Quantitation of Human Immunodeficiency Virus Provirus and Circulating Virus: Relationship with Immunologic Parameters

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Virologic and seroimmunologic parameters were determined in 56 persons infected with human immunodeficiency virus (HIV). The provirus level varied from 10 to $100,000/10^6$ $CD4^+$ lymphocytes, and genomic HIV RNA was detectable in 39 of 56 patients at a relative concentration varying from 10 to >250 copies/mL of serum. Provirus expressed as copies per 10^6 $CD4^+$ lymphocytes and as circulating virus per milliliter of serum increased with disease progression and decrease of $CD4^+$ cell concentration. The mean provirus concentration expressed per milliliter of blood varied little among categories of patients with various levels of $CD4^+$ cells, but there was a progressive increase of circulating HIV genomic RNA. These virologic data suggest that during the course of HIV infection, an increasing proportion of the remaining $CD4^+$ lymphocytes harbor the HIV genome and produce infectious virus. Finally, there was a marked correlation between increased provirus and genomic RNA concentration and three seroimmunologic markers: decrease in $CD4^+$ cell count, p24 antigenemia, and disappearance of antibodies to HIV core antigen.

Human immunodeficiency virus (HIV) induces a persistent infection that progresses slowly from asymptomatic infection to AIDS. Antiretroviral therapy may delay progression. The identification of factors that correlate with progression or regression of HIV infection is important for prognosis and for the monitoring of treatment.

$CD4^+$ lymphocytes are the main target of HIV infection [1], and the decline in $CD4^+$ cell concentration is the best predictive factor for progression to AIDS. Other markers with predictive value are p24 antigenemia, antibodies to HIV core antigen (anti-core), and serum levels of neopterin and β_2-microglobulin [2, 3]. Previous studies [4–6] have attempted to detect HIV itself in plasma and in peripheral blood mononuclear cells (PBMC) and demonstrated, using appropriate coculture techniques, that viremia is present in most infected patients and that the virus load increases with disease progression. In clinical trials, the biologic effects of antiviral therapy are assessed mainly by counting $CD4^+$ lymphocytes and by measuring β_2-microglobulin and p24 antigenemia [7–9]. Quantitative determination of infectious HIV in PBMC and plasma is expensive and technically demanding; it has therefore been done in only a few investigations, in which viremia usually decreased after zidovudine therapy [4, 5].

The polymerase chain reaction (PCR) offers a simple method to quantify proviral HIV DNA and genomic HIV RNA. The aim of this study was to determine virologic parameters in a large group of patients with various clinical presentations and to analyze the relationship between virologic parameters, such as provirus and circulating virus, and seroimmunologic parameters.

Patients and Methods

Study population. Fifty men and 6 women with serum antibodies to HIV-1 participated. Eleven were asymptomatic (Centers for Disease Control [CDC] stage II); 21 had persistent generalized lymphadenopathy (CDC stage III); 12 had CDC stage IV disease (10, IV-C-2; 2, IV-D, analyzed together); and 12 had CDC stage IV-C-1 disease [10]. The mean $CD4^+$ lymphocyte counts in these four subgroups were 497, 481, 254, and 45/μL, respectively. Negative control samples were collected from 20 blood donors with no antibodies to HIV-1. A number of participants were on zidovudine treatment: 2 with CDC stage II disease (55 and 33 days), 1 with stage III (165 days), 3 with stage IV-C-2 or IV-D (48, 65, and 766 days), and 7 with stage IV-C-1 (83, 120, 139, 308, 386, 572, and 659 days).

Seroimmunologic data. EDTA-treated blood (10–20 mL) and native blood (10 mL) were collected from the participants by venipuncture and processed within 4 h. Native blood samples were centrifuged at 1500 g for 10 min, and sera were aliquoted and frozen at −70°C. Complete blood count was done, and the absolute concentrations of CD3, CD4, and CD8 lymphocytes were determined by flow cytometry (Coulter EPICS IV; Instrumente Gesellschaft, Basel, Switzerland) using fluoresceinated OKT3, OKT4a, and OKT8 monoclonal antibodies (Orthomune; Ortho Diagnostics, Raritan, NJ). HIV-1–specific antibod-

ies were determined by EIAs (Abbott recombinant HIV-1/HIV-2 EIA and Envacor, Abbott Laboratories, Abbott Park, IL) and Western blot (Du Pont, Geneva). Levels of p24 antigen were measured in duplicate, and positive samples were neutralized with antibodies to HIV-1 (HIVAG-1; Abbott). Serum β_2-microglobulin levels were determined using a commercial assay (IMX β_2-Microglobulin; Abbott). All the EIAs were done in batch testing.

Semiquantitation of HIV provirus. PBMC were isolated by Ficoll-Paque (Pharmacia, Dubendorf, Switzerland). The pellet containing 1–20 × 10⁶ PBMC was incubated overnight at 37°C in a lysis buffer containing 100 mM NaCl, 50 mM TRIS-HCl (pH 7.5), 1 mM EDTA, 0.5% SDS, and 100 µg/mL proteinase K. After phenol-chloroform extraction, DNA was precipitated with ethanol and resuspended in TE buffer (10 mM TRIS-HCl, pH 7.5, 1 mM EDTA). The LAV-8E5 cell line (National Institute of Allergy and Infectious Diseases, AIDS Research and Reference Reagent Program, Bethesda, MD) containing one copy of HIV-1 provirus per cell was grown in culture as recommended [11]. DNA was extracted from a pellet of 50 × 10⁶ cells and serially diluted in HIV-negative human genomic DNA to obtain the equivalent of 1–1000 LAV-8E5 cells in 1 µg of human genomic DNA/10 µL. For provirus amplification, a 238-bp segment of a conserved region of the HIV-1 *pol* gene was selected and amplified [12]. All samples were tested in duplicate using 1 µg of genomic DNA corresponding to 150,000 cells/test. The reaction mixture of 100 µl containing 1 µg of genomic DNA and 2.5 units of DNA polymerase (Amplitaq; Perkin-Elmer Cetus, Norwalk, CT) was adjusted to 10 mM TRIS-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 0.5 mM of each deoxynucleoside triphosphate (dNTP), 0.5 µM of POL 1 and POL 2 primers (POL 1,5′-TGGGTACCAGCACACAAAGG, 3734–3753 in HIV BRU; POL 2,5′-ACTTGTCCATGCATGGCTTC, 3953–3972 in HIV BRU) [13]. The reaction was cycled 37 times (94°C, 1 min; 56°C, 1.5 min; 72°C, 1 min) using a DNA thermal cycler (model 480; Perkin-Elmer Cetus) [12]. Five samples of human genomic DNA containing 1, 3, 10, 100, and 1000 copies of HIV provirus from the LAV-8E5 cells were included in each experiment.

The denatured PCR product was applied to nylon membrane (GeneScreenPlus; NEN, Boston) using a vacuum filtration apparatus (Minifold II; Schleicher & Schuell, Keene, NH). Filters were incubated for 1 h at 45°C in prehybridization solution (4× SSC [standard sodium citrate], 5× Denhardt's solution, 5% SDS, 20 mM sodium phosphate [pH 7], 100 µg/mL sheared and denatured salmon sperm DNA) and hybridized to a ³²P-labeled probe (5′-AATTGGAGAGCAATGGCTAGTGA, 3863–3885 in HIV BRU) by incubation at 50°C overnight (specific activity, 10⁸ cpm/µg). Then filters were washed 15 min at 50°C, once with 3× SSC and 0.1% SDS, once with 0.5× SSC and 0.1% SDS, then twice with 0.1× SSC and 0.1% SDS. The radioactivity of each spot applied to the nylon membrane was determined using the PhosphorImager system (Molecular Dynamics, Basel, Switzerland).

The number of HIV proviral copies was calculated in relation to the standard curve generated by using DNA from defined cell equivalent of the LAV-8E5 cell line. To relate the number of proviral copies to 10⁶ CD4 cells, the number of copies measured

in 1 µg of DNA corresponding to 150,000 cells was multiplied by a correcting factor (6.66) and further divided by the ratio of CD4 cells per microliter to total lymphocytes per microliter. We have shown previously that the sensitivity of proviral HIV DNA detection is on the order of one proviral copy per sample [12, 13]. The one-copy samples were detected in 6 of 14 experiments and three copies in at least one of the two duplicate amplifications in all assay runs. Negative samples (serum from HIV-seronegative blood donors included in each run) gave numbers (pixels) nearly the same as the background value measured on the membrane in the absence of DNA. Reference samples with the equivalent of one proviral copy gave numbers at least twice the background value. The coefficient of correlation between points of the standard curve is typically ∼0.98. Interassay variation, checked using 10 different samples in three different experiments, was 30%; intraassay variation was 19%.

HIV RNA semiquantitation. Circulating genomic HIV RNA was detected after immunocapture of the virus, reverse transcription (RT), and cDNA amplification. To prepare the immunoabsorbent, IgG was purified from 35 mL of pooled sera from 10 patients with a high titer of anti–HIV-1 antibodies and no circulating antigen by ammonium sulfate precipitation [14] and diethylaminoethyl cellulose chromatography. IgG (200 mg) was coupled to 5 g of cyanogen bromide–activated Sepharose 4B (Pharmacia) according to the manufacturer's recommendations. The final step was extensive washing with 2 L of 2% Nonidet P-40 (NP40) in TE buffer. The coupling efficacy was 75%. Sepharose was stored in PBS plus 0.02% NaN₃ at 4°C.

Immunocapture of circulating virus was achieved as follows: 100 µL of frozen serum or dilutions was adjusted to 200 µL with 150 mM NaCl and incubated on a rotator overnight at 4°C with 20 µL of 5% Sepharose suspension (a threefold excess as determined in preliminary experiments). The Sepharose particles were pelleted, washed twice with 150 mM NaCl, suspended in 5 µL of 1% NP40, and heated at 95°C for 5 min. RT was done in 20 µL containing 50 mM TRIS-HCl (pH 8.3), 20 mM KCl, 6 mM MgCl₂, 1 mM of each dNTP, 25 units of RNase inhibitor (Boehringer, Mannheim, Germany), 100 pmol of dN₆ random primers (Boehringer), and 200 units of Moloney murine leukemia virus reverse transcriptase (BRL, Bethesda, MD). The mixture was incubated 10 min at room temperature and 1 h at 42°C, heated to 95°C for 15 min, then 2 µl of 0.05 mg/mL solution of proteinase K (Sigma, St. Louis) was added. The mixture was incubated at 55°C for 30 min, heated to 95°C for 15 min, and cooled to 4°C. Next, cDNA was amplified as described above for provirus amplification.

Circulating genomic HIV RNA was measured semiquantitatively by using serial dilutions of serum in the absorption step. All sera were tested at least twice without dilution (100 µL of serum) and when diluted 10 and 25 times with 150 mM NaCl. Samples positive without dilution were diluted twice and tested singly. Identical results were obtained with duplicate tests for all but 3 samples; these 3 were retested three times, and the result used was the mean of the five determinations. Each set of experiments included a negative control (serum from a blood donor), two known weakly reactive sera, and, for the PCR step, dilutions of LAV-8E5 DNA. The two weakly positive samples (RNA of <10 but >1 copy/mL) included in each run gave positive results

% per subclass

	RNA undetect	RNA >10/ml	RNA >100/ml	RNA >=250/ml
log >= 4 ■	1	6	3	8
log = 3	7	8	2	1
log = 2	7	8	0	0
log = 1	2	3	0	0
	n=17	n=25	n=5	n=9

Figure 1. HIV provirus levels in relation to circulating genomic RNA. Provirus is expressed as log of provirus/10^6 CD4$^+$ lymphocytes. Participants are divided into four groups on basis of level of RNA molecules detected/ mL of serum. Number of participants with various levels of provirus for each category is shown at bottom.
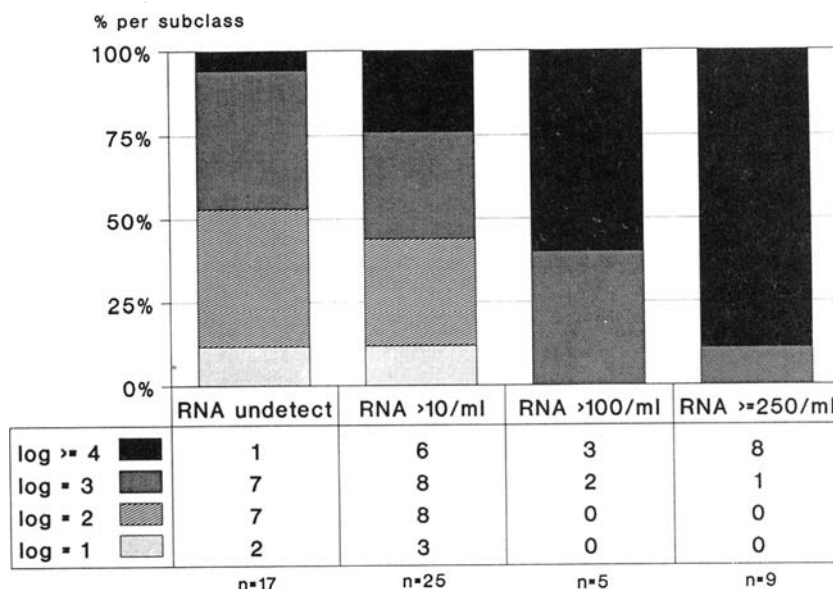
in 14 of 15 experiments. The results of RNA copy number are measured as a function of the dilutions of LAV-8E5 DNA and are expressed as undetectable, >10 copies/mL (positive signal using 100 μL of serum and negative signal using 10 μL of serum), >100 copies/mL, and >250 copies/mL. As a control, serum of each participant was absorbed on Sepharose, and the pellet was amplified without the reverse transcriptase step; none gave a positive signal. For determining circulating HIV RNA, $-70°$C frozen stored sera were tested within 3 months of collection. No decline of HIV RNA level has been observed when serial unthawed samples have been tested repeatedly over 4–6 months.

Statistical analysis. Groups were compared using the χ^2 test with Yates's correction or the two-tailed Fisher's exact test.

Results

Figure 1 presents the relationship between number of proviral copies and concentration of genomic viral RNA in serum. The provirus was detectable in all participants, whereas genomic RNA was detectable in 39 (70%) of 56. In general, persons with a high number of proviral copies also had high RNA levels. High numbers of proviral copies ($\geq$1000 copies/10^6 CD4$^+$ lymphocytes) were detected in all participants with >100 copies of genomic RNA/mL of serum compared with 22 (52%) of 42 participants with <100 copies/mL (P = .008).

In figure 2, provirus and circulating virus are shown in relation to clinical stages. A high number of proviral copies (log $\geq$3) was found in 20 (83%) of 24 participants in CDC stages IV-C-2, IV-C-1, or IV-D compared with 16 (50%) of 32 participants in CDC stages II and III (P = .02). More than 100 RNA copies/mL were found in 11 (46%) of 24 participants with stage IV disease compared with 3 (9%) of 32 participants with stage II or III (P = .005).

The concentration of CD4$^+$ lymphocytes in the peripheral blood is often used as a prognostic marker. In figure 3, results are expressed in relation to four categories of CD4$^+$ cell concentration. Concentrations of provirus per 10^6 CD4$^+$ lymphocytes and of circulating genomic RNA both increase when the CD4$^+$ cell count decreases. More than 1000 proviral copies were found in 25 (96%) of 26 participants with CD4$^+$ cell counts <200/μL compared with 11 (37%) of 30 participants with CD4$^+$ cell counts >200/μL (P < .001). More than 100 RNA copies/mL were found in 13 (50%) of 26 participants with CD4$^+$ cell counts <200/μL compared with 1 (3%) of 30 participants with CD4$^+$ cell counts >200/ μL (P < .001).

Figure 4 shows concentrations (values) of provirus (DNA) and circulating genomic RNA and the ratio between them for individual subjects. The values are expressed per milliliter of blood, to determine the virus load, and are presented for four categories of CD4$^+$ concentration. It appears that individual values of provirus vary to a greater extent within each category than do the mean values of the four categories. Furthermore, this does not seem to be linked to zidovudine treatment. In contrast, the percentage of patients with circulating genomic HIV RNA increases markedly in the two groups of participants with CD4$^+$ cell counts < 200/μL compared with those with >200/μL.

The ratio of provirus to circulating genomic RNA (also shown in figure 4) indicates that the concentration of provirus exceeds the concentration of circulating virus until late in the disease (participants with CD4$^+$ cell counts <50/μL).

In figures 5–7, concentrations of provirus and circulating genomic RNA are reported in relation to presence or absence of p24 antigen, to high or medium level of or absence of anti-p24 antibodies, and to $\geq$3 or <3 mg/mL β_2-micro-globulin. Associations were found between virologic parame-

Log proviral DNA/10^6 CD4

log	stage II	stage III	st. IVc2/IVd	stage IVc1
log >= 4	1	4	3	10
log = 3	3	8	5	2
log = 2	5	8	2	0
log = 1	2	1	2	0

HIV RNA in serum

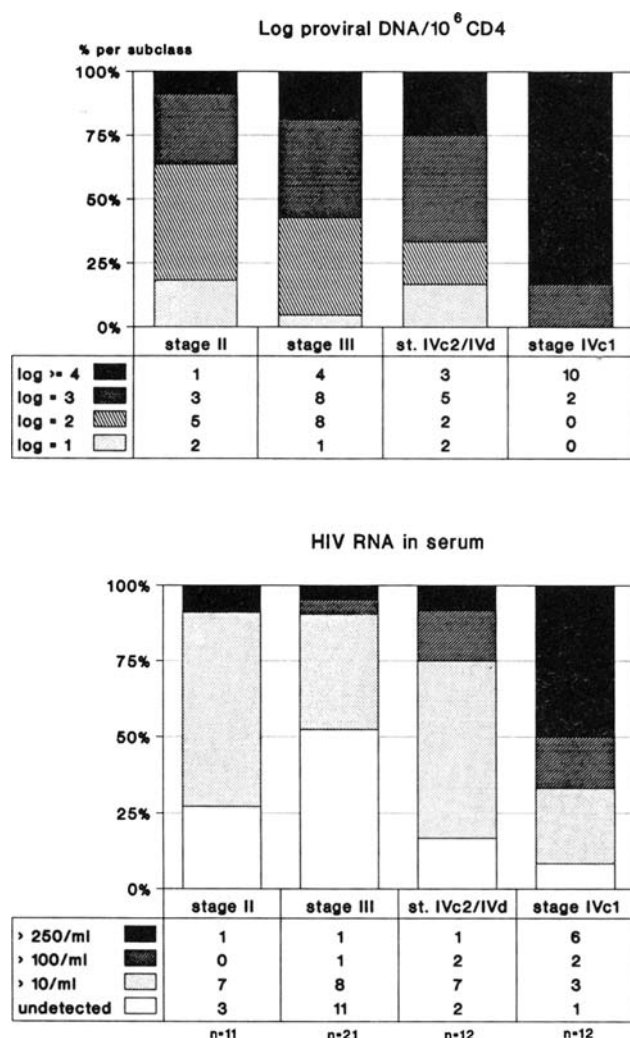|	stage II	stage III	st. IVc2/IVd	stage IVc1
> 250/ml	1	1	1	6
> 100/ml	0	1	2	2
> 10/ml	7	8	7	3
undetected	3	11	2	1
	n=11	n=21	n=12	n=12

Figure 2. HIV load in relation to disease stage according to Centers for Disease Control (CDC) classification. Top, number of proviral copies in different CDC stages. Results of provirus (DNA) are expressed as log of provirus per 10^6 CD4$^+$ lymphocytes. Bottom, number of RNA molecules/mL of serum for different CDC stages. Results of circulating genomic HIV RNA are reported as undetectable, >10 molecules/mL, >100 molecules/mL, and >250 molecules/mL. Number of participants is shown at bottom of each graph.

ters and both p24 antigen and anti-p24 antibodies but not β_2-microglobulin. As shown in figure 5, >1000 copies of HIV provirus/10^6 CD4$^+$ lymphocytes were detected in 20 (91%) of 22 participants with p24 antigen compared with 16 (47%) of 34 participants without p24 antigen (P = .002). More than 100 RNA copies/mL were found in 11 (50%) of 22 participants with p24 antigen compared with 3 (9%) of 34 participants without p24 antigen (P = .002). As shown in figure 6, >1000 copies of provirus were found in 17 (94%) of 18 participants without anti-p24 antibodies compared with 19 (50%) of 38 participants with anti-p24 antibodies (P = .006). More than 100 RNA copies/mL were found in 9

(50%) of 18 participants without anti-p24 antibodies compared with 5 (13%) of 38 participants with anti-p24 antibodies (P = .007). However, the associations were far from perfect; for example, 50% of p24 antigen–negative subjects had detectable circulating genomic RNA, and some participants with high levels of anti-core antibodies had high levels of provirus and circulating RNA.

Only limited follow-up was available after these tests (between September 1990 and May 1991). Five subjects have died (21 days, 91 days, 105 days, 7 months, and 8 months) since completion of this investigation. All had >250 copies/mL circulating genomic RNA (of 9 total subjects with >250 copies/mL) and >10^4 copies of provirus/10^6 CD4$^+$ cells (of 18 total subjects with that level). Their CD4$^+$ cell counts at the time of the investigation were 2, 7, 8, 16, and 71/μL.
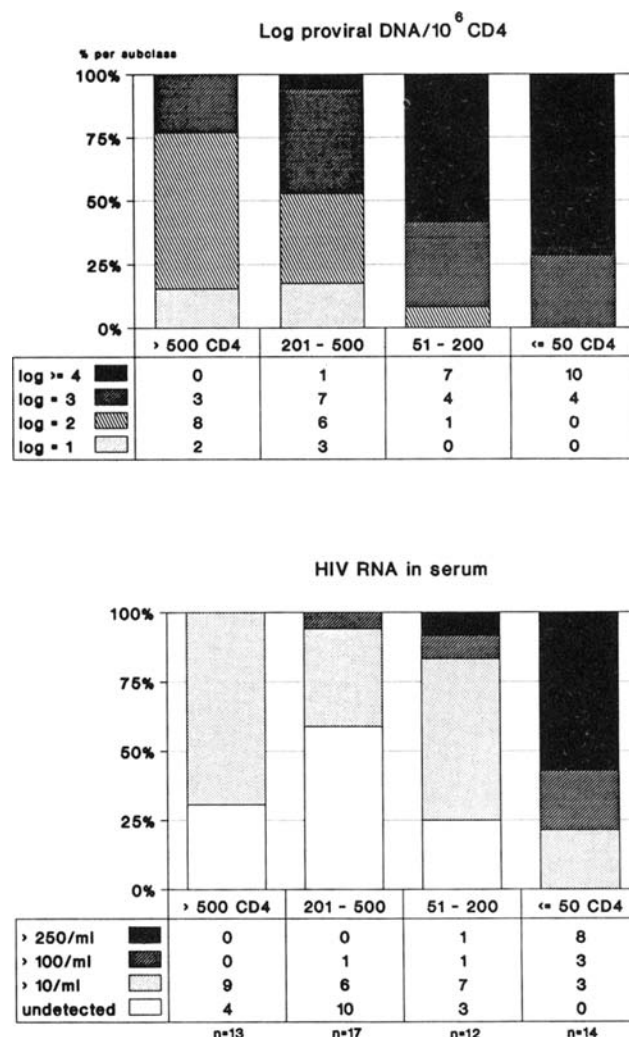
Log proviral DNA/10^6 CD4

log	> 500 CD4	201 - 500	51 - 200	<= 50 CD4
log >= 4	0	1	7	10
log = 3	3	7	4	4
log = 2	8	6	1	0
log = 1	2	3	0	0

HIV RNA in serum

	> 500 CD4	201 - 500	51 - 200	<= 50 CD4
> 250/ml	0	0	1	8
> 100/ml	0	1	1	3
> 10/ml	9	6	7	3
undetected	4	10	3	0
	n=13	n=17	n=12	n=14

Figure 3. HIV load in relation to CD4$^+$ lymphocytes/μL. Participants are divided into four groups according to concentration of CD4$^+$ in peripheral blood. Number of CD4$^+$ cells in each category is shown at bases of columns. Results of provirus and circulating HIV RNA are expressed as in figure 2.

Figure 4. HIV provirus copies (DNA) and circulating HIV RNA/mL of blood and ratio of provirus to HIV RNA. Values of circulating HIV RNA have been adjusted for hematocrit of 40% (RNA/mL values divided by 2.5). For determination of ratio of DNA to RNA, value of 1 (1 copy of RNA/mL of blood, concentration of RNA not detectable in our system) has been attributed to samples with undetectable circulating RNA. Participants are grouped according to CD4+ concentration. O, participants not treated with zidovudine; ●, participants treated with zidovudine; ▲, ratio of provirus divided by HIV RNA; horizontal bars, mean value.
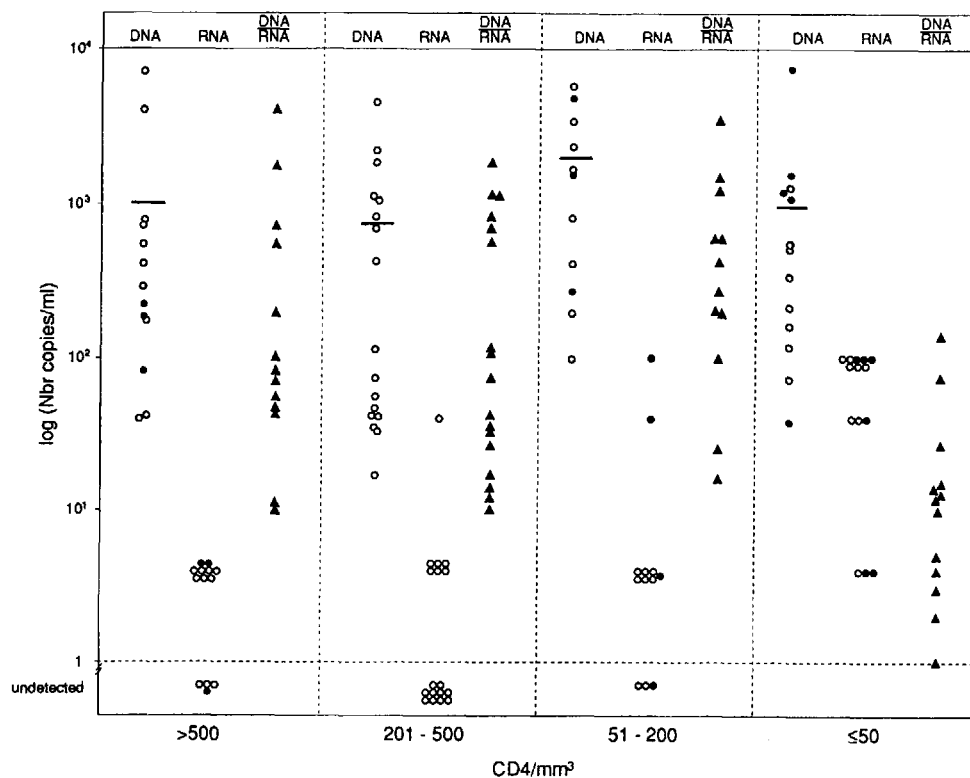
Discussion

In this study, a close association was observed between virologic parameters and seroimmunologic markers commonly used for the evaluation of HIV infection, such as CD4+ lymphocytes, p24 antigenemia, and anti-core antibodies.

The virologic parameters selected explore different aspects. Semiquantitation of the provirus is related to the reservoir of HIV, although cell types not represented in the peripheral blood (such as Langerhans cells) may be an important reservoir of HIV [15]. Circulating genomic RNA reflects viremia and is linked not only to viral replication but also to viral clearance, which may be more efficient in early stages of HIV infection. The result of these two factors (replication and clearance of HIV) as determined by circulating genomic RNA is a sensitive index of the quantity of HIV available for propagation of the infection.

The virologic parameters measured in this investigation provided semiquantitative information. The choice of a *pol* gene segment for HIV DNA amplification was based on the low number of base substitution among HIV isolates in these parts of the *pol* gene [12, 13]. A method based on immunocapture of the virus rather than RNA purification was selected for the detection of circulating HIV. For the capture of circulating virus, we used polyclonal (rather than monoclonal) antibodies to HIV to avoid biases linked to sequence variations of gp120 among HIV isolates. The absorption step

is convenient in terms of rapidity, simplicity, specificity (only the virus is absorbed), and suitability for quantitation by limiting dilutions. The main limitation of the quantitation of RNA is linked to the RT step, whose efficacy is ~5% [16] (in contrast, DNA amplification can detect a single copy of provirus [12, 17, 18]). For this reason, it may be more appropriate to express the results of circulating RNA in arbitrary units rather than in number of RNA molecules per milliliter. However, for simplicity of expression and to avoid artificial manipulations of the data, results are expressed in terms of relative concentration of RNA molecules per milliliter. Although they are relative, RNA results are reproducible; they are therefore suitable for comparative studies.

When the provirus measurements were regarded in relation to 10^6 CD4+ lymphocytes, we observed, as have others, an increase of proviral copies with progression of HIV infection and with the decrease in CD4+ cell concentration [3, 4, 17, 19–21]. Only a few copies of provirus are detectable in each infected PBMC [17], so our results suggest there is a progressive increase in the percentage of cells carrying the provirus that may reach 10%. The other mode of expressing the number of proviral copies per milliliter of blood reflects the absolute concentration of the provirus and is a better estimate of the HIV reservoir in CD4+ lymphocytes. During the progression of HIV infection, the mean concentration of provirus per milliliter of blood increases by only a factor of three for subjects with the lowest concentrations of CD4+ lymphocytes. Greater variations are observed among individ-
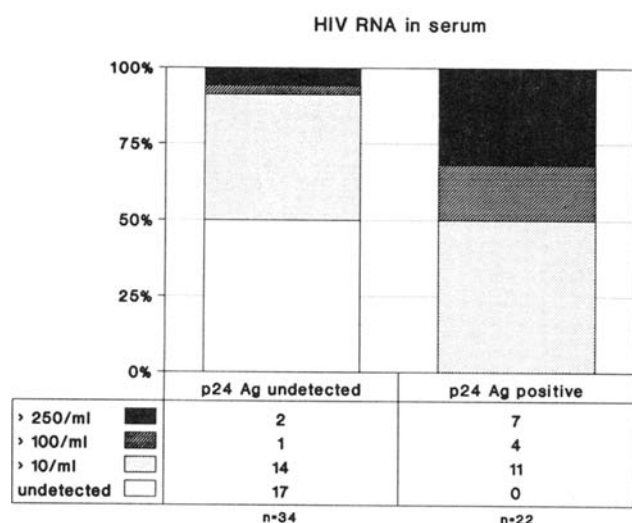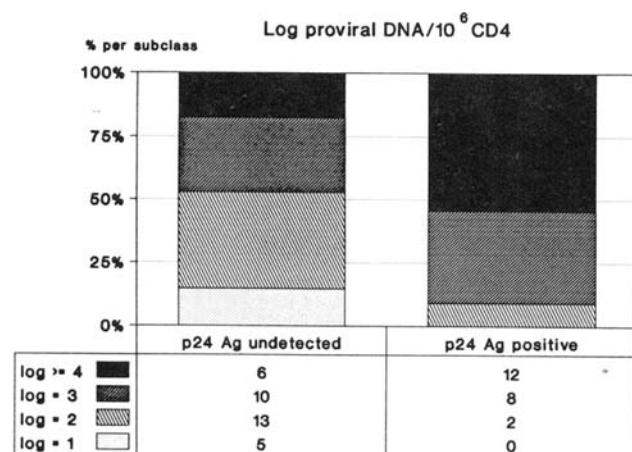
Log proviral DNA/10⁶ CD4

% per subclass

	p24 Ag undetected	p24 Ag positive
log >= 4	6	12
log = 3	10	8
log = 2	13	2
log = 1	5	0

HIV RNA in serum

	p24 Ag undetected	p24 Ag positive
> 250/ml	2	7
> 100/ml	1	4
> 10/ml	14	11
undetected	17	0
	n=34	n=22

Figure 5. HIV load in relation to p24 antigenemia (p24 Ag). Participants are divided into those without and those with p24 Ag. Results of provirus and circulating HIV RNA are expressed as in figure 2.

tion system [3, 4, 6, 19, 22]. For example, in one large study, the percentage of plasma coculture positivity varied from 23% in asymptomatic participants to 82% in patients with AIDS [3]. The marked variation in concentration of infectious virus reported by different groups using HIV culture [3, 4, 19] underlines the need of alternative approaches for the analysis of virologic parameters. Recently, Zang et al. [16], using an RNA PCR assay, detected circulating genomic RNA in 100% of individuals with AIDS-related complex and in 60% of asymptomatic individuals. Other investigators have reported a correlation between disease stage and viral RNA in serum [22, 23], as was seen in the present study.

Participants with CD4⁺ counts >200/μL can be divided into two subgroups: those without detectable circulating

Log proviral DNA/10⁶ CD4

% per subclass

	Undetected	1=<anti-Core<10	anti-Core >= 10
log >= 4	10	4	4
log = 3	7	2	9
log = 2	1	8	6
log = 1	0	0	5

HIV RNA in serum

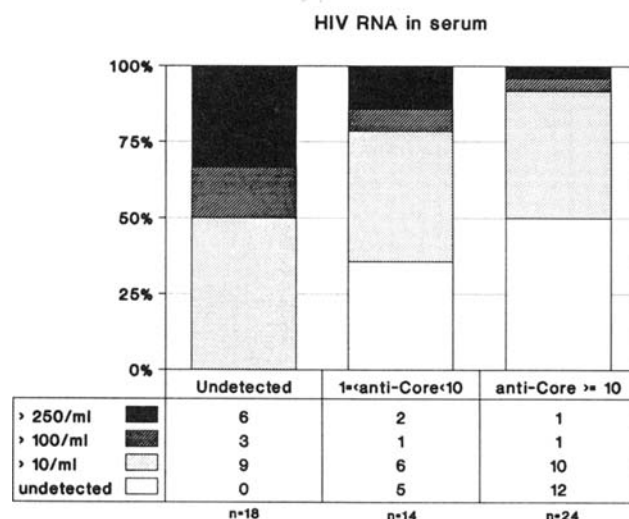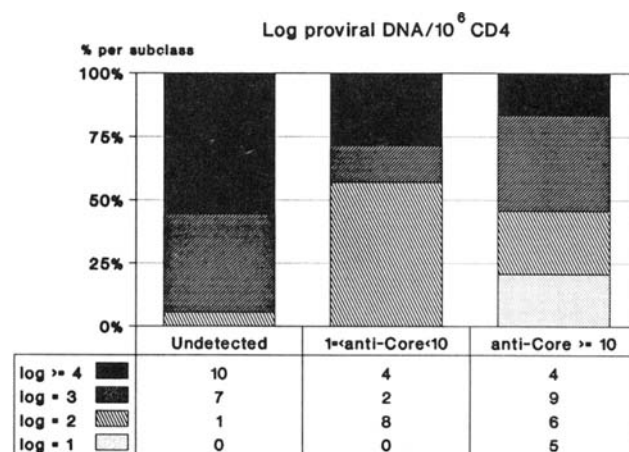|	Undetected	1=<anti-Core<10	anti-Core >= 10
> 250/ml	6	2	1
> 100/ml	3	1	1
> 10/ml	9	6	10
undetected	0	5	12
	n=18	n=14	n=24

Figure 6. HIV load in relation to antibodies to HIV core antigen (anti-core). Participants are divided into those without detectable anti-core antibodies (undetected) and those with low (index, 1–10) and high levels of anti-core antibodies (index, ≥10). Results of provirus and circulating HIV RNA are expressed as in figure 2.
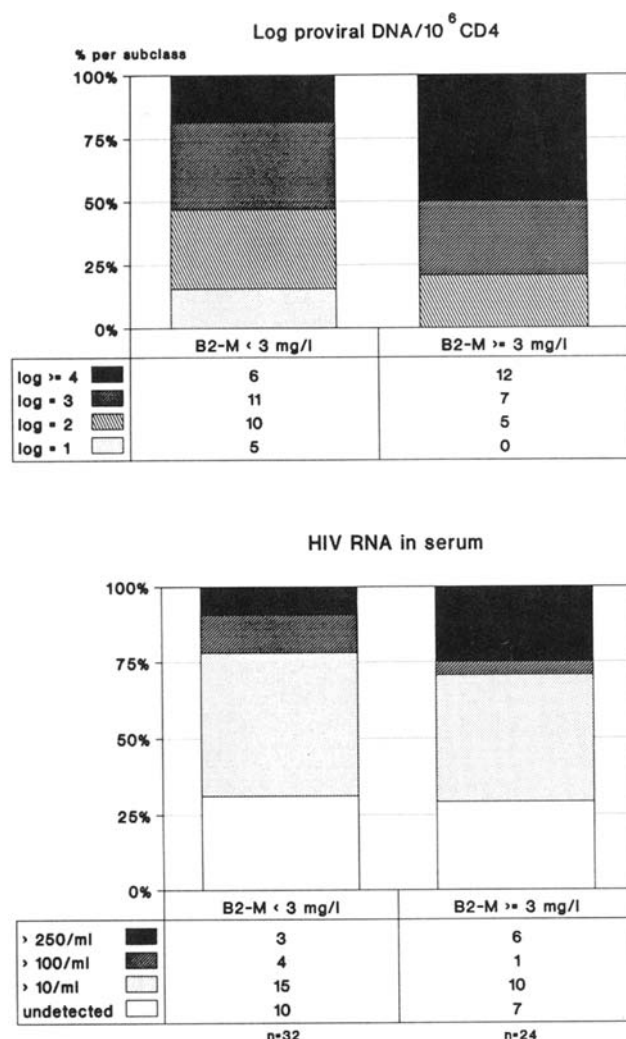
uals, and these are relatively independent of their CD4⁺ cell counts. All together, these data suggest that in peripheral blood the increase in percentage of cells carrying the provirus is counterbalanced by a decrease in the concentration of cells susceptible to HIV infection during the progression of HIV infection. In addition, in the present investigation, values determined in participants on zidovudine (most of them on long-term zidovudine, mean duration of 261 days) did not differ significantly from those not on zidovudine.

Circulating genomic RNA was detected in 70% of the participants. The proportion of subjects with detectable genomic RNA and the amount of genomic RNA increased with progression of HIV infection. These results are compatible with those of earlier investigations using a coculture detec-

Log proviral DNA/10⁶CD4

	B2-M < 3 mg/l	B2-M >= 3 mg/l
log >= 4	6	12
log = 3	11	7
log = 2	10	5
log = 1	5	0

HIV RNA in serum

	B2-M < 3 mg/l	B2-M >= 3 mg/l
> 250/ml	3	6
> 100/ml	4	1
> 10/ml	15	10
undetected	10	7
	n=32	n=24

Figure 7. HIV load in relation to β_2-microglobulin. Participants are grouped according to level of β_2-microglobulin; limit of 3 mg/L was arbitrarily selected and corresponds to 250% of mean value of healthy adults. Results of provirus and circulating HIV RNA are expressed as in figure 2.

RNA (14 participants) and those with RNA [16]. It is tempting to speculate that the patients with circulating RNA are at higher risk for rapid progression of HIV infection and that they would benefit most from antiviral treatment. Verification of this hypothesis will require long prospective studies. Ours was a cross-sectional study with only 9 months of follow-up. During this period, 5 subjects with >250 circulating RNA molecules/mL died, emphasizing the negative prognostic value of high levels of circulating genomic RNA.

A positive association between circulating genomic HIV RNA and p24 antigenemia was observed; this indirectly validates the techniques used for RNA determination. All p24 antigen–positive but only 50% of p24 antigen–negative subjects were found to be RNA-positive. As expected, an inverse

association is observed with anti-core antibodies. The detection of genomic HIV RNA in participants with high levels of anti-core antibodies is not surprising, since immunocapture is designed to capture intact virus whose surface component is gp160 and its process products. The absence of association with β_2-microglobulin, whose increase has been shown to correlate with progression of AIDS [24, 25], may be due to a preferential link of β_2-microglobulin increase with cell activation rather than with HIV multiplication itself. In this context, β_2-microglobulin levels may be more influenced by opportunistic infections than by HIV load.

In conclusion, the present investigation demonstrated a closed association of virologic parameters with CD4⁺ concentration, p24 antigen, and anti-core antibodies. It also shows that a high proportion of participants with CD4⁺ cell counts $>200/\mu L$ already have high levels of provirus and circulating genomic RNA; these individuals may benefit most from early antiviral therapy. Finally, the technique of semiquantitation of circulating genomic HIV RNA is specific and relatively simple and should be of value in monitoring the effect of antiviral drugs.

Acknowledgments

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