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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 (PB C) 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 \( r_p \) = .71, \( P < .001 \)) and during antiviral treatment (\( r_p = .78, P < .001 \)). 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 PB C 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 on AIDS (ACS). Fifteen subjects were receiving zidovudine and didanosine combination therapy or ritonavir and had measurable CD4 T cell counts before 10.2–13.7 years and had serum and CD4 T cell counts of >400/μL at least once until year 9 of follow-up.

Group B consisted of 22 persons who were already seroconvertin at the first visit and evaluated of an antiviral therapy.

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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 correla correllation 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 correlation 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 one person, the median of the paired measurements was analyzed using Pearson’s correlation coefficient (r). In cases in which the number of samples was small, Spearman’s correlation coefficient (r_s) was used. The two-tailed Fisher’s exact test 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 patients. Analysis of the median of paired measurements of all participants revealed a statistically significant correlation between HIV-1 RNA levels in serum and the frequency of productively infected cells (n = 54, r_p = .52, P < .001) (figure 1A). Analysis of subgroups of samples, stratified by race and age of disease, indicated that both measures of virus load were highly correlated in the period between the first 18 months of follow-up and AIDS diagnosis (n = 43, r_p = .71, P < .001) and during treatment (n = 26, r_p = .78, P < .001). However, neither measure of virus load correlated in the period after an AIDS diagnosis (n = 8, r_p = -.05, P = .9).

Stratification of the samples by the absence or presence of SI variants showed a similar correlation between the two measures of virus load in persons harboring both non-SI and SI variants (n = 24, r_p = .53, P = .007) and in persons with only non-SI variants (n = 42, r_p = .40, P = .008). Both measures of virus load were higher in persons with SI variants (figure 1B). These data confirm the existence of an association between viral phenotype and cellular infectivity load [3] and also show a similar association 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 infectivity load were observed in the 23 group A patients. After the first 16 months (range, 2–28) of follow-up until AIDS diagnosis or the end of follow-up (mean, 18 months),...
Figure 2. Longitudinal analysis of HIV-1 RNA levels in sera and frequencies of productively infected CD4 T cells (TCID/10⁶ CD4 T cells) during natural course of infection (A) and during an antiviral remission (B). A. For each pattern described in ex., 1 representative 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 to 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). \( \nabla \) = time of AIDS diagnosis; \( \nabla \) = time syncytium-inducing variants appeared.

B. Represents persons reared 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 <10⁴ RNA copies/mL of serum or <10 TCID/10⁶ 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 yet moderate high levels (10⁵–10⁶ RNA copies/mL of serum and 50–70 TCID/10⁶ 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 to 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) reared with different antiretroviral drugs (group B), changes in serum RNA load and cellular infectious load were similar. For both patients reared 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 ive a en ry was higher in group 1; as a resul , he mean ime point of analysis in rela ion o he es ina ed seroonversion 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-
ion 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 signifiancy wi h respec o he chance of progressing o AIDS (odds ra io = 12.6, 95% confidence in erval = 1.2–134.0, P = .03) and wi h respec 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 correla ion be wen early RNA levels and disease progression (odds ra io = 17.5, 95% confidence in erval = 1.6–192.1, P = .02) and ime o AIDS (P = .04).

Discussion

In he presen sudy, cross-sec ional analysis revealed a s rong correla ion be wen serum HIV-1 RNA levels and cellular infec ious load in he 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 udied during he na ural course of infec ion and he majori 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 rele ed, virus compar men s. Moreover, he simul aneou occurre nce of rebound o baseline levels in serum RNA and frequenies of produc ively infec ed cells in mos rea ed men 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-
turnover kine ics in he produc ively infec ed and he o al infec ed cell popula ions, which may be due o a longer half-
ife of cells carrying defec ive HIV-1. The no ion ha he kine ics of he o al virus popula ion lag behind hose of he infec-
iou us virus popula ion is suppor ed by he finding ha he viral quasispecies, which predomina es af er cocul iva ion of pa i
PBMC, only represen s a minor frac ion o he o al virus popula ion in he same PBMC. However, his quasispecies is he major sequence in he o al virus popula ion presen in PBMC isola ed 6 mon hs la er in infec ion [11]. Whe her a any mo-
men in ime he infec ious virus popula ion in PBMC is iden i-

cal o he virus popula ion in RNA is curre nly under inves ig-

In accordance wi he previously described correla ion be wen 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 o he asym poma ic phase.

The s rong correla ion be wen cellular infec ious load and RNA load in serum subs an he use of RNA qua n a ion in moni oring disease progression and herapy. Quan i a ion of cellular infec ious load, however, would provide addi ional relevan 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 he period and high fluc ua ions in RNA load seen early in infec ion in some per-
sons migh con ribu e to he appearance of rela ively increased infec ed virus in peripheral blood generally coincide sugges s los [12], which migh resul in leakage of infec ed cells from

A s able RNA load in serum in he presen sudy of an increasing cellular infec ious load in he periphery migh re flec a change in he ra io o noninfec ious versus infec ious virus par icles, wi h he appearance of rela ively increased infec ious virus in he er s ages of infec ion. The increase in infec ed cells in peripheral blood migh also resul from an al era ion in lymphocyte dis ribu ion. During infec ion, he lymph node archi ec ure is The finding ha changes in RNA load in serum RNA load and 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 ery [13, 14] are ex-
presed in differen qua i res on T cells [15, 16], evolu ion o

HIV-1 varian s wi h al ered corecep or usage and also evolu ion o varian s wi h hore 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 usage coinciding wi h a more cy opa hic pheno ype. This would resul in a higher frequenies of produc ively infec ed cells ye simul aneou 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) o he par icipan s wi h progressive disease, while ei her virus RNA load in serum or bo h RNA load and cellular
infectious load remained stable at moderate to high levels. Conversely, we found that some persons who main ained stable and high CD4 T cell counts over prolonged periods had either increasing cellular infectous load and stable, moderately high infe cion: progression of disease is associated with monocyto- tropic T-cell– tropic virus populations. J Virol 1992;66:1354–60.


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