HIV-1 p24 May Persist During Long-Term Highly Active Antiretroviral Therapy, Increases Little During Short Treatment Breaks, and Its Rebound After Treatment Stop Correlates With CD4+ T Cell Loss

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Summary: The dynamics of HIV-1 RNA during structured treatment interruptions (STIs) are well established, but little is known about viral proteins like p24. We studied 65 participants of an STI trial. Before the trial, continuous highly active antiretroviral therapy (HAART) had suppressed their viral load to <50 copies/mL during 6 months. They then interrupted HAART during weeks 1 through 2, 11 through 12, 21 through 22, 31 through 32, and 41 through 52. The p24 was measured by boosted enzyme-linked immunosorbent assay of plasma pretreated by efficient virus disruption and heat denaturation. At time point 0, p24 was measurable in 22 patients (34%), who had maintained a viral load <50 copies/mL for 25.4 months (median, range: 6.2–38.9 months) under HAART. Viral rebounds during 2-week STIs led to a mean p24 increase of only 0.08 to 0.19 $\log_{10}$ (ie, 20%–60%). Pre-HAART viral load and p24 at time 0 independently predicted p24 rebounds during the 4 2-week STIs. The p24 at time 0 and HIV-1 RNA rebound during weeks 41 through 52 independently determined the concomitant p24 rebound. An increase of p24 but not viral load during the first 8 weeks of the long STI correlated significantly with concomitant CD4+ T cell loss. Persisting p24 despite successful HAART may reflect virus replication in reservoirs not represented by plasma viral load and has implications for the concept of therapeutic vaccination.

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Highly active antiretroviral therapy (HAART) reduces HIV-1 RNA in plasma to <50 copies/mL in most patients but cannot eradicate HIV-1. Virus rebounds rapidly after treatment cessation and stabilizes at levels comparable to those seen before treatment.[4,5] Investigations in patients with controlled discontinuation of treatment or during treatment-free intervals of structured treatment interruptions (STIs) have demonstrated that the viral rebound occurs within days.[6,7]

Although the dynamics of the HIV-1 RNA rebound in plasma are thus well established, little is known about the behavior of viral proteins, among which the p24 antigen (p24) is measured easily. During the past decade, p24 measurement has been highly improved by measures that include heat denaturation of interfering antibodies, signal amplification, and use of an efficient virus disruption buffer.[8] These improvements have rendered the test more accurate and more sensitive.[9–15] The p24 concentration is as predictive of disease progression in early and late disease as is HIV-1 RNA.[16,17] The measurement of p24 has potential for antiretroviral treatment monitoring in adult and pediatric HIV-1 infection.[9–14]

Of note, extravirally located p24 has been measured in some patients with stably suppressed viremia, and the changes in concentrations of p24 and CD4+ T cells in such patients were inversely correlated.[18] Here, we have investigated the dynamics of p24 during 52 weeks of an STI trial.[19] Patients interrupted treatment 4 times for a 2-week period, each followed by 8 weeks of retreatment and, finally, for a period of at least 12 weeks, which permitted the virus to rise to a new set point.[20–23] We relate the changes in concentrations of p24 to those observed for HIV-1 RNA and CD4+ T cells.

MATERIALS AND METHODS

Patients and Specimens

We investigated patients of the Swiss-Spanish Intermittent Treatment Trial (SSITT).[19] They had started HAART during

chronic HIV infection, had been taking 2 classes of drugs (nonnucleoside reverse transcriptase inhibitors excluded) for >6 months, and had presented for >6 months with an HIV-1 RNA level <50 copies/mL in plasma and a $CD4^+$ T-cell count of >300 cells/μL. During the SSITT, the patients underwent 4 cycles consisting of a 2-week treatment interruption followed by an 8-week retreatment phase with the identical drug combination used before the trial. At the fifth and final cycle (week 41 onward) treatment was suspended for >12 weeks if no adverse effects occurred. Weeks 1 through 42 of the trial were used for studying the reappearance of p24 during 5 2-week treatment-free intervals spanning weeks 1 through 2, 11 through 12, 21 through 22, 31 through 32, and 41 through 42. Weeks 39 through 52 were used to study associations between p24, HIV-1 RNA, and $CD4^+$ T cells in a situation of prolonged viral rebound up to a new set point. Patients were included in the present investigation if (1) they had been treated at one of the participating Swiss centers, (2) they had completed the first 4 STIs and started the fifth long STI, and (3) frozen plasma was available for p24 testing. Of the total 133 participants of the SSITT, 65 patients (48.9%) met all criteria; they had a follow-up interval of 44 weeks. One patient each dropped out after weeks 44, 48, and 50. Sixty-two of the patients studied here thus completed the study up to week 52.

Tests

$CD4^+$ T cell counts were determined prospectively at weeks 0, 9, 19, 29, 39, 44, 48, and 52. HIV-1 RNA concentration in plasma determined prospectively by the ultrasensitive Amplicor Monitor version 1.5 test, with a quantification limit of 50 copies/mL, was available from weeks 0, 2, 9, 12, 19, 22, 29, 32, 39, 42, 44, 46, 48, 50, and 52. HIV-1 RNA concentration before initiation of HAART was available from the Swiss HIV Cohort Study database. Concentrations of cell-associated unspliced HIV-1 RNA and proviral DNA at week 0 were available from study files and had been determined batchwise as described elsewhere.[24,25] The p24 was tested retrospectively at the same time points at which HIV-1 RNA had been tested. All remaining plasma samples, stored at −70°C, were analyzed after heat denaturation by a signal amplification–boosted enzyme-linked immunosorbent assay (ELISA).[8] All specimens of a given patient were tested on the same ELISA plate, thus minimizing test-associated variation. The HIV-1 p24 Ag Ultra kit (Perkin Elmer Life Sciences) was used according to the manufacturer's instructions except for the following crucial modification of sample pretreatment: 100 μL of plasma was added to an Eppendorf tube containing 50 μL of a mixture comprising 30 mM of Tris/HCl pH 7.2, 450 mM of NaCl, 1.5% Triton X-100, 1.5% deoxycholic acid (sodium salt), 0.3% sodium dodecylsulfate, and 10 mM of ethylenediamine tetra-acetic acid (EDTA), mixed well and left for 10 minutes at room temperature. After further dilution with 450 μL of the kit's prediluted Dissociation Solution, the sample was boiled for 5 minutes on a Techne dry heat block preheated to 100°C. After cooling down to room temperature, 250 μL of the mixture was tested in duplicate wells according to the manufacturer's instructions. Concentrations were deduced by end point reading from a 3-fold dilution series of standards extending from 170 to 10,000,000 fg/mL. Frozen aliquots of prediluted standards were used in all experiments to reduce intertest variation. Reactivity in the test was related to a quantitative cutoff based on 4 negative controls run on every assay plate. As in earlier studies this cutoff equaled the mean plus 3 standard deviations (SDs) of these negative controls.[9,17,18]

Evaluation and Statistics

For statistical analysis, the StatView 5.0 program for Macintosh (SAS Institute, Cary, NC) was employed. The Wilcoxon signed-rank test and paired *t* tests were used for analyzing differences in concentrations. The Spearman rank correlation, Pearson correlation, and multivariate linear regression analysis were used for assessing correlations. All concentrations were evaluated as $\log_{10}$-transformed values. HIV-1 RNA <50 copies/mL was set to 50 copies/mL. The p24 concentrations were entered as calculated based on the standard curve, irrespective of whether reactivity was above or below the cutoff. This is justified because the concentrations directly represent the recorded absorbance readings (ie, experimentally determined values) and because many of the statistical analyses remained significant even when only p24 concentrations below the cutoff were considered, as detailed in the Results section. HIV-1 RNA was always above the limit of quantification when analyzed together with p24.

RESULTS

We investigated 65 patients, 39 men and 26 women, with a median age of 40 years (range: 22–68 years), who had received antiretroviral treatment for 27.0 months (median, range: 8.5–44.6 months) and had presented with <50 copies/mL of plasma viremia for 24.7 months (median, range: 6.2–42.8 months). On entry into the study, these patients interrupted their individual treatment during weeks 1 through 2, 11 through 12, 21 through 22, 31 through 32, and, finally, for a 12-week period beginning with week 41. p24 persists during long-term suppressed viremia. At time point 0 of the study, whereas HIV-1 RNA was <50 copies/mL in all patients, p24 reactivity was above the cutoff in 22 (34%) patients. At time point 0, p24 exhibited no dependence on the pre-HAART viral load, the total duration of HAART or of viremia <50 copies/mL under HAART, the gain in $CD4^+$ cells while receiving HAART, the concentrations of peripheral blood mononuclear cell (PBMC)–associated proviral DNA or HIV-1 RNA at time point 0, age, or gender (all correlations <0.2; $P > 0.15$). Positive results were found in patients who had maintained <50 copies/mL during a median time of 25.4 months (range: 6.2–38.9 months). The fraction with p24 reactivity above the cutoff remained similar at the end of each retreatment period (ie, at weeks 9, 19, 29, and 39) (Fig. 1).

p24 Increases Little During Structured Treatment Interruptions

During all 5 STIs, the concentrations of p24 rose little compared with HIV-1 RNA (Fig. 2). During the first STI, mean viral RNA rose from <50 copies/mL or <1.7 $\log_{10}$ to 2.36 $\log_{10}$ (ie, by >0.66 $\log_{10}$). In contrast, mean p24 increased by only 0.19 ± 0.37 $\log_{10}$. For weeks 12, 22, 32, and 42, the respective mean increases of p24 were 0.11 ± 0.36, 0.19 ± 0.31,

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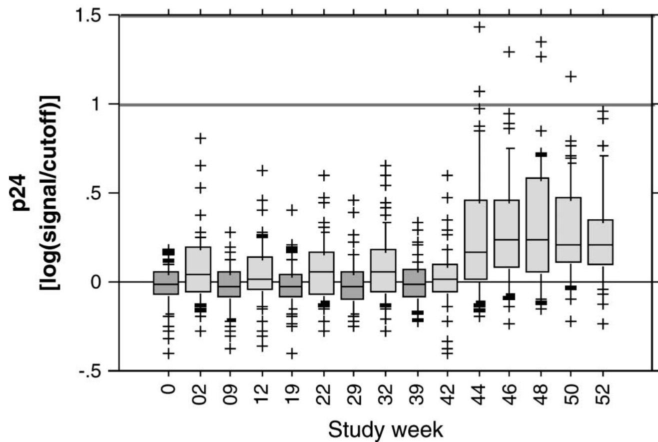

FIGURE 1. Signal/cutoff ratio of p24 during structured treatment interruptions (log-transformed values; a value of 0 represents a positive result). In the box plots, the boxes indicate the median and 25th and 50th percentiles, whereas the horizontal bars closest to the box represent the 10th and 90th percentiles, respectively. Outliers are plotted individually. The darker shaded boxes indicate measurements while on antiretroviral treatment.

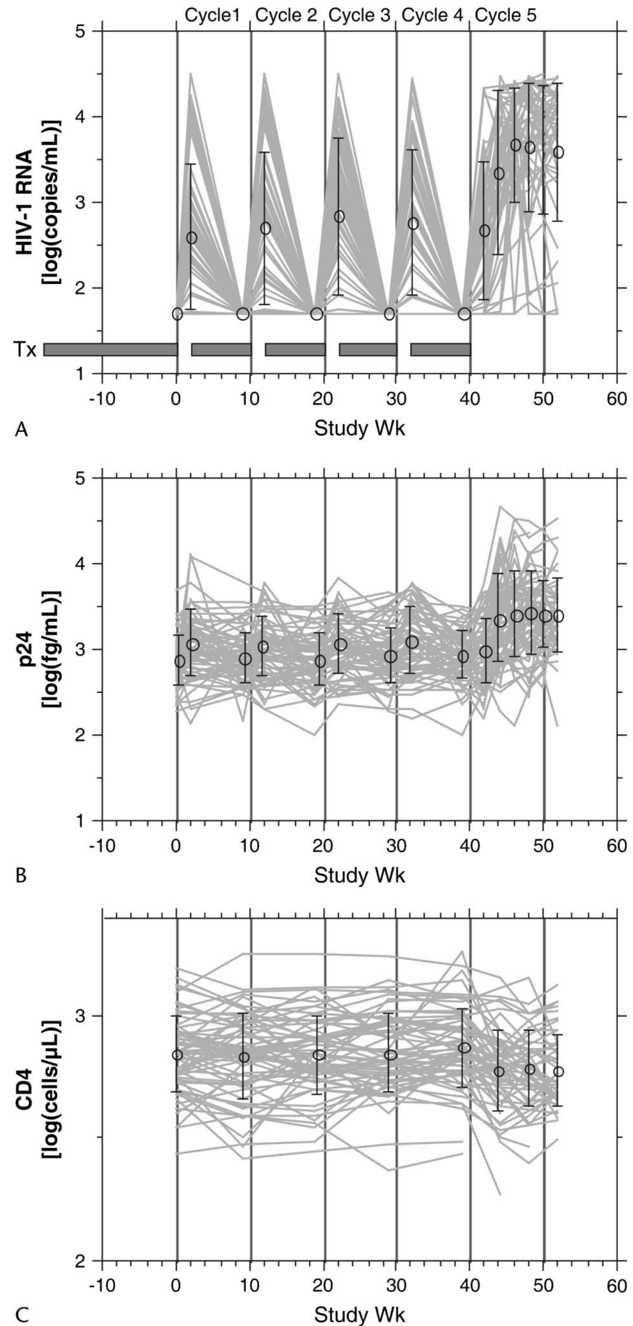

FIGURE 2. Concentrations of HIV-1 RNA (A), p24 (B), and $CD4^{+}$ cells (C) of all patients during the study. Circles indicate means ± SD. Treated and treatment-free intervals are represented by the horizontal bars in A.

0.17 ± 0.26, and 0.08 ± 0.30 $\log_{10}$. The resulting ratios by which the increases of HIV-1 RNA exceeded those of p24 were >5.2, >5.6, >4.7, and >4.8 (average: >4.6 ± 1.0). Despite their low magnitude, the p24 increases during successive 2-week STIs were statistically significant in cycles 1, 3, and 4 (Wilcoxon test, $P < 0.0001$ for all 3 cycles) and weakly significant for the first 2 weeks of cycle 5 ($P = 0.042$). The p24 increase during the first STI but not the subsequent short STI correlated significantly with the corresponding HIV-1 RNA peak (Spearman ρ: 0.393; $P = 0.005$). Concentrations of p24 at many time points off therapy, namely, at weeks 12, 22, 32, 44, 46, 48, and 50, correlated significantly with those of HIV-1 RNA before initiation of HAART (Spearman ρ: 0.26–0.48; $P =$ 0.0003 to $P = 0.05$). Multivariate linear regression analysis indicated that p24 at time point 0 and pre-HAART viral load were independent predictors of p24 levels at weeks 2, 12, 22, and 32 (Table 1A). The influence of p24 at time point 0 was always stronger than that of the pre-HAART viral load.

The p24 also increased less during the long STI than did the HIV-1 RNA (see Fig. 2). Mean HIV-1 RNA rose rapidly from <50 copies/mL (<1.7 $\log_{10}$) at week 39 and reached a peak of 3.6 $\log_{10}$ at weeks 46 through 48. The mean increase amounted to >1.9 $\log_{10}$ (ie, >80-fold). For p24, the overall mean increase was approximately 0.5 $\log_{10}$ (3.2-fold). The relative increase of p24 was at least 25-fold lower than that of HIV-1 RNA. Nevertheless, p24 and HIV-1 RNA concentrations correlated significantly at all time points (Spearman ρ: 0.33–0.61; $P = 0.001$ to $P = 0.05$). The p24 levels during this period were, however, also markedly influenced by the p24 concentration at time point 0. Indeed, p24 at time point 0 was correlated significantly or at least showed a trend of correlation with all subsequently measured p24 concentrations up to and including week 52 (Spearman ρ: 0.27–0.53; $P < 0.001$ to $P <$ 0.07). This even remained the case if only patients with a p24 level below the cutoff at time point 0 were analyzed, indicating that even p24 levels below the cutoff represented true concentrations rather than chance values.

We investigated by multivariate linear regression analysis how p24 concentration at different time points during the final long STI was influenced by the concomitant concentration of HIV-1 RNA and p24 at time point 0 (see Table 1B). All models were adjusted for age, gender, and other parameters listed at the bottom of the table. The p24 at time point 0 was

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TABLE 1. Correlates of P24 Concentrations Achieved During the 5 Structured Treatment Interruptions (STI). Multivariate Linear Regression Analysis

Short Treatment Interruption	1st STI (Week 2) n = 59		2nd STI (Week 12) n = 57		3rd STI (Week 22) n = 53		4th STI (Week 32) n = 61	
	Coeff. (95% CI)	*P*	Coeff. (95% CI)	*P*	Coeff. (95% CI)	*P*	Coeff. (95% CI)	*P*
Pre HAART Viral Load [log (copies/mL)]	0.166 (0.029 to 0.302)	0.0181	0.189 (0.070 to 0.307)	0.0025	0.191 (0.078 to 0.304)	0.0014	0.144 (0.019 to 0.270)	0.0246
p24 at day 0 [log (fg/mL)]	0.561 (0.247 to 0.875)	0.0007	0.556 (0.290 to 822)	0.0001	0.658 (0.412 to 0.903)	<0.0001	0.546 (0.250 to 0.842)	0.0005
Long Treatment Interruption	**Week 42 n = 41**		**Week 44 n = 23**		**Week 48 n = 33**		**Week 52 n = 30**	
	Coeff. (95% CI)	***P***	**Coeff. (95% CI)**	***P***	**Coeff. (95% CI)**	***P***	**Coeff. (95% CI)**	***P***
Concomitant HIV-1 RNA [log (cc/mL)]	0.150 (0.051 to 0.255)	0.0063	0.176 (0.046 to 0.306)	0.0118	0.274 (0.103 to 0.446)	0.0031	0.251 (0.011 to 0.491)	0.0413
p24 at day 0 [log (fg/mL)]	0.764 (0.474 to 1.053)	<0.0001	0.667 (0.329 to 1.006)	0.0009	0.799 (0.459 to 1.140)	<0.0001	0.705 (0.228 to 1.181)	0.0058

All models also adjusted for age, duration of ART, duration of <50 copies/mL viral load prior to study entry, pre-ART viral load, cell-associated DNA at study entry, cell-associated HIV-1 RNA at study entry, CD4+ at study entry.

a stronger correlate of p24 during viral rebound than was the concomitant HIV-1 RNA, which was also independently significant at all time points however. Pre-HAART viral load had no significance.

The Rebound of p24 After Treatment Stop Correlates with $CD4^+$ T Cell Loss

As seen from Figure 2, there was a noticeable drop in $CD4^+$ cell counts during weeks 39 through 44 ($-0.094\ log_{10}$; Wilcoxon signed-rank test, $P < 0.0001$). Subsequent changes lacked significance despite considerable individual change. The pattern of $CD4^+$ cell count changes in the patients studied here was in accordance with that seen in the whole SSITT.[19]

We investigated whether $CD4^+$ cell counts or their changes during the final STI correlated with the respective concentrations or changes of the 2 viral markers. $CD4^+$ cell counts at weeks 44, 48, or 52 did not correlate with HIV-1 RNA at any time point. There was also no correlation of the $CD4^+$ cell count changes between any of these time points with the concomitant or preceding HIV-1 RNA changes (not shown). In contrast, there were multiple correlations between $CD4^+$ cell counts and p24 at different time points (Table 2).

Multivariate linear regression analysis was used to investigate the factors that determined the $CD4^+$ cell count changes in the final STI (Table 3). The models investigate how the $CD4^+$ cell count changes during 3 time intervals were influenced by the concentrations of $CD4^+$ cells and p24 at the beginning of the respective interval and by the p24 changes during the interval. We also investigated a reported effect of the gain in $CD4^+$ cells during uninterrupted HAART preceding the study on the $CD4^+$ cell loss after treatment cessation.[26] All models were also adjusted for gender, age, and time of HIV-1 RNA <50 copies/mL during uninterrupted HAART. The $CD4^+$ cell count drop observed during weeks 39 through 44 was independently associated with the $CD4^+$ count at week 39 and the p24 increase during weeks 42 through 44 (model 1). Further changes in $CD4^+$ cell counts during weeks 44 through 48 were associated with $CD4^+$ cell counts at week 44 and with p24 at week 44 and its change during weeks 44 through 48 (model 2). These factors remained significant when the entire interval spanning weeks 39 through 48 was examined (model 3). No significance was found for the $CD4^+$ cell count gained during uninterrupted HAART or for any variable listed at the bottom of the table. Similar findings were seen for the change in nontransformed $CD4^+$ cell numbers (not shown). None of

TABLE 2. Correlation of CD4 Counts and P24 During the Final Treatment Interruption

Variables	n	Pearson's Correlation (R)	95% CI	*P*
CD4+ [log_{10} (cells/μL)] at week 44				
p24, week 44	50	−0.254	−0.497 to 0.26	0.076
p24, change wks 39–44	49	−0.282	−0.522 to −0.000	0.050
p24, change wks 42–44	43	−0.379	−0.610 to −0.088	0.012
CD4+[log_{10} (cells/μL)] at week 48				
p24, week 48	48	−0.431	−0.637 to −0.168	0.002
p24, change wks 42–44	38	−0.332	−0.589 to −0.014	0.041
p24, change wks 39–48	47	−0.415	−0.628 to −0.145	0.003
CD4+[log_{10} (cells/μL)] at week 52				
p24, week 48	44	−0.361	−0.594 to −0.072	0.016
p24, week 50	44	−0.261	−0.518 to 0.039	0.088
p24, change wks 39–48	43	−0.371	−0.604 to −0.079	0.014
p24, change wks 42–48	39	−0.353	−0.601 to −0.042	0.027

TABLE 3. Multivariate Linear Regression Analysis of CD4+ Change in Dependence of Concentrations of CD4+ Cells and p24 at the Beginning of Various Time Intervals and of Changes in p24 Concentration During the Time Intervals

	Difference in CD4 Counts [$\log_{10}$(cells/μL)]					
	Model 1 Weeks 39–44 (n = 42)		Model 2 Weeks 44–48 (n = 41)		Model 3 Weeks 39–48 (n = 36)	
Variable	Regr. Coeff. (95% CI)	*P*	Regr. Coeff. (95% CI)	*P*	Regr. Coeff. (95% CI)	*P*
CD4+ T cells at week 39 [$\log_{10}$(cells/μL)]	−0.358 (−0.627 to −0.088)	0.011			−0.494 (−0.788 to −0.201)	0.002
p24 at week 39 [$\log_{10}$(fg/mL)]	0.21 (−0.124 to 0.167)	0.766			−0.045 (−0.226 to 0.137)	0.617
p24 change weeks 39–42 [$\log_{10}$(fg/mL)]	0.053 (−0.101 to 0.208)	0.487			−0.010 (−0.181 to 0.161)	0.907
p24 change weeks 42–44 [$\log_{10}$(fg/mL)]	−0.121 (−0.220 to −0.021)	0.019			−0.153 (−0.285 to −0.021)	0.024
CD4+ T cells at week 44 [$\log_{10}$(cells/μL)]			−0.223 (−0.390 to −0.057)	0.010		
p24 at week 44 [$\log_{10}$(fg/mL)]			−0.113 (−0.182 to −0.044)	0.002		
p24 change weeks 44–48 [$\log_{10}$(fg/mL)]			−0.100 (−0.172 to −0.029)	0.007	−0.127 (−0.247 to −0.008)	0.038
CD4+ change during uninterrupted HAART prior to study [$\log_{10}$(cells/μL)]	−0.149 (−0.311 to 0.014)	0.072	0.027 (−0.081 to 0.135)	0.714	−0.068 (−0.250 to 0.113	0.446

All three models also adjusted for gender, age, and duration of VL <50 copies/mL prior to study ($P > 0.10$ in all).

the parameters was significant regarding the $CD4^+$ cell count change in the interval during weeks 48 through 52. Thus, during the first 8 weeks of the final STI, p24 was a significant correlate of $CD4^+$ count change.

DISCUSSION

We used an STI trial to study the dynamics of HIV-1 p24 on treatment withdrawal. We found that (1) p24 in plasma persisted in one third of patients after a median of 2 years of stably suppressed viremia (see Fig. 1), (2) levels of persisting p24 were affected little during short STIs (see Fig. 2), (3) the concentrations of the persisting p24 and rebounding RNA determined the concentration of the p24 rebound after final treatment cessation (see Table 1), and (4) the rebounding p24 correlated inversely with the $CD4^+$ cell count changes seen during the first 8 weeks of the final STI (see Tables 2, 3).

STI was originally proposed as an "autovaccination" concept by which the immune system of patients with suppressed virus replication under HAART would be boosted by brief intervals of virus replication. Results of STI studies have shown, however, that virus control in the absence of HAART is rare.[19] The autovaccination concept implies that patients successfully treated by HAART lack viral antigen and that sufficient viral immunogen is produced during STI. Our findings suggest that neither expectation was met in the SSITT. At least 1 important protein, p24, remained measurable in many patients (see Fig. 1), and it is likely that other viral proteins persisted as well. The p24 concentrations at time point 0 clearly did not represent random values or technical artifacts because they were firmly correlated with p24 at multiple other time points (see Table 1). The persistence of p24 in patients with HIV-1 RNA <50 copies/mL under HAART has recently been confirmed by preliminary results of studies using immuno-polymerase chain reaction (PCR)[27] or new ultrasensitive nanoparticle technology.[28] The latter study also identified persisting nucleocapsid p7, which is cleaved from the same precursor Pr55Gag as p24.

Although more sensitive tests may detect residual HIV-1 RNA in a similarly high proportion,[29–32] the p24 detected in our patients cannot be the structural p24 contained in the few virus particles present in plasma.[33] Based on an estimate of 2000 to 4000 p24 molecules contained in 1 virus particle,[34] 6150 to 12,500 particles (or 12,500–25,000 copies of viral RNA) are needed for 1 pg of particle-associated p24. The mean concentration of p24 at week 0, approximately 1 pg, exceeded the maximum of particle-associated p24 present in a plasma with <50 copies/mL by 3 orders of magnitude.

Ultracentrifugation experiments have shown that in contrast to HIV-1 RNA and reverse transcriptase, most of the p24 present in chronically infected patients cannot be pelleted and is located outside virus particles as immune-complexed protein or free protein of essentially unknown origin.[35] The absence of a correlation between p24 at time point 0 and the pre-HAART viral load, the duration of HAART, or the duration of viremia <50 copies/mL in this study suggests that this p24 does not represent protein remaining from the time before HAART. Lack of correlation with PBMC-associated HIV-1 RNA or DNA at time point 0 also suggests that PBMCs are not a major source. The p24 may instead be derived from ongoing virus replication in reservoirs not represented by HIV-1 RNA in plasma. Persisting viral protein may also have implications for the concept of therapeutic vaccination in such patients. The persistence of viral protein after prolonged HAART is in agreement with the increased $CD8^+$ T-cell activation state of such patients compared with controls negative for HIV-1.[36,37] It is tempting to speculate that this residual immune activation is attributable to p24 and other persisting viral antigens. This hypothesis is testable but exceeds the scope of this paper.

With regard to the dose aspect of the autovaccination concept, it is evident that the viral rebounds during short STIs, although leading to sizable increases in HIV-1 RNA,[7] had a limited additive effect, with average p24 increases of only 0.08 to 0.2 $\log_{10}$ (ie, 20%–60%) (see Fig. 2), thus rendering a pronounced strengthening of the antiviral immune response unlikely. Indeed, during the 4 short STIs, the $CD8^+$ T-cell

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responses in half of the patients of the SSITT appeared with a delay or not at all, although many patients exhibited stronger responses after the final STI, when more virus was produced. Thus, the short STI may indeed have produced a suboptimal dose of viral immunogen.[38] It would be of interest to analyze the $CD8^+$ T-cell responses individually with respect to the p24 concentrations present; however, again, this exceeds the scope of our study. We finally found that the changes in $CD4^+$ cells observed during the first 8 weeks after the final treatment stop correlated inversely with the respective changes in p24 concentration. This is in agreement with an evaluation of 115 patients from 3 prospective STI trials in which the increase in HIV-1 RNA during the first 4 weeks of the final STI was also found to be inversely correlated with the early decline in $CD4^+$ cells,[39] although such a correlation was not found in the present small study. Our findings contrast sharply with those of another STI study, which found a lack of longitudinal intrapatient correlation between p24 and HIV-1 RNA; it did not find a significant increase of p24 during short or long STIs, which lasted for up to 12 months.[40] The discrepancies are likely attributable to the fact that we used an external virus disruption buffer for sample pretreatment, whereas Prado et al[40] used the kit's 0.5% Triton X-100 buffer. Previous work has demonstrated the effectiveness of our new sample pretreatment procedure.[18,41,42]

In conclusion, the results of this study suggest that p24 is a viral marker that has distinct properties, although it correlates with HIV-1 RNA. The presently unclear meaning of p24 persistence under successful long-term HAART should be elucidated and eventually assessed for its relevance to treatment success. The persistence of viral protein would also seem to be of relevance to therapeutic vaccination studies. The possibility of an influence of persisting viral protein on the outcome of such studies should be assessed.

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REFERENCES

1. Finzi D, Hermankova M, Pierson T, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science*. 1997; 278:1295–1300.
2. Wong JK, Hezareh M, Gunthard HF, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science*. 1997;278:1291–1295.
3. Chun TW, Stuyver L, Mizell SB, et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci USA*. 1997;94:13193–13197.
4. Harrigan PR, Whaley M, Montaner JS. Rate of HIV-1 RNA rebound upon stopping antiretroviral therapy. *AIDS*. 1999;13(Suppl):F59–F62.
5. Hatano H, Vogel S, Yoder C, et al. Pre-HAART HIV burden approximates post-HAART viral levels following interruption of therapy in patients with sustained viral suppression. *AIDS*. 2000;14:1357–1363.
6. Ruiz L, Carcelain G, Martinez-Picado J, et al. HIV dynamics and T-cell immunity after three structured treatment interruptions in chronic HIV-1 infection. *AIDS*. 2001;15(Suppl):F19–F27.
7. Fischer M, Hafner R, Schneider C, et al. HIV RNA in plasma rebounds within days during structured treatment interruptions. *AIDS*. 2003;17: 195–199.
8. Schupbach J. Viral RNA and p24 antigen as markers of HIV disease and antiretroviral treatment success. *Int Arch Allergy Immunol*. 2003;132: 196–209.
9. Schupbach J, Boni J, Flepp M, et al. Antiretroviral treatment monitoring with an improved HIV-1 p24 antigen test: an inexpensive alternative to tests for viral RNA. *J Med Virol*. 2001;65:225–232.
10. Steindl F, Armbruster C, Pierer K, et al. A simple and robust method for the complete dissociation of HIV-1 p24 and other antigens from immune complexes in serum and plasma samples. *J Immunol Methods*. 1998;217: 143–151.
11. Boni J, Opravil M, Tomasik Z, et al. Simple monitoring of antiretroviral therapy with a signal-amplification boosted HIV-1 p24 antigen assay with heat-denatured plasma. *AIDS*. 1997;11(Suppl):F47–F52.
12. Nadal D, Böni J, Kind C, et al. Prospective evaluation of amplification-boosted ELISA for heat-denatured p24 antigen for diagnosis and monitoring of pediatric HIV-1 infection. *J Infect Dis*. 1999;180:1089–1095.
13. Pascual A, Cachafeiro A, Funk ML, et al. Comparison of an assay using signal amplification of the heat-dissociated p24 antigen with the Roche Monitor human immunodeficiency virus RNA assay. *J Clin Microbiol*. 2002;40:2472–2475.
14. Ribas SG, Ondoa P, Schupbach J, et al. Performance of a quantitative human immunodeficiency virus type 1 p24 antigen assay on various HIV-1 subtypes for the follow-up of human immunodeficiency type 1 seropositive individuals. *J Virol Methods*. 2003;113:29–34.
15. Sutthent R, Gaudart N, Chokpaibulkit K, et al. p24 Antigen detection assay modified with a booster step for diagnosis and monitoring of human immunodeficiency virus type 1 infection. *J Clin Microbiol*. 2003;41: 1016–1022.
16. Ledergerber B, Flepp M, Boni J, et al. Human immunodeficiency virus type 1 p24 concentration measured by boosted ELISA of heat-denatured plasma correlates with decline in CD4 cells, progression to AIDS, and survival: comparison with viral RNA measurement. *J Infect Dis*. 2000; 181:1280–1288.
17. Sterling TR, Hoover DR, Astemborski J, et al. Prognostic value of heat denatured HIV-1 p24 antigen and correlation with plasma HIV-1 viral load and CD4+ T-lymphocyte level in adults. *J Infect Dis*. 2002;186:1181–1185.
18. Schupbach J, Boni J, Bisset LR, et al. HIV-1 p24 antigen is a significant inverse correlate of CD4 T-cell change in patients with suppressed viremia under long-term antiretroviral therapy. *J Acquir Immune Defic Syndr*. 2003;33:292–299.
19. Fagard C, Oxenius A, Gunthard H, et al. A prospective trial of structured treatment interruptions in human immunodeficiency virus infection. *Arch Intern Med*. 2003;163:1220–1226.
20. Henrard DR, Phillips JF, Muenz LR, et al. Natural history of HIV-1 cell-free viremia. *JAMA*. 1995;274:554–558.
21. Jurriaans S, Van Gemen B, Weverling GJ, et al. The natural history of HIV-1 infection: virus load and virus phenotype independent determinants of clinical course? *Virology*. 1994;204:223–233.
22. Mellors JW, Rinaldo CR Jr, Gupta P, et al. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma [see comments]. *Science*. 1996;272:1167–1170.
23. Schacker TW, Hughes JP, Shea T, et al. Biological and virologic characteristics of primary HIV infection. *Ann Intern Med*. 1998;128:613–620.
24. Fischer M, Wong JK, Russenberger D, et al. Residual cell-associated unspliced HIV-1 RNA in peripheral blood of patients on potent antiretroviral therapy represents intracellular transcripts. *Antivir Ther*. 2002;7: 91–103.
25. Fischer M, Trkola A, Joos B, et al. Shifts in cell-associated HIV-1 RNA but not in episomal HIV-1 DNA correlate with new cycles of HIV-1 infection in vivo. *Antiviral Ther*. 2003;8:97–104.
26. Tebas P, Henry K, Mondy K, et al. Effect of prolonged discontinuation of successful antiretroviral therapy on CD4+ T cell decline in human immunodeficiency virus-infected patients: implications for intermittent therapeutic strategies. *J Infect Dis*. 2002;186:851–854.
27. Barletta JM, Edelman DC, Constantine NT. Lowering the detection limits of HIV-1 viral load using realtime immuno-PCR for HIV-1 p24 antigen. *Am J Clin Pathol*. 2004;122:20–27.
28. Kim EY, Lee KB, Nam JM, et al. Ultrasensitive detection of HIV-1 p24CA and p7NC antigens in plasma by nanoparticle-based bio-bar code amplification testing [abstract 735]. Presented at: 12th Conference on

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Retroviruses and Opportunistic Infections; 2005; Boston. Available at: http://www.retroconference.org/2005/cd/Abstracts/25109.htm.
29. Fischer M, Gunthard HF, Opravil M, et al. Residual HIV-RNA levels persist for up to 2.5 years in peripheral blood mononuclear cells of patients on potent antiretroviral therapy. *AIDS Res Hum Retroviruses*. 2000; 16:1135–1140.
30. Havlir DV, Bassett R, Levitan D, et al. Prevalence and predictive value of intermittent viremia with combination HIV therapy. *JAMA*. 2001;286: 171–179.
31. Günthard HF, Fiscus S, Zhang Z-Q, et al. Residual human immunodeficiency virus (HIV) type 1 RNA and DNA in lymph nodes and HIV RNA in genital secretions and in cerebrospinal fluid after suppression of viremia for 2 years. *J Infect Dis*. 2001;183:1318–1327.
32. Dornadula G, Zhang H, Van Uitert B, et al. Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy. *JAMA*. 1999;282:1627–1632.
33. Yerly S, Günthard HF, Fagard C, et al. Proviral HIV-DNA predicts viral rebound and viral setpoint after structured treatment interruptions. *AIDS*. 2004;18:1951–1953.
34. Coffin JM. Retroviridae: the viruses and their replication. In: Fields BN, Knipe DM, Howley PM, eds. *Virology*. 3rd ed. Philadelphia: Lippincott-Raven; 1996:1767–1847.
35. Schupbach J. Measurement of HIV-1 p24 antigen by signal-amplification-boosted ELISA of heat denatured plasma is a simple and inexpensive alternative to tests for viral RNA. *AIDS Rev*. 2002;4:83–92.
36. Beran O, Holub M, Spala J, et al. CD38 expression on CD8+ T cells in human immunodeficiency virus 1-positive adults treated with HAART. *Acta Virol*. 2003;47:121–124.
37. Al-Harthi L, Voris J, Patterson BK, et al. Evaluation of the impact of highly active antiretroviral therapy on immune recovery in antiretroviral naive patients. *HIV Med*. 2004;5:55–65.
38. Trkola A, Kuster H, Leemann C, et al. Humoral immunity to HIV-1: kinetics of antibody responses in chronic infection reflects capacity of immune system to improve viral set point. *Blood*. 2004;104:1784–1792.
39. Fagard C, Bandelier CY, Ananworanich J, et al. Biphasic decline of CD4 cell count during scheduled treatment interruptions. *AIDS*. 2005;19:439–441.
40. Prado JG, Shintani A, Bofill M, et al. Lack of longitudinal intrapatient correlation between p24 antigenemia and levels of human immunodeficiency virus (HIV) type 1 RNA in patients with chronic HIV infection during structured treatment interruptions. *J Clin Microbiol*. 2004;42: 1620–1625.
41. Jennings CL, Danilovic AD, Burgess DL, et al. A comparison of two non-RNA-based assays for the quantitation of HIV [poster 03-A-3803-ASM]. Presented at: 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy; 2003; Chicago.
42. Cachafeiro A, Taha TE, Hall C, et al. Evaluation of heat-denatured HIV-1 p24 antigen assays and a reverse transcriptase assay in patients infected with HIV-1 subtypes B and C [poster 957]. Presented at: 11th Conference on Retroviruses and Opportunistic Infections; 2004; San Francisco. Available at: http://www.retroconference.org/2004/cd/Abstract/957.htm.

APPENDIX

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