Virus and host determinants of HIV-1 infection and AIDS pathogenesis
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Chapter 6

'Temporal relationship between HIV-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

Cross-sectional analysis of 252 paired serum and peripheral blood mononuclear cell (PBMC) samples derived from 54 human immunodeficiency 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 \( r = 0.71, P < 0.001 \)) and during antiviral treatment (\( r = 0.78, P < 0.001 \)). To gain insight into the temporal relationship between both measures of virus load, longitudinally obtained samples from 23 persons with varying clinical course (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 stage of disease in human immunodeficiency type 1 (HIV-1)-infected persons is associated with the level of virus in serum or plasma and the level of cell-associated virus. In addition, plasma levels of HIV-1 RNA early in infection are predictive of the rate of progression. This latter finding, together with the fact that RNA load in plasma directly reflects viral replication, favored plasma RNA load as a progression marker in HIV-1 infection and as a marker for initiation and evaluation of antiviral therapy.

Although both HIV-1 RNA levels in serum or plasma and frequencies of productively infected cells correlate with CD4+ T cell decline and disease progression, their temporal relationship is not fully understood. Therefore, we longitudinally compared virus RNA load in sera and infectious load in peripheral CD4+ T cells from a heterogeneous group consisting of long-term survivors of HIV-1 infection (LTS) and slow or rapid progressors to AIDS, either in the absence or presence of syncytium-inducing (SI) variants. In addition, the effect of treatment on virus RNA load in serum and on cellular infectious load was analyzed for patients receiving antiviral therapy.

SUBJECTS AND METHODS

Group A consisted of 23 participants of the Amsterdam Cohort Studies on AIDS (ACS). Fifteen subjects entered ACS while still seronegative for HIV-1 antibodies. Eight participants were already seropositive at their first visit, and the seroconversion date of these persons was estimated to be 18 months before entry into ACS. Fifteen participants progressed to AIDS with an asymptomatic phase ranging from 2.8 to 11.3 years. Eight subjects, classified as long-term survivors (LTS) were still asymptomatic after 10.2 to 13.7 years and had stable CD4+ T cell counts of >400/μl at least until year 9 of follow-up.

Group B consisted of 22 patients who were participating in ACS or visiting the Academic Medical Centre AIDS clinic. Changes in serum HIV-1 RNA levels and in frequencies of productively infected cells were monitored during treatment with zidovudine (n = 10), didanosine (n = 6), a combination of zidovudine and didanosine (n = 2), or ritonavir (n = 4).

Data were available for all participants as a result of ongoing research in our laboratory, and no specific selection was made.

The biological phenotype of HIV-1 and frequencies of productively infected CD4+ T cells were determined by cocultivation of patient peripheral blood mononuclear cells (PBMC) with fresh, phytohemagglutinin-stimulated, healthy donor peripheral blood lymphocytes under limiting-dilution conditions. The frequency of productively infected cells was calculated from the fraction of negative cultures (\( F = \frac{-ln(Fo)}{d} \)) and was expressed as TCID/10^6 CD4+ T cells [49,260].

Serum RNA levels for untreated subjects and subjects receiving zidovudine or didanosine were determined by use of a nucleic acid-based amplification assay (NASBA; HIV-1 RNA QT; Organon Teknika, Boxtel, The Netherlands). RNA levels for subjects receiving zidovudine and didanosine combination therapy or ritonavir were analyzed using the reverse-transcriptase polymerase chain reaction (Amplicor HIV-1 monitor assay; Roche Molecular Systems, Branchburg, NJ). RNA levels in plasma and in serum that are measured by NASBA correlate very well, with RNA levels in plasma being, on average, 0.5 log higher than those in serum [497]. For...
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 [450].

The correlation between serum RNA load and the 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 was determined for each person, and the correlation between the log-transformed load values was analyzed by use of the Pearson's correlation coefficient ($r_p$). In cases in which the number of samples was small, the Spearman's correlation coefficient ($r_s$) was used. The two-tailed Fisher's exact test and the Mann-Whitney U test 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 and time to AIDS diagnosis.

**RESULTS**

**Cross-sectional analysis of RNA load and cellular infectious load.** Virus load was analyzed in 252 paired serum and PBMC samples derived 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, $r_p$ = 0.52; $P < 0.001$) (Fig. 1a). Analysis of subgroups of samples, stratified by treatment and stage 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, $r_p$ = 0.71; $P < 0.001$) and during treatment (n = 26, $r_p$ = 0.78; $P < 0.001$). However, neither measures of virus load correlated in the first 18 months of follow-up (n = 18, $r_p$ = 0.06; $P = 0.8$) or in the period after an AIDS diagnosis (n = 8, $r_p$ = -0.05; $P = 0.9$).

Stratification of the samples by the absence or presence of SI variants showed a similar correlation between both measures of virus load in persons harboring both NSI and SI variants (n = 24, $r_p$ = 0.53; $P = 0.007$) and in persons with only NSI variants (n = 42, $r_p$ = 0.40, $P = 0.008$). Both measures of virus load were higher in the persons with SI variants (Fig. 1b). These data confirm the existence of an association between viral phenotype and cellular infectious load [208] 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 infectious load were observed in the 23 group A participants. After the first 16 months (range, 2 to 28) of follow-
up until AIDS diagnosis or the end of follow-up (mean time, 5.3 years; range, 2.0 to 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) (Fig. 2a).

Maintenance of low levels of both measures of virus load was associated with long-term survival (5/5 were long-term survivors). Stable yet moderate to high levels (10^4 to 10^6 RNA copies/ml of serum and 50 to 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 to high levels while the cellular infectious load increased, 2 were long-term survivors and 3 progressed to AIDS within 2.8 to 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 (Fig. 2b).

### Predictive value of early virus load measures.

In 22 participants from group A, cellular infectious load was measured at least once between follow-up months 10 and 26. These persons were classified into 2 groups according to their cellular infectious load during this period. Persons in group 1 (n = 12) had ≤10 TCID/10^6 CD4^+ T cells; individuals in group 2 (n = 10) had ≥10 TCID/10^6 CD4^+ T cells.

The number of participants who were seropositive at entry was higher in group 1; as a result, the mean time point of analysis in relation to the estimated seroconversion date was later in group 1 (30 months; range, 16 to 42) than in group 2 (18 months; range, 10 to 34). In group 1, 7 persons still had not progressed to AIDS after 10 to 12 years of follow-up. Of the 5 persons in group 1 who progressed to AIDS, the mean incubation time was 7.2 years (range, 5.2 to 11.3). Nine of 10 persons in group 2 progressed to AIDS within a mean of 4.4 years (range, 2.8 to 5.9). The 2 groups differed significantly with respect to the chance of progressing to AIDS (odds ratio = 12.6, 95% confidence interval = 1.2 to 134.0; P = 0.03) and with respect to the mean time to an AIDS diagnosis in those individuals who progressed to AIDS (P = 0.03).

Similarly, stratification by virus RNA levels below or above 10^4 copies/ml of serum showed a correlation between early RNA levels and disease progression (odds ratio = 17.5, 95% confidence interval = 1.6 to 192.1; P = 0.02) and time to AIDS (P = 0.04).

### DISCUSSION

In the present study, cross-sectional analysis...
revealed a strong correlation between serum HIV-1 RNA levels and cellular infectious load in the period after 18 months follow-up until AIDS diagnosis and during antiviral therapy. Moreover, the kinetics of changes in both measures of virus load were similar in the majority (78%) of persons studied during the natural course of infection and the majority (82%) of persons undergoing treatment.

The finding that changes in RNA load in serum and cellular infectious load in peripheral blood generally coincide suggests that PBMC and serum represent the same, or at least closely related, virus compartments. Moreover, the simultaneous occurrence of rebound to baseline levels in serum RNA and frequencies of productively infected cells in most treated persons suggests that the turnover kinetics of cellular infectious load are similar to those reported for viral RNA in plasma.23,24

This seems to contrast with the finding that mutations in HIV-1 in RNA precede the appearance of these mutations in proviral DNA. However, the absence of a response to antiviral treatment in the proviral DNA load in PBMC suggests different turnover kinetics in the productively infected and the total infected cell populations, which may be due to a longer half-life of cells carrying defective HIV-1. The notion that the kinetics of the total virus population lag behind those of the infectious virus population is supported by the finding that the viral quasispecies, which predominates after cocultivation of patient PBMC, only represents a minor fraction of the total virus population in the same PBMC. However, this quasispecies is the major sequence in the total virus population present in PBMC isolated 6 months later in infection. Whether at any moment in time the infectious virus population in PBMC is identical to the virus population in RNA is currently under investigation.

In accordance with the previously described correlation between the rate of progression and plasma RNA load early in infection, we found that both the cellular infectious load and serum RNA load in the first 1 to 2 years of follow-up were predictive for the length of the asymptomatic phase.

The strong correlation between cellular infectious load and RNA load in serum substantiates the use of RNA quantitation in monitoring disease progression and therapy. Quantitation of cellular infectious load, however, would provide additional relevant information because it reveals the presence of minor variants and the contribution of distinct variants to the virus load even when the load is very low.

In some persons, distinct patterns in both measures of virus load were observed. In all these cases, the cellular infectious load gradually increased, while the RNA load reached moderate to high levels within 12 to 18 months of follow-up and subsequently remained stable. The resulting large discrepancies between both measures of virus load in this period and high fluctuations in RNA load seen early in infection in some persons might contribute to the absence of a correlation between both measures of virus load in the first 18 months of follow-up.

A stable RNA load in serum in the presence of an increasing cellular infectious load in the periphery might reflect a change in the ratio of noninfectious versus infectious virus particles, with the appearance of relatively increased infectious virus in later stages of infection. The increase in infected cells in peripheral blood might also result from an alteration in lymphocyte distribution. During infection, the lymph node architecture is lost, which might result in leakage of infected cells from the lymph nodes. Furthermore, an increase in the cellular infectious load in peripheral blood might be explained by an increase in the number of target cells. Since the different chemokine receptors used as coreceptors for HIV-1 entry are expressed in different quantities on T cells, evolution of HIV-1 variants with altered coreceptor usage and also evolution of variants with higher coreceptor affinity might result in an increased target cell population. In this light, a distinct pattern in serum RNA load and cellular infectious load can be envisioned to result from altered coreceptor usage coinciding with a more cytopathic phenotype. This would result in a higher frequency of productively infected cells yet simultaneously in a decreased half-life of infected cells and the amount of virus produced per cell.

We were surprised to find declining CD4+ T cell counts in half (7/15) of the participants with progressive disease, while either virus RNA load in serum or both RNA load and cellular infectious load remained stable at moderate to high levels. Conversely, we found that some persons who maintained stable and high CD4+ T cell counts over prolonged periods had either increasing cellular infectious load and stable, moderately high virus...
RNA load or an increase in both measures of load (3/8). From these 10 persons, we observed that, despite the previously described inverse correlation\(^{26,208,268}\), virus load and CD4\(^+\) T cell counts do not necessarily inversely correlate during the entire course of infection at the individual level. Therefore, additional markers should be used to monitor disease progression.

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The access to contrast with the finding that mutations in HIV-1 in RNA precede the appearance of these mutations in primate DNA. However, the absence of a response to antiretroviral treatment in the primate DNA leads in PMAC suggests different turnover kinetics in the productively infected and the total infected cell population, which may be due to a longer half-life of cells carrying defective HIV-1. The notion that the kinetics of the total virus population may be behind those of the infectious virus population is supported by the finding that the viral quasispecies which predominates after conditioning of patient PMAC, only represents a minor fraction of the total virus population in the same PMAC. However, this mutation is in the same sequence in the total virus population present in PMAC and must have a longer half-life before it can be detected. Whether or not the viral quasispecies population in PMAC is identical to the virus population in RNA cannot be determined under investigation.

In accordance with the sequential mutations in the correlation between the rate of progression of cell-free RNA load, early in the infected cell population and the rate of progression of cell-free RNA load later in the infected cell population, we have been able to show that the cellular infections load and total RNA load in the first 1 to 2 years of follow-up, were as high as in the later years of the asymptomatic phase.

The strong correlation between cellular multiplication load and RNA load in certain studies suggests the use of RNA quantitation in monitoring disease progression and therapy. Quantitation of quasispecies load, however, would provide additional relevant information because it reflects the progression of disease.