Temporal relationship between human immunodeficiency virus type 1 RNA levels in serum and cellular infectious load in peripheral blood

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Temporal Relationship between Human Immunodeficiency Virus Type 1 RNA Levels in Serum and Cellular Infectious Load in Peripheral Blood

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Cross-sectional analysis of 252 paired serum and peripheral blood mononuclear cell (PBMC) samples derived from 54 human immunodeficiency virus type 1 (HIV-1)–infected persons revealed a correlation between HIV-1 RNA load in serum and infectious load in peripheral CD4 T cells after 18 months of follow-up and before an AIDS diagnosis (Pearson’s correlation coefficient \( r_p = 0.71 \), \( P < 0.001 \)) and during antiviral treatment (\( r_p = 0.78 \), \( P < 0.001 \)). To gain insight into the temporal relationship between both measures of virus load, longitudinally obtained samples from 23 persons with various clinical courses (slow or rapid disease progression, long-term survival) and 22 persons undergoing antiviral therapy (zidovudine or didanosine, or both, or ritonavir) were analyzed. In general, the kinetics of changes in both measures of virus load were similar in the natural course of infection (78% of study participants) and during treatment (82% of participants). These findings suggest that PBMC and serum represent closely related, if not the same, viral compartments.

The age of disease in human immunodeficiency virus type 1 (HIV-1)–infected persons is associated with level of virus in serum or plasma and level of cell-associated virus [1–4]. In addition, plasma levels of HIV-1 RNA early in infection are predictive of rate of progression [5]. This latter finding, together with the fact that HIV-1 RNA load in plasma directly reflects viral RNA load in plasma in directly infected cells with no fully understood. Therefore, we longitudinally compared virus RNA load in serum and infected cells in peripheral CD4 T cells from a heterogeneous group consisting of individuals with various clinical courses (slow or rapid disease progression, long-term survival) and 22 persons undergoing antiviral therapy (zidovudine or didanosine, or both, or ritonavir) were analyzed. In general, the kinetics of changes in both measures of virus load were similar in the natural course of infection (78% of study participants) and during treatment (82% of participants). These findings suggest that PBMC and serum represent closely related, if not the same, viral compartments.

Subjects and Methods

Group A consisted of 23 participants of the Amsterdam Cohort Study (ACS). Fifteen subjects entered ACS while still viral replicating [6,7], favored plasma RNA load as a progression marker in HIV-1-infected individuals and as a marker for initiation and evaluation of antiviral therapy (zidovudine or didanosine). Fifteen participants were already seropositive at the time of entry, and the seroconversion date of these individuals was assessed using PCR (HIV-1 RNA QT; Oragenics, Germany) and had stable CD4 T cell counts of >400/µL at least until year 9 of follow-up.

Group B consisted of 22 participants who entered ACS while still viral replicating [8,9]. Changes in serum HIV-1 RNA levels and in frequencies of productively infected cells were monitored during antiretroviral therapy. Productively infected CD4 T cells were defined as cells with HIV-1 RNA load in serum or plasma and frequencies of productively infected cells correlating with CD4 T cell decline and disease progression, even in the absence of a clinically significant change in viral load. Therefore, we longitudinally compared virus RNA load in serum and infected cells in peripheral CD4 T cells from a heterogeneous group consisting of individuals with various clinical courses (slow or rapid disease progression, long-term survival) and 22 persons undergoing antiviral therapy (zidovudine or didanosine, or both, or ritonavir) were analyzed. In general, the kinetics of changes in both measures of virus load were similar in the natural course of infection (78% of study participants) and during treatment (82% of participants). These findings suggest that PBMC and serum represent closely related, if not the same, viral compartments.

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Wien ari informed consent was obtained from all participants. In clinical research, human experiment involves in vivo testing of in vivo testing of in vivo testing. This study was supported in part by the European Community through the European Programme against AIDS Research (90413 and 90419). Reprints or correspondence: Dr. H. Schuitemaker, Dep. of Clinical Viroimmunology, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.

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navir were determined by use of reverse-transcriptase polymerase chain reaction (Amplicor HIV-1 monitor assay; Roche Molecular Systems, Branchburg, NJ). RNA levels in plasma and in serum were measured by NASBA corona very well, with RNA levels in plasma being, on average, 0.5 log higher than those in serum [8]. For the participants receiving zidovudine and didanosine combination therapy, the number of proviral HIV-1 DNA copies in PBMC was determined using a competitive quantitative polymerase chain reaction [9].

The correla ion between serum RNA load and cellular infectious load was analyzed in 252 paired serum and cryopreserved PBMC samples derived from the subjects in groups A and B and from 9 additional persons, from whom only samples from a single time point were analyzed (n = 54). To avoid bias caused by repeated measurements for 1 person, the median of the paired measurements for each person, and the correla ion between log-transformed load values was analyzed by use of Pearson’s correla ion coefficient (r). In cases in which fewer samples were small, Spearman’s correla ion coefficient (r) was used. The two-tailed Fisher’s exact and the Mann-Whitney U tests were used to analyze the relationship between the frequency of productively infected CD4 T cells or serum RNA copies early in infection and the occurrence of an AIDS diagnosis.

**Results**

**Cross-sectional analysis of RNA load and cellular infectious load.** Virus load was analyzed in 252 paired serum and PBMC samples from 54 participants. Analysis of the median of paired measurements of all par participants revealed a statistically significant correla ion be een HIV-1 RNA levels in serum and the frequency of productively infected cells (n = 54, r = .52, P < .001) (figure 1A). Analysis of subgroups of samples, stratified by stage and age of disease, indicated that both measures of virus load were highly correlated in the period when the first 18 mon hs of follow-up and AIDS diagnosis (n = 43, r = .71, P < .001) and during rea men (n = 26, r = .78, P < .001). However, neither measure of virus load correlated in the period after the first 18 mon hs of follow-up (n = 18, r = .06, P = .8) or in the period before an AIDS diagnosis (n = 8, r = -.05, P = .9).

Stratification of the samples by absence or presence of SI variants showed a similar correla ion between both measures of virus load in persons harboring both non-SI and SI variants (n = 24, r = .53, P = .007) and in persons with only non-SI variants (n = 42, r = .40, P = .008). Both measures of virus load were higher in persons with SI variants (figure 1B). These data confirm the existence of an associa ion between viral phenotype and cellular infect load [3] and also show a similar associa ion between viral phenotype and RNA load in serum.

**Virus load during the natural course of HIV-1 infection and during treatment.** Three different profiles in serum RNA load and cellular infect load were observed in the 23 group A par participants. After the first 16 mon hs (range, 2–28) of follow-up un il AIDS diagnosis or the end of follow-up (mean, ime,

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**Figure 1.** Cross-sectional analysis of HIV-1 RNA levels in serum and frequencies of productively infected CD4 T cells (TCID/10⁶ CD4 T cells). A, Virus load in serum and peripheral blood mononuclear cell samples for 54 persons a 252 identical time points during the natural course of infection and during treatment. B, Samples depic ed in A, the absence or presence of SI variants.
Figure 2. Longitudinal analysis of HIV-1 RNA levels in sera and frequencies of productively infected CD4 T cells (TCID/10^6 CD4 T cells) during natural course of infection (A) and during an antiretroviral regimen (B). A. For each pattern described in ex, 1 represents a rise is given: I, Both measures of virus load remain stable at low levels (n = 5); II, both measures of virus load remain stable at moderate or high levels (n = 4); III, both measures of virus load increase (n = 9); and IV, RNA levels in sera remain stable and frequencies of productively infected cells increase (n = 5). \( \text{\(V\)} \) = time of AIDS diagnosis; \( \text{\(\nabla\)} \) = time syncytium-inducing variant appeared. B. Represents persons treated with zidovudine (I; n = 10), didanosine (II; n = 6), ritonavir (III; n = 4), or zidovudine and didanosine in combination (IV; n = 2). In persons receiving both zidovudine and didanosine, proviral DNA load in CD4 T cells was also measured.

5.3 years; range, 2.0–9.4), (1) both measures remained stable (<1 log increase and/or <10^4 RNA copies/mL of serum or <30 TCID/10^6 CD4 T cells at the end of follow-up; n = 9); (2) both measures increased (>1 log; n = 9); or (3) the cellular infectious load increased while the RNA load remained stable (n = 5; figure 2A).

Mainenance of low levels of both measures of virus load was associated with long-term survival (5/5 were long-term survivors). Stable levels or high levels (10^3–10^5 RNA copies/mL of serum and 50–70 TCID/10^6 CD4 T cells) or an increase in both measures of virus load was associated with a progressive clinical course (12/13 were progressors). Of the 5 persons in whom RNA load remained stable at moderate or high levels while the cellular infectious load increased, 2 were long-term survivors and 3 progressed to AIDS within 2.8–5.5 years. In most patients (18/22) treated with different anti-HIV-1 drugs (group B), changes in serum RNA load and cellular infectious load were similar. For both persons treated with the combination of zidovudine and didanosine, proviral DNA was additionally quantified but showed no change (figure 2B).

Predictive value of early virus load measures. In 22 patients from group A, cellular infectious load was measured a
leas once be ween follow-up mon hs 10 and 26. These persons were classified in o 2 groups according o heir cellular infec-
ious load during his period. Persons in group 1 (n = 12) had <10 TCID/10⁶ CD4 T cells; persons in group 2 (n = 10) had ≥10 TCID/10⁶ CD4 T cells.

The number of par icipan s who were seroposi ve a en ry was higher in group 1; as a resul , he mean ime point of analysis in rela ion o he to he es ina ed s e rocon ver sion da e was la er in group 1 (30 mon hs; range, 16–42) han in group 2 (18 mon hs; range, 10–34). In group 1, 7 persons s ill had no progressed o AIDS af er 10–12 years of follow-up. Of he 5 persons in group 1 who progressed o AIDS, he mean incuba-

tion ime was 7.2 years (range, 5.2–11.3). Nine of 10 persons in group 2 progressed o AIDS wi hin a mean of 4.4 years (range, 2.8–5.9). The 2 groups differed signif ican ly wi h re-

spect o he chance of progressing o AIDS (odds ra io = 12.6, 95% confidence in eral = 1.2–134.0, P = .03) and wi h re-

spect o he mean ime o an AIDS diagnosis in hose who progressed o AIDS (P = .03).

Similarly, s ra ifica ion by virus RNA levels below or above 10⁴ copies/mL of serum showed a cor rela ion be ween early RNA levels and disease progression (odds ra io = 17.5, 95% confidence in eral = 1.6–192.1, P = .02) and ime o AIDS (P = .04).

Discussion

In he presen s udy, cross-sec ional analysis revealed a s rong cor rela ion be ween serum HIV-1 RNA levels and cellular infec ious load in a period af er 18 mon hs follow-up un il AIDS diagnosis and during an iviral herapy. Moreover, he kine ics of changes in bo h measures of virus load were similar over ime in he majori y (78%) of persons s adied during he na ural course of infec ion and he maj ori y (82%) of persons undergoing rea men.

The finding ha changes in RNA load in serum and cellular infec ious load in peripheral blood generally coincide sugges s ha PBMC and serum represen he same, or a leas closely rela ed, virus compar men s. Moreover, he sim ulaneous oc-

currence of rebound o baseline levels in serum RNA and frequencies of produc ively infec ed cells in mos rea ed men-
P sugges s ha he urnover kine ics of cellular infec ious load are similar o hose repor ed for viral RNA in plasma [6, 7].

This seems o con ras wi h he finding ha mu a ions in HIV-1 RNA precede he appearance of hee mu a ions in pro-
viral DNA [10]. However, he absence of a response o an iviral rea men in he proviral DNA load in PBMC sugges s differ-
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PBMC, only represen s a minor frac ion of he o al virus popu-

la ion in he same PBMC. However, his quasispecies is he major sequence in he o al virus popula ion presen in PBMC iso-

la ed 6 mon hs la er in infec ion [11]. Whe he a any mo-

men in ime he infec ious virus popula ion in PBMC is iden i-
c al o he virus popula ion in RNA is curren ly under inves iga-

tion.

In accordance wi h he previously described cor rela ion be-

ween he ra e of progression and plasma RNA load early in infec ion [5], we found ha bo h he cellular infec ious load and serum RNA load in he firs 1–2 years of follow-up were predic ive for he leng h of he asymp oma ic phase.

The s rong cor rela ion be ween cellular infec ious load and RNA load in serum subs an a es he use of RNA quan i a ion in mon i oring disease progression and herapy. Quan i a ion of cellular infec ious load, however, would provide addi ional rele van informa ion because i reveals he presence of minor varian s and he con ribu ion of dis inc varian s o he virus load even when he load is very low.

In some persons, dis inc pa erns in bo h measures of virus load were observed. In all hese cases, he cellular infec ious load gradually increased, while he RNA load reached moder a e o high levels wi hin 12–18 mon hs of follow-up and subse-

quen ly remained s able. The resul ing large discrepancies be-

ween bo h measures of virus load in his period and high fluc ua ions in RNA load seen early in infec ion in some per-

sons migh con ribu e o he absence of a correla ion be ween serum HIV-1 RNA levels and disease progression (odds ra io

= 3.7, 95% confidence in eral = 0.96–12.6) and ime o an AIDS diagnosis in hose who progressed o AIDS (P = .03).

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Discussion

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sons migh con ribu e o he absence of a cor rela ion be ween bo h measures of virus load in he firs 18 mon hs of follow-

up.

A s able RNA load in serum in he presence of an increasing cellular infec ious load in he periphery migh reflec a change in he ra io of noninfec ious versus infec ious virus par icles, wi h he appearance of rela ively increased infec ious virus in he la er s ages of infec ion. The increase in infec ed cells in peripheral blood migh also resul from an al era ion in lymphocy e dis ribu ion. During infec ion, he lymph node archi ec ure is is los [12], which migh resul in leakage of infec ed cells from he lymph nodes. Fur hermore, an increase in he cellular infec ious load in peripheral blood migh be explained by an increase in he number of arge cells. Since he differen chemokine recep ors used as cofac ors for HIV-1 en ry [13, 14] are expressed in differen quan i a ed on T cells [15, 16], evolu ion of HIV-1 varian s wi h al ered corecep or usage and also evolu ion of varian s wi h higer corecep or or affini y migh resul in an increased arge cell popula ion. In his ligh , a dis inc pa ern in serum RNA load and cellular infec ious load can be envi-

sioned o resul from al ered corecep or or usage coinciding wi h a more cy opa hic pheno ype. This would resul in a higher frequency of produc ively infec ed cells ye simul aneously in a decreased half-life of infec ed cells and he amoun of virus produced per cell.

We were surprised o find declining CD4 T cell coun s in half (7/15) of he par icipan s wi h progressive disease, while ei her virus RNA load in serum or bo h RNA load and cellular
infected load remained stable at moderate levels. Conversely, we found that some persons who maintained stable and high CD4 T cell counts over prolonged periods had either increasing cellular infected load and stable, moderately high infection: progression of disease is associated with a shift from monocytic to T-cell– tropic virus populations. J Virol 1992;66:1354–60.

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References


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