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

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Temporal Relationship between Human Immunodeficienc Virus T pe 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.

The s age of disease in human immunodeficiency virus ype 1 (HIV-1)–infec ed persons is associa ed wi h he level of virus in serum or plasma and he level of cell-associa ed virus [1–4]. In addi ion, plasma levels of HIV-1 RNA early in infec ion are predic ive of he ra e of progression [5]. This la er finding, oge her wi h he fac ha RNA load in plasma direc ly reflec s viral replica ion [6, 7], favored plasma RNA load as a progression marker in HIV-1 infec ion and as a marker for ini ia ion and evalua ion of an iviral herapy.

Al hough bo h HIV-1 RNA levels in serum or plasma and frequencies of produc ively infec ed cells correla e wi h CD4 T cell decline and disease progression, heir emporal rela ionship is no fully unders ood. Therefore, we longi udinally compared virus RNA load in sera and infec ious load in peripheral CD4 T cells from a he erogenous group consis ing of long-erm survivors of HIV-1 infec ion and slow or rapid progressors o AIDS, ei her in he absence or presence of syncy ium-inducing (SI) HIV-1 varian s. In addi ion, he effec of rea men on virus RNA load in serum and on cellular infec ious load was analyzed for pa ien s receiving an iviral herapy.

Subjects and Methods

Group A consis ed of 23 par icipan s of he Ams erdam Cohor S udies on AIDS (ACS). Fif een subjec s en ered ACS while s ill seronega ive for HIV-1 an ibodies. Eigh par icipan s were already seroposi ive a heir firs visi , and he seroconversion da e of hese persons was es ima ed o be 18 mon hs before en ry in o ACS. Fif een par icipan s progressed o AIDS af er an asymp oma ic phase ranging from 2.8 o 11.3 years. Eigh subjec s classified as long- erm survivors were s ill asymp oma ic af er 10.2–13.7 years and had s able CD4 T cell coun s of >400/μL a leas un il year 9 of follow-up.

Group B consis ed of 22 pa ien s who were par icipa ing in ACS or visi ing he Academic Medical Cen re AIDS clinic. Changes in serum HIV-1 RNA levels and in frequencies of produc ively infec ed cells were moni ored during rea men wi h zidovudine ($n = 10$), didanosine ($n = 6$), a combina ion of zidovudine and didanosine ($n = 2$), or ri onavir ($n = 4$).

Da a were available for all par icipan s as a resul of ongoing research in our labora ory, and no specific selec ion was made.

The biologic pheno ype of HIV-1 and frequencies of produc ively infec ed CD4 T cells were de ermined by cocul iva ion of pa ien peripheral blood mononuclear cells (PBMC) wi h fresh, phy ohemagglu inin-s imula ed, heal hy donor peripheral blood lymphocy es under limi ing dilu ion condi ions. The frequency of produc ively infec ed cells was calcula ed from he frac ion of nega ive cul ures (F_0) using he formula for Poisson dis ribu ion ($F = -\ln[F_0]$) and was expressed as TCID/10^6 CD4 T cells [3, 4].

Serum RNA levels for un rea ed subjec s and subjec s receiving zidovudine or didanosine were de ermined by use of a nucleic acid–based amplifica ion assay (NASBA; HIV-1 RNA QT; Organon Teknika, Box el, The Ne herlands). RNA levels for subjec s receiving zidovudine and didanosine combina ion herapy or ri o-

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Wri en informed consen was ob ained from all par icipan s. In he conduc of clinical research, human experimen a ion guidelines of he au hors' ins i u-ions were followed.

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navir were de ermined by use of reverse- ranscrip ase polymerase chain reac ion (Amplicor HIV-1 moni or assay; Roche Molecular Sys ems, Branchburg, NJ). RNA levels in plasma and in serum ha are measured by NASBA correla e very well, wi h RNA levels in plasma being, on average, 0.5 log higher han hose in serum [8]. For he par icipan s receiving zidovudine and didanosine combina ion herapy, he number of proviral HIV-1 DNA copies in PBMC was de ermined using a compe i ive quan i a ive polymerase chain reac ion [9].

The correla ion be ween serum RNA load and cellular infec ious load was analyzed in 252 paired serum and cryopreserved PBMC samples derived from he subjec s in groups A and B and from 9 addi ional persons, from whom only samples from a single ime poin were analyzed ($n = 54$). To avoid bias caused by repea ed measuremen s for 1 person, he median of he paired measuremen s was de ermined for each person, and he correla ion be ween he log- ransformed load values was analyzed by use of Pearson's correla ion coefficien (r_p). In cases in which he number of samples was small, Spearman's correla ion coefficien (r_s) was used. The wo- ailed Fisher's exac es and he Mann-Whi ney U es were used o analyze he rela ionship be ween he frequency of produc ively infec ed CD4 T cells or serum RNA copies early in infec ion and he occurrence of and ime o 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 pa ien s. Analysis of he median of paired measuremen s of all par icipan s revealed a s a is ically significan correla ion be ween HIV-1 RNA levels in serum and he frequency of produc ively infec ed cells ($n = 54$, $r_p = .52$, $P < .001$) (figure 1A). Analysis of subgroups of samples, s ra ified by rea men and s age of disease, indica ed ha bo h measures of virus load were highly correla ed in he period be ween he firs 18 mon hs of follow-up and AIDS diagnosis ($n = 43$, $r_p = .71$, $P < .001$) and during rea men ($n = 26$, $r_p = .78$, $P < .001$). However, nei her measure of virus load correla ed in he firs 18 mon hs of follow-up ($n = 18$, $r_s = .06$, $P = .8$) or in he period af er an AIDS diagnosis ($n = 8$, $r_s = -.05$, $P = .9$).

S ra ifica ion of he samples by he absence or presence of SI varian s showed a similar correla ion be ween bo h measures of virus load in persons harboring bo h non-SI and SI varian s ($n = 24$, $r_p = .53$, $P = .007$) and in persons wi h only non-SI varian s ($n = 42$, $r_p = .40$, $P = .008$). Bo h measures of virus load were higher in persons wi h SI varian s (figure 1B). These da a confirm he exis ence of an associa ion be ween viral pheno ype and cellular infec ious load [3] and also show a similar associa ion be ween viral pheno ype and RNA load in serum.

Virus load during the natural course of HIV-1 infection and during treatment. Three differen profiles in serum RNA load and cellular infec ious load were observed in he 23 group A par icipan s. Af er he firs 16 mon hs (range, 2–28) of follow-up un il AIDS diagnosis or o he end of follow-up (mean ime,

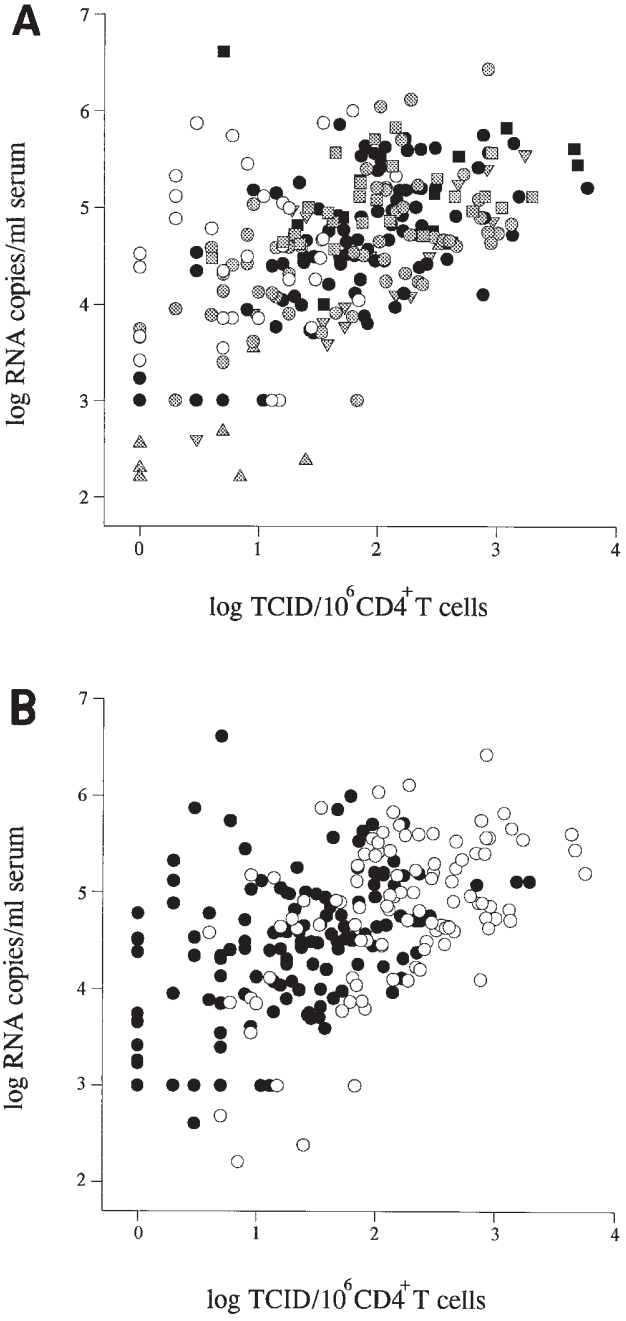

Figure 1. Cross-sec ional analysis of HIV-1 RNA levels in serum and frequencies of produc ively infec ed CD4 T cells (TCID/10^6 CD4 T cells). **A**, Virus load in serum and peripheral blood mononuclear cell samples for 54 persons a 252 iden ical ime poin s during na ural course of infec ion and during rea men . Resul s for 7 subgroups are depic ed: un rea ed (or prior o rea men) persons be ween firs 18 mon hs of follow-up and AIDS diagnosis (●), un rea ed persons before 18 mon hs of follow-up (○), un rea ed persons af er AIDS diagnosis (■), persons rea ed wi h zidovudine (s ippled circles), persons rea ed wi h didanosine (s ippled squares), persons rea ed wi h zidovudine-didanosine combina ion (s ippled riangles), persons rea ed wi h ri onavir (s ippled inver ed riangles). **B**, Samples depic ed in **A** s ra ified by absence (●) or presence (○) of syncy ium-inducing varian s.

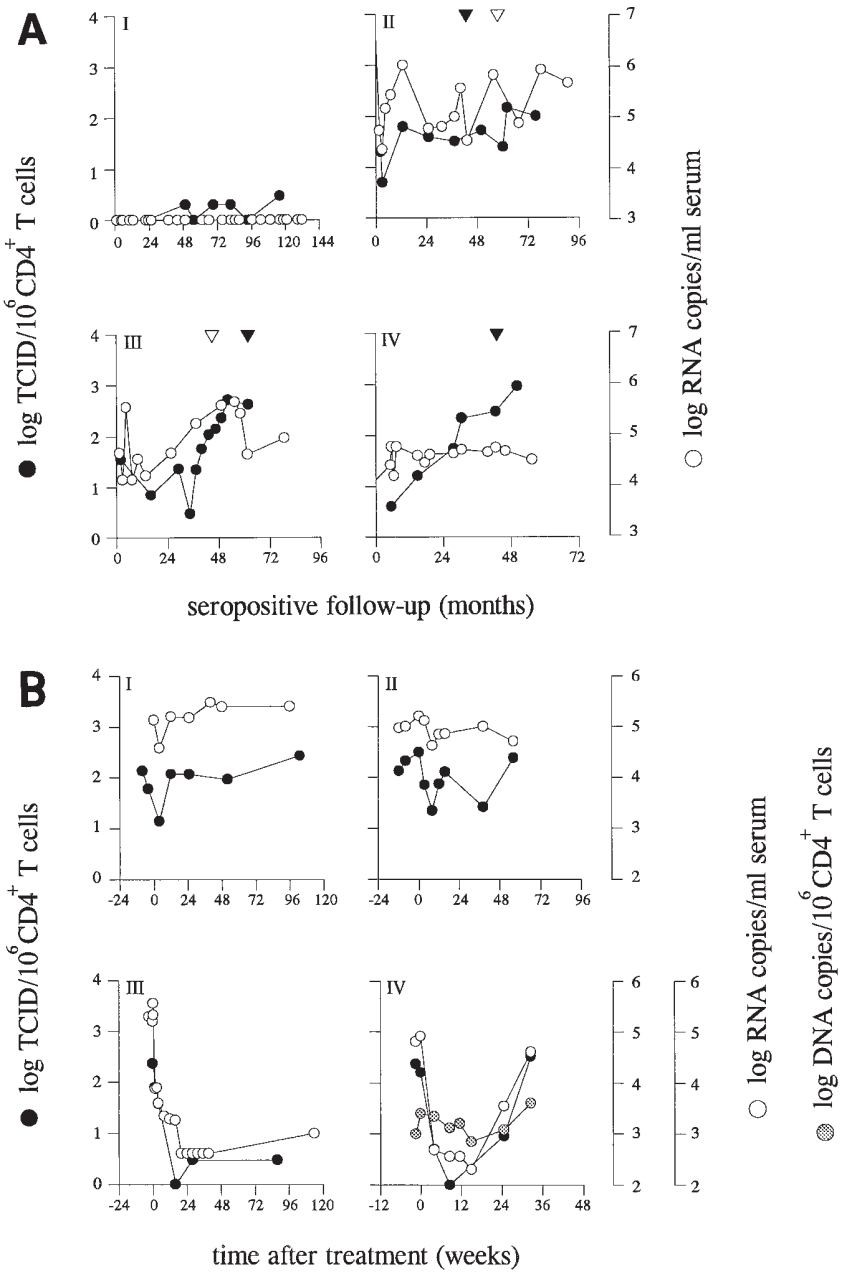

Figure 2. Longi udinal analysis of HIV-1 RNA levels in sera and frequencies of produc- ively infec ed CD4 T cells (TCID/10^6 CD4 T cells) during na ural course of infec ion (**A**) and during an iviral rea men (**B**). **A**, For each pa ern described in ex , 1 represen a ive is given: I, Bo h measures of virus load remain s able a low levels ($n = 5$); II, bo h measures of virus load remain s able a modera e o high levels ($n = 4$); III, bo h measures of virus load increase ($n = 9$); and IV, RNA levels in sera remain s able and frequencies of produc ively infec ed cells increase ($n = 5$). ▼ = ime of AIDS diagnosis; ▽ = ime syncy ium-inducing varian s appeared. **B**, Represen s persons rea ed wi h zidovudine (I; $n = 10$), didanosine (II; $n = 6$), ri onavir (III; $n = 4$), or zidovudine and didanosine in combina ion (IV; $n = 2$). In persons receiving bo h zidovudine and dida- nosine, proviral DNA load in CD4 T cells was also measured.

5.3 years; range, 2.0–9.4), (1) bo h measures remained s able (<1 log increase and/or $<10^4$ RNA copies/mL of serum or <30 TCID/10^6 CD4 T cells a he end of follow-up; $n = 9$); (2) bo h measures increased (>1 log; $n = 9$); or (3) he cellular infec ious load increased while he RNA load remained s able ($n = 5$; figure 2A).

Main enance of low levels of bo h measures of virus load was associa ed wi h long- erm survival (5/5 were long- erm survivors). S able ye modera e o high levels (10^4–10^6 RNA copies/mL of serum and 50–70 TCID/10^6 CD4 T cells) or an increase in bo h measures of virus load was associa ed wi h a progressive clinical course (12/13 were progressors). Of he 5 persons in whom RNA load remained s able a modera e o high levels while he cellular infec ious load increased, 2 were long- erm survivors and 3 progressed o AIDS wi hin 2.8–5.5 years. In mos pa ien s (18/22) rea ed wi h differen an i–HIV-1 drugs (group B), changes in serum RNA load and cellular infec ious load were similar. For bo h persons rea ed wi h he combina ion of zidovudine and didanosine, proviral DNA was addi ionally quan ified bu showed no change (figure 2B).

Predictive value of early virus load measures. In 22 par ici- pan s from group A, cellular infec ious 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/10^6 CD4 T cells; persons in group 2 ($n = 10$) had $\geqslant 10$ TCID/10^6 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 poin of analysis in rela ion o he es ima ed seroconversion 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 significan ly wi h re- spec 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 10^4 copies/mL of serum showed a correla ion be ween 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 s udy, cross-sec ional analysis revealed a s rong correla ion be ween serum HIV-1 RNA levels and cellu- lar 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 rela ed, virus compar men s. Moreover, he simul aneous oc- currence of rebound o baseline levels in serum RNA and frequencies of produc ively infec ed cells in mos rea ed per- sons 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 hese 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 differen urnover 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- life 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- ious virus popula ion is suppor ed by he finding ha he viral quasispecies, which predomina es af er cocul iva ion of pa ien 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 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 curren ly under inves iga- ion.

In accordance wi h he previously described correla 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 correla ion be ween cellular infec ious load and RNA load in serum subs an ia es he use of RNA quan i 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 modera 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 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 la er s ages of infec ion. The increase in infec ed cells in periph- eral 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 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 ex- pressed in differen quan i ies 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 higher corecep 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 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

infec ious load remained s able a modera e o high levels. Conversely, we found ha some persons who main ained s able and high CD4 T cell coun s over prolonged periods had ei her increasing cellular infec ious load and s able, modera ely high virus RNA load or an increase in bo h measures of load (3/8). From hese 10 persons, we observed ha , despi e he previously described inverse correla ion [1–3], virus load and CD4 T cell coun s do no necessarily inversely correla e during he en ire course of infec ion a he individual level. Therefore, addi ional markers should be used o moni or disease progression.

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