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Dynamics of Intermittent Viremia during Highly Active Antiretroviral Therapy in Patients Who Initiate Therapy during Chronic versus Acute and Early Human Immunodeficiency Virus Type 1 Infection

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The meaning of viral blips in human immunodeficiency virus type 1 (HIV-1)-infected patients treated with seemingly effective highly active antiretroviral therapy (HAART) is still controversial and under investigation. Blips might represent low-level ongoing viral replication in the presence of drug or simply release of virions from the latent reservoir. Patients treated early during HIV-1 infection are more likely to have a lower total body viral burden, a homogenous viral population, and preserved HIV-1-specific immune responses. Consequently, viral blips may be less frequent in them than in patients treated during chronic infection. To test this hypothesis, we compared the occurrence of viral blips in 76 acutely infected patients (primary HIV infection [PHI] group) who started therapy within 6 months of the onset of symptoms with that in 47 patients who started HAART therapy during chronic infection (chronic HIV infection [CHI] group). Viral blip frequency was approximately twofold higher in CHI patients (0.122 ± 0.12/viral load [VL] sample, mean ± standard deviation) than in PHI patients (0.066 ± 0.09/VL sample). However, in both groups, viral blip frequency did not increase with longer periods of observation. Also, no difference in viral blip frequency was observed between treatment subgroups, and the occurrence of a blip was not associated with a recent change in CD4$^+$ T-cell count. Finally, in PHI patients the VL set point was a significant predictor of blip frequency during treatment.

Since the introduction of highly active antiretroviral therapy (HAART), there has been a dramatic decrease in human immunodeficiency virus type 1 (HIV-1)-related mortality and HIV-1 infection has been transformed from a near-uniformly fatal condition into a chronic or subacute disease in substantial numbers of treated patients (13, 15, 26).

Most HAART-treated patients demonstrate "undetectable" levels of HIV-1 RNA in plasma within a few months after the start of therapy. In diverse cohorts, HAART may lead to plasma viral loads (VLs) of less than 500 copies/ml, with the majority of these individuals reaching less than 50 copies of plasma HIV-1 RNA/ml. However, HIV-1 RNA levels at this level do not imply that viral replication has stopped, as evidenced by ongoing viral sequence evolution (14, 39), the expression of viral mRNA species in peripheral blood mononuclear cells (12, 19, 22), and the presence of low but detectable levels of viral RNA in the plasma of infected subjects (6, 9, 38). Failure to completely suppress viral replication with HAART and the presence of the long-lived reservoir of resting latently infected CD4$^+$ T cells are major hurdles to the eradication of HIV-1 infection in vivo (18).

Clinical trials in which well-suppressed patients were periodically tested for plasma VL with assays with a lower limit of detection of 50 copies/ml have shown that most of these patients demonstrate intermittent positive plasma HIV-1 RNA determinations (viral blips) (9, 33, 38). The source and the meaning of this episodic low-level viremia in the setting of seemingly effective HAART remain unclear.

Nevertheless, achieving low levels of viremia during antiretroviral treatment predicts a sustained virologic response. Kempf et al. reported a strong association between the nadir plasma HIV-1 RNA level and the durability of response to treatment (20). Using a more sensitive PCR assay, Raboud et al. observed that patients whose viremia fell below 20 copies/ml were less prone to virological failure than were those who stayed above this threshold (32). Havlir et al. found an association between viral blips and a higher steady state of viral replication, but not virologic failure over 4.5 years of observation, with virologic failure defined as two consecutive plasma VLs above 200 copies/ml (16). Sklar et al. (36) found that the occurrence of transient viremia did not vary with whether the patient was HAART naive or experienced or was currently taking protease inhibitors or not. Also, these transient episodes of low-level plasma viremia did not appear to affect the risk of developing lasting viremia. Whether the emergence of drug-resistant virions is associated with viral blips during treatment is still controversial (5, 17) and deserves further and prompt investigation. Finally, it has been reported that an increased frequency of blips correlates with slower decay of latently infected cells harboring replication-competent provirus (33).

It was recently shown that the variability in the number of

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TABLE 1. Number of PHI and CHI patients in each treatment substudy

Treatment no.	Drugs[a]	No. of patients	
		PHI	CHI
1	LOP-RIT-TEN-EFV-3TC	4	9
2	RIT-SAQ-ZDV-3TC	12	9
3	ABC-APV-3TC	29	0
4	ABC-APV-ZDV-3TC	18	8
5	NLF-RIT-DDI-D4T	0	11
6	IND-ZDV-3TC	8	0
7	NLF-ZDV-3TC	0	10
8	RIT-ZDV-3TC	5	0
	Total	76	47

[a] Abbreviations: LOP, lopinavir; RIT, ritonavir; TEN, tenofovir; EFV, efavirenz; 3TC, lamivudine; SAQ, saquinavir; ZDV, zidovudine; ABC, abacavir; APV, amprenavir; NLF, nelfinavir; DDI, didanosine; D4T, stavudine; IND, indinavir.

viral blips observed in HAART-treated patients during the period of VL suppression cannot be explained by assuming a common probability distribution of blip amplitudes among patients, as would be expected if blips were simply caused by assay variations (28). Thus, this argues against the hypothesis that viral blips represent assay error or variability and suggests that blips have an underlying biological cause. In a more recent study, there was presented an analysis on the dynamics of viral blips with data obtained from 123 patients treated with eight different protease inhibitor-containing regimens. It was shown that viral blips occur substantially at random, viral blip frequency does not change with longer periods of observation, and blip frequency inversely correlates with the baseline $CD4^+$ T-cell counts, i.e., host-specific factors that precede the period of VL suppression (7).

Of the 123 patients analyzed, 76 were treated during acute and early infection, within 3 to 157 days from the onset of symptoms (primary HIV infection [PHI]). The remaining 47 patients started therapy during chronic HIV infection (CHI). The aim of this study was to retrospectively analyze the frequency of viral blips during the period of VL suppression and to compare blip frequency as it relates to the status of infection, acute or chronic, and the virologic set point (level of viremia) at the start of therapy.

MATERIALS AND METHODS

Patients and sampling. The dynamics of viral blips was studied by analyzing data from a database of patients treated with antiretroviral therapy in eight open-label combination therapy trials (Table 1). VL measurements were obtained with the reverse transcription-PCR assay (Cobas Amplicor HIV-1 Monitor Test [v 1.5] Ultrasensitive Assay; Roche Diagnostics Systems, Pleasanton, Calif.) with a lower threshold of detection of 50 copies/ml.

After the start of therapy VLs declined below the limit of detection in most patients. We define the period of sustained VL suppression as starting when the first two consecutive VL measurements below threshold occur. Although blips subsequently may occur, as long as VLs return to below the limit of detection, we say that the period of suppression continues.

The eight clinical trials contained 175 patients. Patients were eliminated from this analysis if they did not show two consecutive VLs below threshold or if the period of suppression was too poorly sampled (less than four blood tests over 3 months). By use of these criteria, a subgroup of 123 patients was available for viral blip analysis. In 121 patients the period of suppression lasted for the entire period of observation. The remaining two patients showed a sustained rebound (VLs of >1,000 HIV RNA copies/ml) of viremia, and the periods of analysis were terminated at the last VL measurement below threshold preceding the rebound. If the patient abandoned the study or the follow-up ended, the period of VL suppression was terminated at the last VL measurement below threshold. Consecutive blips during the period of VL suppression are counted as independent blips. Given the definition of period of VL suppression, a sequence of consecutive blips is always followed in our study by at least one VL below the threshold of detection. Figure 1 illustrates the definitions of the period of sustained VL suppression, a viral blip, and the frequency of viral blips for a representative patient. Acutely infected patients were enrolled in the study up to 157 days from the onset of symptoms. A precise record of the time from the onset of symptoms of acute infection to the time of initiation of therapy was available for 68 of 76 patients treated during acute and early infection. Since patients in CHI groups were observed longer, in order to have the two groups of patients with a similar number of VL measurements and a similar period of VL suppression, we chose to limit the period of VL suppression in both groups to a maximum of 42 VL measurements. If a patient showed a viral blip at the 42nd VL, then the period of VL suppression was extended to the first VL measurement below threshold following the 42nd VL measurement.

Statistical methods. Change in viral blip frequency over time was analyzed by subdividing the period of VL suppression into nonoverlapping and consecutive time windows (see Fig. 4). Given the definition of period of VL suppression, the left extreme of the first time window was fixed at the third VL measurement of the period of suppression. For each group of patients, the equality of the blip frequency means across time windows was tested for significance by a nonparametric repeated measurements analysis of variance (Friedman test) (24).

The correlation between the baseline VL (log number of copies per milliliter) or T-cell count (cells per microliter) at day zero (start of therapy) and frequency of viral blips during the period of VL suppression was tested for significance with the Spearman rank correlation test. Equality of Spearman rank correlations between the PHI and CHI groups was tested with a bootstrap method. With this method, pseudogroups of 76 and 47 patients were created by randomly selecting patients with replacement from the PHI and CHI groups, respectively. From the pseudogroups, Spearman rank correlations were calculated and their difference was formed. This maneuver was repeated 10,000 times, and the standard deviation of these 10,000 differences was calculated. The ratio of the original difference to the bootstrap standard deviation forms the test statistic, which is standard normal on the null hypothesis. To test equality of the two correlations from data in a single group, a similar procedure was used; here for each pseudogroup the difference of the absolute values of the correlations was calculated.

If the increase in virions during a blip impairs the recovery of CD4 count or causes a decrease in CD4 count, the relative change in CD4 counts on two successive visits, starting with a nonblip and ending with a blip, should tend to be lower than the analogous relative change ending in a nonblip. Thus, the difference in these relative changes should tend to be negative. If each patient produced a single difference, a simple *t* test could be calculated, but each patient provides many differences, one for each pair of successive visits. Multiple outputation was used to accommodate the multiple differences (11). For each

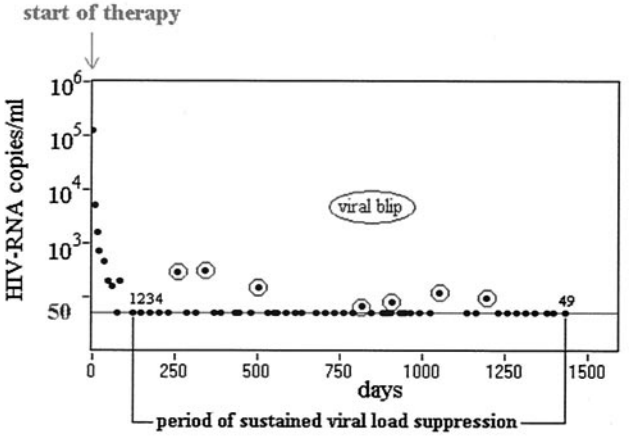

FIG. 1. Definition of the period of sustained VL suppression, viral blip, and frequency of viral blips during the period of VL suppression. During the period of sustained VL suppression this patient showed seven viral blips out of 49 VL measurements. Thus, the frequency of viral blips was $7/49 = 0.143$ sample^{-1}.

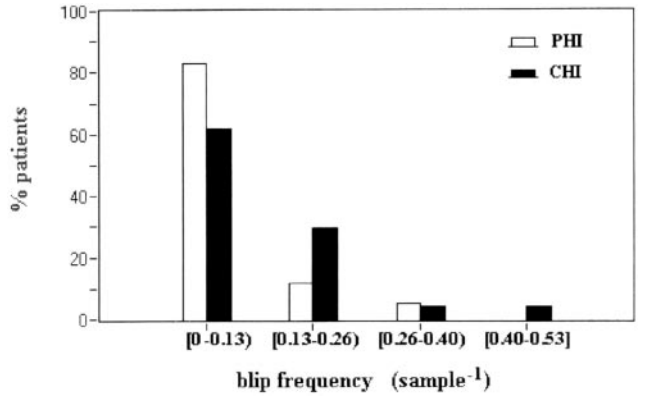

FIG. 2. Distribution of viral blip frequency among primary HIV-infected patients (PHI) and chronically HIV-infected patients (CHI). The histograms were obtained by subdividing the range of observed viral blip frequencies per patient in four subintervals and by calculating the percentage of patients showing a viral blip frequency within a given subinterval (*y* axis).

patient a difference was selected at random and the mean and variance of these differences over all patients were calculated. This was done 10,000 times, and the associated means and variances were combined to form a test statistic that is standard normal on the null hypothesis. Because a CD4 change may be delayed relative to the occurrence of a blip, we also tested whether the blip pattern predicted a change in CD4 count with a delay of one visit. Thus, for example, if the blip pattern is based on visits 3 and 4, we then examined the relative $CD4^+$ T-lymphocyte change based on visits 3 and 5.

Differences for viral blip frequencies between PHI and CHI groups were tested for significance by the nonparametric Mann-Whitney test.

RESULTS

The general pattern of VL decay observed after initiation of HAART consisted of a fast first phase followed by a slower second phase, consistent with previous observations (30). For all patients, in approximately 2 to 6 months, antiretroviral therapy reduced the viral load below the threshold of the reverse transcription-PCR assay adopted in this study, i.e., 50 copies/ml.

Viral blips in the PHI group. The PHI group consisted of 76 patients observed for a period of 737 ± 355 days (mean ± standard deviation). VL measurements (VL) were obtained on average every 33 ± 22 days, yielding a total of 23 ± 11 VL measurements per patient. A total of 108 blips were observed in the PHI group out of a total of 1,773 VL measurements. The mean and median amplitude of blips were 131 ± 105 and 86 copies/ml, respectively. The distribution of the percentage of patients versus blip frequency is shown in Fig. 2. In the PHI group, 32 patients (45%) did not show viral blips during the period of sustained VL suppression. The distribution decreases with higher values of viral blip frequencies. Thus, most patients have low viral blip frequencies and few patients have high frequencies. The mean and median frequencies were 0.066 ± 0.09 and 0.04 sample^{-1}, respectively.

Patients in the PHI group were treated with six different HAART regimens (Table 1). The mean viral blip frequency within each treatment group is shown in Fig. 3. No statistically significant difference between means with respect to frequency was detected by analysis of variance (Kruskal-Wallis test, $P = 0.36$) or between two treatment groups ($P > 0.05$, Mann-Whitney test followed by Bonferroni's correction). Figure 4 shows the mean frequency of viral blips observed in nonoverlapping and consecutive time windows of the period of VL suppression. The analysis was restricted to patients who were observed more than 2 years during the period of sustained VL suppression, with each time window fixed to 6 months and the analysis limited to the first 2 years (Fig. 4a), or to patients who were observed more than 3 years during the period of sustained VL suppression, with each subinterval fixed to 9 months and the analysis limited to the first 3 years (Fig. 4b). Viral blip frequency did not increase with longer periods of observation (2 years: $n = 41$, Friedman test, $P = 0.59$; 3 years: $n = 14$, Friedman test, $P = 0.82$). The lack of statistically significant changes in viral blip frequency was also confirmed in other smaller subgroups of patients with different time window lengths or periods of observation as well as in subgroups of patients who started therapy with higher VLs (data not shown).

A positive correlation was observed between the VL at the start of therapy (V_0) and the frequency of viral blips (*f*) during the period of suppression. A weaker negative correlation was found between *f* and the $CD4^+$ T-cell count ($CD4_0$) at the start

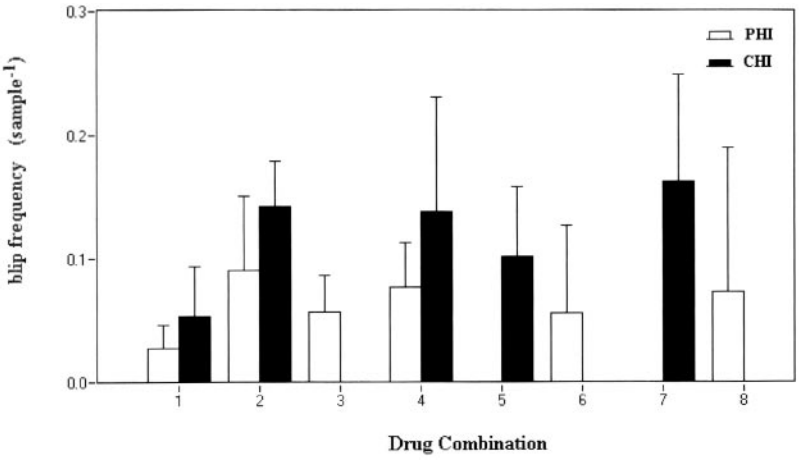

FIG. 3. Mean (±2 standard errors) viral blip frequency observed with each of the eight drug combinations listed in Table 1.

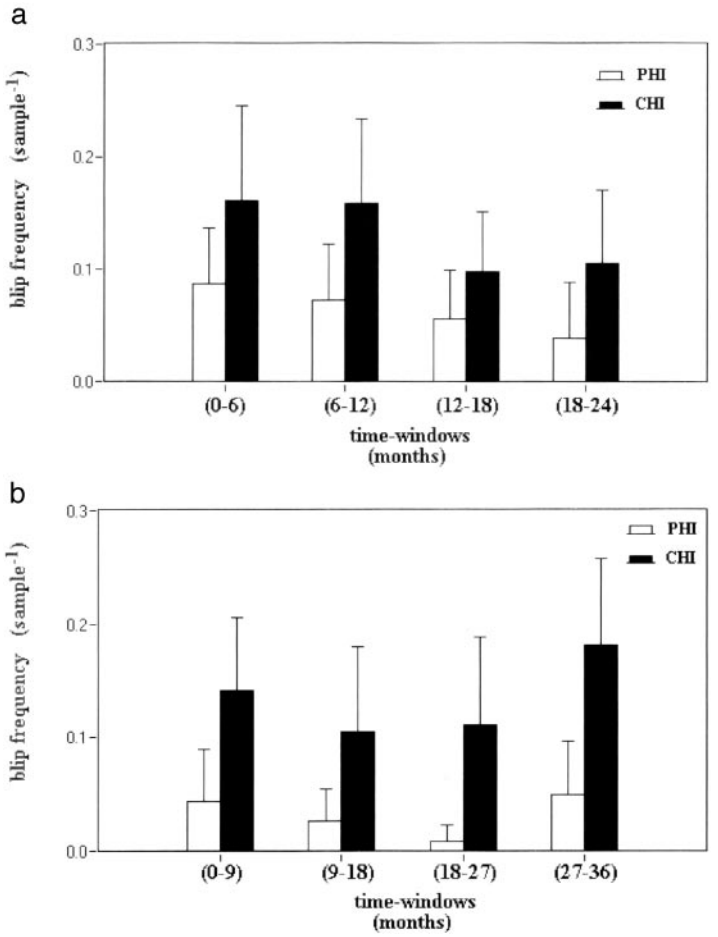

FIG. 4. (a) Mean (±2 standard errors) viral blip frequency for each 6-month period during the first 2 years of sustained VL suppression. The analysis was restricted to patients who were observed more than 2 years (during the period of sustained VL suppression). $n = 41$ for PHI group (empty bars); $n = 31$ for CHI group (filled bars). (b) Mean (±2 standard errors) viral blip frequency for each 9-month period during the first 3 years of sustained VL suppression. The analysis was restricted to patients who were observed more than 3 years (during the period of sustained VL suppression). $n = 14$ for PHI group (empty bars); $n = 19$ for CHI group (filled bars).

of therapy, but no correlation was observed with the baseline $CD8^+$ T-cell count ($CD8_0$) (Table 2). As shown in Fig. 5, by grouping patients in subgroups based on quartiles of the baseline $\log_{10}$ VL, $\log_{10} V_0$, a highly significant difference between the mean viral blip frequency in each subgroup was detected using a nonparametric analysis of variance (Kruskal-Wallis test, $P = 0.0008$). Statistical analysis based on multiple outputation (11) (see Materials and Methods) did not reveal any associations between the occurrence of a blip and relative changes in either $CD4^+$ or $CD8^+$ T-cell counts at the time of the occurrence of a blip or with a delay of one visit from the occurrence of a blip ($P > 0.05$).

The number of days between onset of symptoms compatible with PHI and the initiation of therapy (time S) could be esti-

TABLE 2. Correlation of baseline characteristics with blip frequency[b] (f)

Baseline characteristic	Correlation coefficient (ρ) (P)			
	f			S^c (PHI; $n = 68$)
	PHI		CHI ($n = 47$)	
	$n = 76$	$n = 68$		
V_0	0.47 (9.1×10^{-6a})	0.51 (4.0×10^{-6a})	0.24 (0.048^a)	−0.57 (2.4×10^{-7a})
$CD4_0$	−0.34 (0.010^a)	−0.34 (0.002^a)	−0.23 (0.063)	0.04 (0.38)
$CD8_0$	−0.04 (0.37)	−0.001 (0.47)	0.12 (0.22)	−0.24 (0.024^a)

[a] Significant ($P < 0.05$).
[b] Abbreviations: f, blip frequency; V_0, baseline VL; $CD4_0$, baseline $CD4^+$ T-cell count; $CD8_0$, baseline $CD8^+$ T-cell count; S, time of therapy start.
[c] S could be identified only in a subgroup of 68 PHI patients.

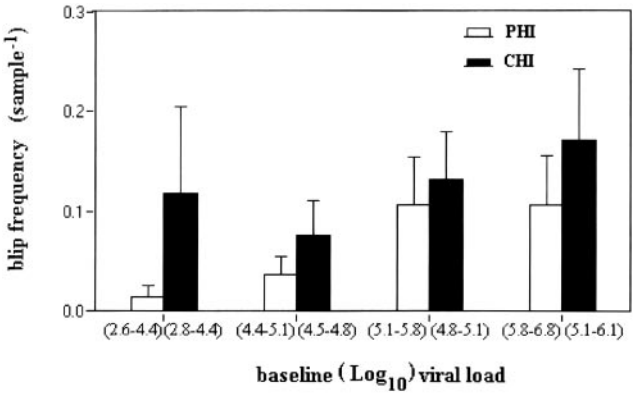

FIG. 5. Mean (±2 standard errors) viral blip frequency in four subgroups of patients who started therapy during PHI (empty bars) and CHI (filled bars). Patients were grouped based on increasing interquartile ranges of $\log_{10}$ VL at the day of start of therapy (V_0). The interquartile ranges of $\log_{10} V_0$ are shown on the x axis.

mated for 68 out of 76 patients based on information recorded from PHI patients when they were enrolled in the study. In this smaller subgroup, the correlation between baseline characteristics and viral blip frequency (f) does not substantially change from that found in analyzing all 76 patients (Table 2). We observed a negative correlation between the VL at the start of therapy and the therapy start time S (Table 2; Fig. 6), which suggests that higher VL values are indicative of the early peak of viremia, whereas lower loads may indicate that the patient has reached the "trough" of viremia or that the acute phase of infection is substantially passed. However, no correlation was found between therapy start time S and blip frequency f, which suggests that V_0 independently correlates with f and time S. The positive correlation between V_0 and f suggests for PHI patients that there are factors preceding the initiation of treatment that determine the patients' tendency to show blips during treatment.

To further confirm this, we divided the PHI patients into early ($S \leq 40$ days, $n = 34$, $S = 21 \pm 10$ days) and late ($S > 40$ days, $n = 34$, $S = 72 \pm 27$ days) subgroups depending on the time at which they started therapy. In the late subgroup we expect V_0 to better reflect the viral set point and we found an increased correlation between V_0 and f ($\rho = 0.72$, $P = 9.4 \times 10^{-7}$) for this subgroup compared with the early subgroup ($\rho = 0.32$, $P = 0.033$) with the difference in Spearman rank correlations being statistically significant ($P = 0.028$). The correlation between blip frequency and $CD4_0$ only slightly increased in the late group over the early group, but this increase was not statistically significant. Moreover, a significant inverse correlation between the baseline VL and time S was observed in the early group ($\rho = -0.46$, $P = 0.003$). The latter correlation confirms that in this subgroup of patients the baseline VL is still close to the peak of viremia of PHI, and the evidence that the correlation is inverse suggests that the majority of early patients started therapy during the decreasing phase of viremia or after the peak. Finally, in the late subgroup the Spearman rank correlation between baseline VL and blip frequency was significantly higher than the correlation between the baseline $CD4^+$ T-cell count and the blip frequency (bootstrap method: ρ_{late} [V_0, f] $\neq$ ρ_{late} [$CD4_0$, f], $P = 0.013$, 95% confidence interval [CI] of the difference of modules [0.08, 0.72]). Similar patterns of correlations were observed when the cutoff of number of days from the onset of symptoms to treatment was chosen as between 30 and 40 days (data not shown), which has been suggested as the minimum time from the peak of viremia to the viral set point (23). This parametric analysis makes evident that the later that patients start therapy from the onset of symptoms, and hence the more that V_0 reflects the viral set point if left untreated, the higher the correlation between V_0 and blip frequency f.

Viral blips in the CHI group. The CHI group consisted of 47 patients observed for a period of 853 ± 426 days (mean ± standard deviation). VL measurements were obtained on average every 32 ± 16 days, yielding a total of 27 ± 14 VL measurements per patient. The total number of blips observed in the CHI group was 171 out of 1,287 VL measurements. The

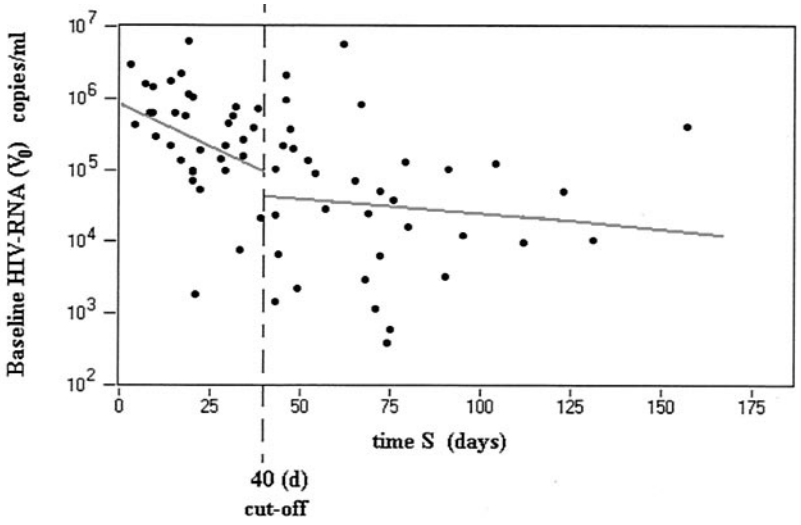

FIG. 6. Baseline VL, V_0, versus time from onset of symptoms to start of therapy, S, in PHI patients. Shown are regression lines for early (left) and late (right) subgroups.

mean and median blip amplitudes were 176 ± 145 and 117 HIV-1 RNA copies/ml, respectively. The distribution of percentage of patients versus blip frequency is given in Fig. 2. Ten patients (21%) did not show viral blips, and the mean and median frequency were 0.12 ± 0.12 and 0.11 sample^{-1}, respectively.

Patients in the CHI group were treated with five different HAART regimens (Table 1). The mean viral blip frequency within each treatment group is shown in Fig. 3. No statistically significant difference between means was detected by analysis of variance (Kruskal-Wallis test, $P = 0.18$) or between two treatment groups ($P > 0.05$, Mann-Whitney test followed by Bonferroni's correction). Viral blip frequency did not increase with longer periods of observation, as shown in Fig. 4 (2 years: $n = 31$, Friedman test, $P = 0.27$; 3 years: $n = 19$, Friedman test, $P = 0.17$). The lack of statistically significant changes in viral blip frequency was also confirmed in other smaller subgroups of patients with different time windows and periods of observations as well as in subgroups of patients who started therapy with higher VLs (data not shown). A positive, weakly significant Spearman rank correlation was observed between the VL at the start of therapy (V_0) and the blip frequency (f) (Table 2), but no significant correlations were observed between the baseline CD4$^+$ T-cell count ($CD4_0$) or the baseline CD8$^+$ T-cell count ($CD8_0$) and the viral blip frequency (Table 2). When patients were grouped in subgroups based on quartiles of the baseline VL, V_0, no statistically significant difference among the mean viral blip frequencies in each subgroup was detected (Kruskal-Wallis test, $P = 0.16$). We did not observe any associations between the occurrence of a blip and relative changes in either CD4$^+$ or CD8$^+$ T-cell counts at the time of the occurrence of a blip or with a delay of one visit from the occurrence of a blip ($P > 0.05$).

Comparison between PHI and CHI groups. The mean viral blip amplitude differed between the two groups (175 ± 145 HIV-1 RNA copies/ml for CHI versus 131 ± 105 HIV-1 RNA copies/ml for PHI; $P = 0.001$, Mann-Whitney test), being higher in the chronic infection group. The frequency of viral blips was also significantly higher in CHI than in PHI patients (0.122 ± 0.12 for CHI group versus 0.066 ± 0.09 for PHI group; $P = 0.001$, Mann-Whitney test). Thus, patients who started therapy during chronic infection show approximately a twofold-higher tendency to show viral blips than do patients who started therapy during acute infection. In both groups, the percentage of patients with a given blip frequency decreased as the blip frequency increased. However, Fig. 2 makes evident that in CHI the frequency distribution is flatter than that in PHI, as confirmed by the different distance between the mean and the median for each group. Thus, the frequency of viral blips is more homogenous among chronically infected patients than among acutely infected patients. In both groups, the mean blip frequency did not differ between treatment subgroups. Nonparametric stratum-specific comparisons (21) between PHI and CHI groups were also performed by limiting the analysis to treatments 1, 2, and 4 (Table 1), which were given to both PHI and CHI patients, or by grouping antiretroviral regimens (strata) characterized by the same number of drugs (treatments 1, 2 + 4, and 3 + 6 + 8 in PHI group and 1, 2 + 4 + 5, and 7 in CHI group). Both analyses showed a statistically significant lower mean blip frequency in the PHI group than in the CHI group of patients ($P = 0.034$ and 0.002, respectively), suggesting that the lower tendency to show blips in PHI than in CHI patients is independent of drug regimens.

Also, viral blip frequencies did not increase with longer periods of observation. We did not observe any associations between the occurrence of a blip and relative changes in either CD4$^+$ or CD8$^+$ T-cell counts at the time of the occurrence of a blip or with a delay of one visit from the occurrence of a blip in both groups of patients. The viral blip frequency correlates with the baseline VL and the CD4$^+$ T-cell count at the start of therapy in both groups, but not with the baseline CD8$^+$ T-cell count. The ability of baseline VL to predict blip frequency appears higher in the PHI group than in the CHI group; however, this increased correlation is not statistically significant when tested with a bootstrap method (Spearman rank correlation: $\rho_{PHI}[V_0, f] = 0.47$, $P = 9.1 \times 10^{-6}$, $n = 76$; $\rho_{CHI}[V_0, f] = 0.24$, $P = 0.048$, $n = 37$; bootstrap method: $\rho_{PHI}[V_0, f] \neq \rho_{CHI}[V_0, f]$, $P = 0.21$, 95% CI of the difference [−0.13, 0.58]). Finally, the ability of baseline VL, but not $CD4_0$ (data not shown), to predict blip frequency is statistically significantly higher in the late subgroup of PHI patients, i.e., those who started therapy after 40 days from the onset of symptoms, than in the CHI group of patients (bootstrap method: $\rho_{late}[V_0, f] \neq \rho_{CHI}[V_0, f]$, $P = 0.012$, 95% CI of the difference [0.09, 0.83]).

DISCUSSION

The inability of present regimens to suppress ongoing viral replication as well as the existence of a stable and long-lived reservoir of proviral DNA-positive cells contributes to HIV-1 persistence in infected individuals (9, 12, 38). Many patients with "undetectable" VL in plasma by standard assays with a lower limit of detection of 50 copies/ml have persistent levels of viremia in plasma that can be detected by more sensitive assays (6, 8, 38). In all 22 HAART patients studied by Dornadula et al. (8) with VLs in plasma below 50 copies/ml, HIV-1 RNA could be detected at a mean level of 17 copies/ml. The existence of low viral steady states was suggested by the work of Furtado et al. (12), in which constant low levels of *tat*, *rev*, and *gag* mRNA were detected in HAART-treated patients with VLs in plasma below 50 copies/ml, and is predicted by dynamic models of HIV-1 infection and treatment in which drug sanctuaries exist (2). In this latter study the basic model of HIV dynamics (29, 31) was extended to consider the interaction between peripheral blood and other compartments with different levels of drug penetrance. Thus, VL fluctuations over 50 copies/ml might represent transient perturbations of a new (drug-induced) low steady state.

In this study the frequency of viral blips was analyzed during the period of VL suppression in a group of 76 patients who started therapy during acute and early HIV-1 infection (PHI) and in a group of 47 patients who started therapy during chronic infection (CHI). All these patients achieved VL suppression below the limit of detection of 50 copies/ml. Subsequently, a few patients showed a very high tendency to show viral blips, whereas other patients showed a lower frequency of blips and other patients did not exhibit any blips during the period of observation. In the patients studied, all in clinical trials, it appears that the blip frequency does not increase with time on therapy, suggesting that reduced adherence as a result

of treatment fatigue with prolonged therapy is not a major factor in generating the observed blips. Also, there were no significant differences in the frequency of viral blips as a function of treatment regimen. Thus, the viral blip frequency appears to be different between patients but approximately constant for each patient. The viral blip frequency is significantly higher in CHI patients than in PHI patients, suggesting that the duration of infection prior to the initiation of therapy (or perhaps the stage of infection pretherapy) is, to some extent, predictive of the tendency to show blips.

The possible benefits of "hitting early" include the limitation of viral spread during PHI (10, 27), the preservation of immune functions (1, 3), and a reduced number of viral quasispecies, some of which may carry drug-resistant mutations (4, 35). Here we have shown that starting therapy early also leads to a reduced viral blip frequency. While it is unclear whether drug-resistant strains are required to generate blips (5, 17), the lack of an increase in viral blip frequency with longer periods of observation as well as the lack of rebounds of viremia in patients showing blips supports the hypothesis that blips are generated by archival drug-sensitive virions (17). Thus, the lower blip frequency in PHI patients may be indicative of other biological phenomena.

The limitation of viral spread due to early intervention might suggest a lower undetectable viral level in PHI patients than in CHI patients. A lower VL may have an impact on the ability of blips to exceed the threshold for detection. If viral blips are thought of as transient elevations of VL of the same order of magnitude in PHI and CHI, then the sum of VL steady state and the blip may be different between the two groups of patients if their steady states during therapy are different. A lower mean residual viremia has been reported in PHI than in CHI patients in a study where a supersensitive assay with a threshold of 3 copies/ml was adopted (37). Thus, the probability of observing a viral blip in blood would ultimately be higher in CHI patients, for whom the VL during therapy is expected to be higher than that in PHI patients.

The difference in blip frequency between PHI and CHI might also be explained by differences in immune function (or perhaps HIV-1-specific immune responses) in the two groups of patients, since the functionality of T-helper cells may be relatively preserved in patients treated early (1). Recently, Ortiz et al. (25) showed that HIV-1-specific $CD8^+$ T cells can significantly increase in number during HAART in subjects treated early after infection who have episodes of viremia, compared to chronically infected subjects.

In both PHI and CHI groups, a negative but weak correlation was observed between the $CD4^+$ T-cell count at the start of therapy and the frequency of viral blips, which suggests that factors beyond the history of infection might play a role in determining the tendency for each patient to show viral blips during therapy. Interestingly, we have observed a positive and highly significant correlation between the VL at the start of therapy and the frequency of viral blips in patients treated during primary infection (Spearman rank correlation: $\rho\,[V_0, f] = 0.47, P = 9.1 \times 10^{-6}, n = 76$). Based on the records of clinical symptoms reported during the week in which these patients were diagnosed and subsequently recruited in the study, we defined a subgroup of patients who started therapy late ($\geq$40 days) after the onset of symptoms (late subgroup). In this subgroup the correlation between baseline VL and blip frequency was stronger than that in the entire population or the complementary early subgroup (Spearman rank correlation: $\rho_{\text{late}}\,[V_0, f] = 0.72, P = 9.4 \times 10^{-7}, n = 34$; $\rho_{\text{early}}\,[V_0, f] = 0.32, P = 0.033, n = 34$). A bootstrap-based method testing the difference in the Spearman rank correlations between the two independent groups of observations showed that the correlations in the early and late subgroups are significantly different, suggesting that the baseline VL of patients who start therapy late is a better predictor of viral blip frequency during treatment than is that of patients who start therapy early. The baseline VL in patients who start therapy relatively later may be more indicative of the set points that each patient would achieve if left untreated and hence might correlate with the steady-state VL that the patients achieve with therapy. (Or in other words, those individuals with yet-undefined host factors or immune responses that predispose to higher levels of viremia in plasma may indeed control viremia less efficiently during therapy.)

In conclusion, from this analysis it appears that viral blip frequency during the period of VL suppression does not increase with longer time of observation, and thus, treatment fatigue is an unlikely explanation for the origin of most blips. We also find that PHI patients have a lower tendency to show viral blips than do CHI patients. Thus, viral blip frequency is somewhat dependent on the history of infection. The different expected level of HAART-induced restoration of the immune system in PHI versus CHI patients might be an explanation for the observed difference in blip frequency between the two groups. However, one is also tempted to speculate that other factors that may characterize infection in PHI patients, such as lower viral diversity, less impairment of helper T-cell function, and possible limitations of viral spread, might ultimately determine a lower steady-state VL on therapy. Having a lower (but undetectable) VL reduces the chance for transient increases in viral replication to push plasma VL over the assay threshold. If this is the case, then lower blip frequency is indicative of low VL and better suppression of viral replication. Here we did not detect any significant differences in blip frequency among different treatment regimens, but our study was not powered to examine such differences. This result is in agreement with the study by Sklar et al. (36), where the authors found that the occurrence of blips was independent of the antiretroviral regimen, but in contrast with the recent observation by Ramratnam et al. (34), where intensification of antiretroviral therapy was shown to reduce the occurrence of blips. Nevertheless, our analysis shows that the higher blip frequency in CHI than in PHI patients is independent of drug regimen, without excluding the possibility that different drug regimens might differentially affect the occurrence of blips. In addition, PHI and CHI patients on the same regimen or on regimens characterized by the same number of drugs may have different blip frequencies, and lower blip frequency may be a surrogate for better tolerance and effectiveness of a particular regimen. Further studies would be required to prospectively test these speculations.

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