HIV-1 viral rebound dynamics after a single treatment interruption depends on time of initiation of highly active antiretroviral therapy

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Objective: An important pending question is whether temporary highly active antiretroviral therapy during primary HIV infection can influence viral rebound dynamics and the subsequently established viral setpoint, through preservation and enhancement of HIV-1-specific immune responses, or through other mechanisms.

Methods: We included all patients from two prospective studies who underwent a single treatment interruption while being well suppressed on highly active antiretroviral therapy. One group started highly active antiretroviral therapy during primary HIV infection, and the other group started it during chronic HIV infection with CD4 cell counts above 350 cells/μl. Data were collected up to 48 weeks from treatment interruption. The median time to viral rebound was analysed for three levels of viraemia: 50, 500 and 5000 copies HIV-RNA/ml plasma.

Results: The median time to viral rebound was significantly longer in primary HIV infection patients ($n = 24$) than in chronic HIV infection patients ($n = 46$): 8 versus 4 weeks ($P < 0.001$ for all three endpoints). In two primary HIV infection patients, no rebound of plasma HIV-1 RNA over 50 copies/ml occurred. In the first 4 weeks after treatment interruption, CD4+ T-cell counts declined with a median of −5.0 cells/μl blood per week in the primary HIV infection group and −45 cells/μl blood per week in the chronic HIV infection group ($P < 0.05$). From week 4 to 48, the decline in CD4+ T-cell count was similar in both groups.

Conclusion: Plasma viral load and CD4 dynamics after a single interruption of highly active antiretroviral therapy were different for primary HIV infection and chronic HIV infection patients. Viral rebound is delayed or absent and early CD4 cell count decline after treatment interruption is less pronounced in primary HIV infection patients.

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Introduction

The HIV-1 viral load in plasma is highly variable among patients, and the plasma viral setpoint is an important prognostic marker of disease during HIV-1 infection [1–4]. An important question still pending is whether temporary highly active antiretroviral therapy (HAART) during primary HIV infection (PHI) can influence the viral setpoint. Early observations showed that robust HIV-1-specific CD4+ T-cell responses were present in patients who underwent early antiretroviral treatment, and that after treatment interruption viral control was achieved in a large proportion of patients [5,6]. Prolonged observation of these patients showed that viral control was not sustained [7]. In a number of cohort studies, it was shown that the viral setpoint was indeed lowered after treatment interruption of initially treated PHI patients as compared with patients who had not received the treatment [6,8–10], but this was not confirmed in other studies [11–14].

The dynamics of plasma HIV-1 RNA rebound in patients who initiate HAART during PHI and then interrupt treatment may also reflect the balance between viral fitness and the immune system. After treatment interruption in patients who started HAART during chronic HIV infection (CHI), plasma viral load returned to its pretreatment setpoint within several weeks [15,16]. In the present study, we compared the dynamics of the plasma viral load after prolonged treatment interruption in two cohorts of patients, those who initiated HAART during PHI versus those during CHI.

Patients and methods

We included patients from two prospective studies that were completed in 2005, who underwent a single treatment interruption while being well suppressed on HAART.

A group of 24 patients initiated HAART during PHI and subsequently interrupted treatment. PHI was defined as having a negative or indeterminate western blot for HIV-1 antibodies in combination with a positive test for either p24 antigen or a detectable HIV-1 RNA concentration, or a negative result on an HIV screening test within 6 months before seroconversion. Patients in the PHI group were treated with an intensive HAART regimen, consisting of two or three nucleoside analogue reverse transcriptase inhibitors (NRTIs), a non-nucleoside reverse transcriptase inhibitor (NNRTI) and a (boosted) protease inhibitor [17,18]. Intensive HAART was commonly simplified after 1 year of treatment. Five patients started mycophenolate mofetil (MMF) concomitantly with their HAART. The maximal duration of MMF use was 100 weeks, MMF was discontinued before HAART in all cases [18].

The other group consisted of 46 patients who had started HAART during CHI with CD4 cell counts of 350 cells/μl or higher, when guidelines still advised to do so. These patients participated in the TRIESTAN study. In 46 of 71 participants in the TRIESTAN study, treatment was stopped and patients were subsequently monitored. All 46 patients had been treated with combinations of three antiretroviral drugs. At the time of treatment interruption, 61% (28 of 46) of these patients used, in addition to two NRTIs, an NNRTI, 21% (10 of 46) a protease inhibitor (boosted with low-dose ritonavir except for nelfinavir), and 17% (eight of 46) used a triple nucleoside-containing regimen [19].

In both groups, after-treatment-interruption visits were planned every 4 weeks up to week 12 and every 12 weeks thereafter, for measurement of plasma HIV-1 RNA and CD4+ T-cell counts. Data were used from time of treatment interruption to the end of follow-up, and censored when HAART was reinitiated. Patients were advised to restart antiretroviral therapy according to the current guidelines.

HIV-RNA was measured with the bDNA 3.0 assay (Bayer Diagnostics, Berkeley, California, USA) or the NucliSens Ultrasensitive assay (BioMerieux, Boxtel, The Netherlands). Low or undetectable plasma viral load results with the NucliSens assay were repeated using the bDNA assay and checked for non-B subtypes.

Statistics

Baseline characteristics of the groups were compared with a chi-squared or Mann–Whitney *U* test. The time to viral rebound was compared between the CHI and PHI groups by Kaplan–Meier plots and tested for significance using the log rank test. Viral rebound was defined as having a single measurement of plasma HIV-1 RNA over 5000, 500 or 50 copies/ml. Relations between the time to rebound and patient characteristics were analysed using Cox regression. Characteristics included the plasma viral load at the start of HAART, the CD4+ T-cell count at the start of HAART as well as at the time of treatment interruption, the presence of human leucocyte antigen (HLA) alleles B27 and B57, the use of MMF and the duration of HAART.

Individual CD4+ T-cell slopes were estimated using linear regression. Differences in the median CD4+ T-cell change per week were tested using a Mann–Whitney *U* test. Statistical analysis was done with SPSS version 12 (SPSS Inc., Chicago, Illinois, USA).

Results

Characteristics of the patients are summarized in Table 1. At the time of initiation of HAART, patients in the PHI group had a higher median plasma viral load ($P < 0.05$). The time between diagnosis of PHI and start of HAART varied between 1 and 46 days. At the time of treatment interruption, PHI patients were shorter on HAART than CHI patients (91 versus 306 weeks, $P < 0.001$) and they had a lower median CD4+ T-cell count ($P < 0.05$). All patients had a plasma HIV-RNA less than 50 copies/ml at the moment of treatment interruption, except for two patients in the PHI group. In one of these patients, plasma HIV-1 RNA was measured with an assay that had a different lower level of quantification. The other patient had a plasma viral load blip of 231 copies HIV-RNA/ml at treatment interruption.

After treatment interruption, all patients in the PHI group remained off therapy for 48 weeks. In the CHI group, five patients reinitiated antiretroviral therapy before week 48, at their own request. Their plasma HIV-1 RNA at the time therapy was reinitiated ranged from 4.1 to 5.0 log copies/ml, the CD4+ T-cell counts from 440 to 1030 cells/μl blood.

Longitudinal plots of plasma HIV-1 RNA after treatment interruption showed different dynamics in patients with PHI versus CHI (Fig. 1a). The median time to a viral rebound in plasma was significantly longer in PHI patients than in CHI patients: 8 versus 4 weeks for all three applied levels of viral rebound (50, 500 and 5000 copies/ml, $P < 0.001$ in all cases) (Fig. 1b–d). Four patients in the PHI group had a plasma HIV-1 RNA concentration below 50 copies/ml at 48 weeks after treatment interruption. Two of them had a transient plasma viraemia of 650 and 2065 copies/ml between the moment of treatment interruption and week 48, the other two remained below the lower limit of quantification of the assay for the entire 48 weeks.

Univariate Cox proportional hazards modelling of all patients showed that no relations existed between the time to rebound over 5000 copies HIV-1 RNA/ml and patient characteristics, including the presence of protective HLA alleles or the use of MMF (data not shown). The start of HAART during CHI as compared with that during PHI was related to a faster rebound [relative hazard 2.3, 95% confidence interval (CI): 1.3–4.1, $P = 0.006$]. A longer duration of HAART before treatment interruption was also related to a faster rebound (relative hazard of 1.003 per week, 95% CI: 1.000–1.005, $P = 0.028$). When the duration of HAART was corrected for CHI or PHI group by multivariate analysis, there was no relation any more between the time on HAART and the time to viral rebound (relative hazard 1.0, $P = 0.8$).

The median overall decline in CD4+ T-cell count after treatment interruption was −3.7 cells/μl blood per week in the PHI group versus −4.8 cells/μl blood per week in the CHI group ($P < 0.05$). In both CHI and PHI patients, CD4+ T-cell decline during the first 4 weeks after treatment interruption was faster than during subsequent follow-up. CD4+ T-cell counts declined from week 0 to 4 with a median of −5.0 cells/μl blood per week in PHI patients and −45 cells/μl blood per week in the group of CHI patients ($P < 0.05$), from week 4 to 48 after treatment interruption the CD4+ T-cell decline was similar in both patient groups (−3.5 cells/μl blood per week in PHI patients versus −2.2 cells/μl blood per week in CHI patients, $P = 0.9$). A further analysis limited to the first 4 weeks after treatment interruption showed a more rapid decline of CD4+ T-cell decline in patients who had a rebound of plasma viral load above 5000 copies HIV-1 RNA (early rebounders), as compared with those who had not (late rebounders) (median decline of −48 cells/μl blood per week versus −7.5 cells/μl blood per week, $P = 0.01$). When this was done for CHI and PHI patients separately, similar results were obtained but statistical significance was lost.

Discussion

Our data show different plasma viral load and CD4 dynamics for PHI and CHI patients after treatment

Table 1. Patient characteristics.

	PHI	CHI	P^a
n	24	46	
Men	22 (92)	39 (85)	0.71
Age at stop HAART (years)	40 (35–48)	44 (39–48)	0.14
Western origin	20 (83)	35 (76)	0.23
MSM	21 (88)	33 (71)	0.23
Year of start HAART	2001 (1999–2002)	1997 (1997–1999)	<0.001
Plasma HIV-1 RNA at start HAART ($\log_{10}$ copies/ml)	5.4 (4.4–6.1)	4.7 (4.4–5.2)	<0.05
CD4+ cell count at start HAART (cells/μl)	500 (410–720)	474 (413–572)	0.37
CD4+ cell count at interrupt (cells/μl)	770 (593–928)	912 (748–1150)	<0.05
Duration of HAART (weeks)	91 (56–112)	306 (214–343)	<0.001
Duration of intensive HAART[b] (weeks)	59 (41–80)	NA	

Data are *n* (%), or medians with interquartile range. CHI, chronic HIV infection; HAART, highly active antiretroviral therapy; MSM, men having sex with men; NA, not available; PHI, primary HIV infection. [a]Chi-squared test or Mann–Whitney *U* test. [b]Intensive HAART defined as treatment with a combination of three classes of antiretroviral drugs.

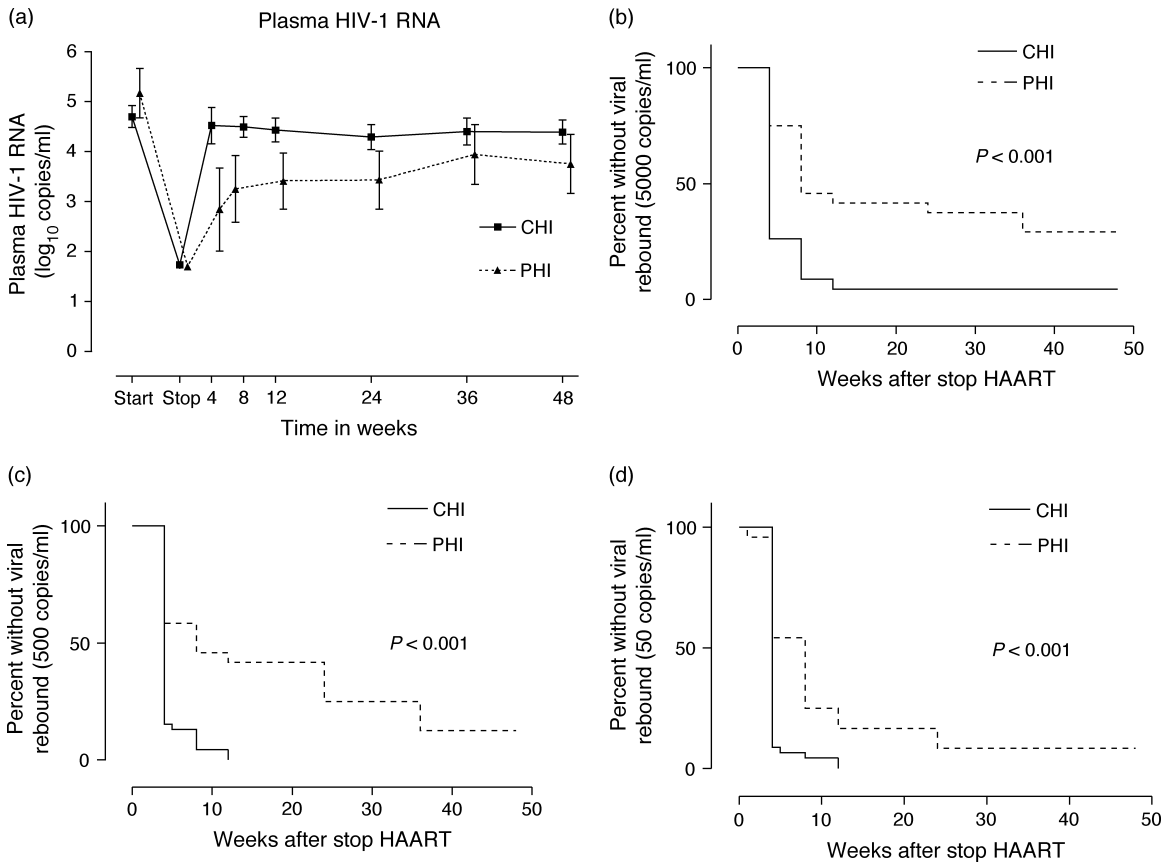

Fig. 1. Longitudinal plots of plasma HIV-1 RNA after treatment interruption. (a) The mean plasma HIV-1 RNA load plotted against the time in weeks after interruption of HAART (stop) for CHI (squares) and PHI (triangles) groups. Error bars represent 95% confidence intervals. Kaplan–Meier plots showing the different time to plasma viral rebound for CHI and PHI groups. Rebound of plasma HIV-1 viral load was defined as a single measurement of plasma HIV-1 RNA above (a) 5000 copies/ml, (b) 500 copies/ml or (c) 50 copies/ml. Differences between the groups were tested using the log rank test. CHI, chronic HIV infection; HAART, highly active antiretroviral therapy; PHI, primary HIV infection.

interruption. CHI patients had a faster rebound of HIV-1 RNA load in plasma and, during the first 4 weeks, a faster decline of CD4+ T cells in blood. In some PHI patients, no rebound of plasma HIV-1 RNA was observed at all. The more rapid loss of CD4+ T cells during the first 4 weeks after treatment interruption, especially in early rebounders, mirrors T-cell dynamics after the initiation of HAART [20,21] and suggests T-cell redistribution following antigen-driven immune activation.

Several factors may contribute to the observed (temporary) viral control after treatment interruption in PHI patients. Initiating HAART during PHI could arrest early viral escape, leaving the virus vulnerable to the building immune response. Preserved or even enhanced immune responses may result from the clearing of antigen during the acute phase of infection [22]. The increase of magnitude and breadth of the CD8+ T-cell response during treatment interruption was related to the time off therapy [7], and data from two previous studies suggested that strong HIV-specific CD4+ T-cell responses were key factors in suppressing plasma viral load after treatment interruption in PHI patients [6,23]. Later studies could not confirm a correlation between these responses and the time to viral rebound [7,24]. A recent study suggested that it is the HIV plasma viral load that determines the nature and magnitude of the HIV-specific responses, rather than the other way around [25].

Another mechanism involved might be that early HAART prevents the establishment of a pool of HIV-specific memory CD4+ T cells and thus leaves less target cells available for viral infection [26,27]. Finally, it might be that the level of CD4+ T-cell depletion in the gastrointestinal tract plays a role.

Pre-existing differences between the CHI and PHI groups could have played a role in the observed differences in the outcome. The CHI group was treated for a longer period of time and had a larger increase in CD4 cell count. The higher CD4 cell count results in a higher number of available target cells, but we found no relationship between the speed of viral rebound and the number of CD4+ T cells at the time of treatment

interruption. The PHI patients were treated for a shorter period of time; however, most of them were initially treated with a quadruple drug, triple class regimen. Neumann *et al.* [28] showed that a very short course of HAART during CHI leads to a rapid viral rebound similar to our data. More importantly, a relation between treatment duration and viral rebound was observed when all our patients were pooled together, but this relation disappeared when both treatment duration and PHI/CHI group were entered in a multivariate model. Furthermore, partial or temporary control of HIV-1 viraemia was observed in other PHI studies as well, where patients were treated during PHI for a relatively short period of time with combinations similar to the ones used here in the CHI group [7,9].

Finally, the reservoir of integrated HIV-1 DNA is established early in infection [29]. When HAART is initiated in PHI patients, the cellular HIV-1 proviral load is decreased in comparison with that initiated in CHI patients [30–32]. The baseline proviral load was related to the established viral setpoint after treatment interruption in CHI [33]. A possible difference in proviral load between both groups cannot explain, however, that no plasma viral rebound occurred in several PHI patients in our study.

Our findings show that, in contrast to treatment of CHI, temporary viral control can be achieved after a treatment period during PHI. The precise mechanism behind the delayed or absent viral rebound after treatment interruption in the PHI group must be further investigated. Whether there is any clinical benefit in the temporary treatment of PHI is currently under investigation in prospective, randomized trials.

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