therapy in persons with any HIV RNA load >40 VC/mL.

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References

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