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A Prospective Trial of Structured Treatment Interruptions in Human Immunodeficiency Virus Infection

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Background: According to the “autovaccination hypothesis,” reexposure to human immunodeficiency virus (HIV) during treatment interruptions may stimulate the HIV-specific immune response and lead to low viremia after withdrawal of highly active antiretroviral treatment (HAART). Many patients who started HAART earlier in their disease course than is currently recommended would like to discontinue, but it is unknown whether it is safe to do so.

Objectives: To determine whether repeated treatment interruptions of HAART (1) stimulated the cytotoxic HIV-specific immune response and whether such stimulation correlated with low viremia off treatment, and (2) were safe with respect to clinical complications, development of viral resistance, and decline in CD4 cell counts.

Design: Interventional study with before-after comparison.

Setting: Outpatient clinics of university hospitals in Switzerland and Spain.

Patients: A total of 133 patients receiving HAART, with a median CD4 cell count of 740/ μ L, and whose viral load had been undetectable for a median of 21 months.

Interventions: HAART was interrupted for 2 weeks, re-started, and continued for 8 weeks. After 4 such cycles, treatment was indefinitely suspended 40 weeks after study entry.

Main Outcome Measures: HIV-specific cytotoxic T-cell responses were evaluated by interferon γ enzyme-linked immunospot analysis. The proportion of “responders” (viral load <5000 copies/mL) was measured at weeks 52 and 96. HIV-related diseases and CD4 cell counts were recorded.

Results: Seventeen percent of patients (95% confidence interval, 11%-25%) were responders at week 52, and 8% at week 96. Low pre-HAART viral load and lack of rebound during weeks 0 to 40 predicted response. HIV-specific CD8⁺ T cells increased between week 0 (median, 343 spot-forming cells per million peripheral blood lymphocytes [SFC/ 10^6 PBL]) and week 52 (median, 1930 SFC/ 10^6 PBL), but there was an inverse correlation between response and the number of spot-forming cells. Eighty-five (64%) of 133 patients stopped therapy for at least 12 weeks, and 55 (41%) for at least 56 weeks. The median CD4 cell count decreased from 792/ μ L to 615/ μ L during the first 12 weeks without treatment, but stabilized thereafter. One patient (0.75%) developed drug resistance necessitating salvage treatment. There were no AIDS-related clinical complications.

Conclusions: Results of this study do not favor the autovaccination hypothesis. Treatment interruptions did not provoke clinical complications, and there was little drug resistance. Comparative trials will have to show what benefit, if any, is associated with intermittent, as opposed to continuous treatment.

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AFTER INTRODUCTION of highly active antiretroviral treatment (HAART), morbidity and mortality of human immunodeficiency virus (HIV) infection have declined.¹ However, many patients find it difficult to comply with long-term HAART,^{2,3} especially if they experience adverse effects.^{4,5}

Treatment interruptions in patients with HIV infections are being studied for

3 main reasons.⁶ (1) An increase in time off drug treatment may improve quality of life and diminish adverse effects and costs. (2) After HIV has become resistant to antiretroviral drugs, treatment interruptions may allow repopulation of plasma with drug-susceptible virus and therefore improve the chances of success of subsequent salvage therapy.^{7,8} (3) Reexposure to viral antigens during treatment interruption may stimulate anti-HIV im-

immune responses and allow withdrawal of drugs with stabilization of viremia at low levels.^{9,10} This is what we call the “autovaccination hypothesis.”

Rosenberg et al¹⁰ described 8 patients who had started treatment within weeks of infection, and whose viral load decreased to below 50 copies/mL while receiving HAART. Following treatment interruptions, 5 of 8 patients remained with plasma viremia below 500 copies/mL after a mean of 6.5 months without therapy. It is unknown whether similar results could be achieved in the more numerous patients who started therapy later, during chronic HIV infection.

To test the autovaccination hypothesis, as well as the feasibility and safety of planned treatment interruptions, we prospectively recruited patients whose HAART, started during chronic HIV infection, had been proven effective. To maximize potential immune stimulation, the Swiss-Spanish Intermittent Treatment Trial (SSITT) included several cycles of treatment interruptions and treatment, before stopping therapy for a longer period of time. The HIV-specific CD8⁺ T-cell responses were measured and correlated with the level of viremia during periods without HAART.

METHODS

INCLUSION AND EXCLUSION CRITERIA

Patients had to have received antiretroviral therapy, with an “undetectable” viral load (<50 or <400 copies/mL, depending on the procedure of the test used) for at least 6 months, and a viral load of less than 50 copies/mL at enrollment. The CD4 cell count had to exceed 300/μL at enrollment. Patients were not eligible for SSITT if they had ever changed treatment because of virologic failure or had ever received nonnucleoside reverse transcriptase inhibitors (NRTIs). By design, half the patients had a pretreatment nadir of CD4 cell counts below 400/μL.

Viremia was measured using the Amplicor Monitor test version 1.5 (Roche Diagnostics, Rotkreuz, Switzerland): in its ultrasensitive procedure (limit of detection, <50 copies/mL) while patients were on treatment, and in the standard format (limit of detection, 200 copies/mL) during treatment interruptions. The pre-HAART viral load was the measurement closest to initiating HAART (66 patients). If more than 1 measurement was available in the 6 months before starting HAART, the average of the last 2 values was taken as the pre-HAART viral load.

TREATMENT SCHEDULE AND DEFINITION OF “RESPONSE”

The treatment schedule is shown in **Figure 1**. HAART was interrupted for 2 weeks, restarted, and continued for 8 weeks, and after 4 such cycles, treatment was indefinitely suspended at week 40 after study entry. If viral load remained above 50 copies/mL after 8 weeks’ retreatment, patients did not undergo further treatment interruptions. At week 40, treatment was also restarted if CD4 cell counts were below 400/μL. Otherwise, at week 40, treatment was stopped. From week 40 to week 52, restarting treatment was recommended if symptoms of acute HIV infection occurred or if viral load exceeded 50 000 copies/mL 3 times, 100 000 copies/mL twice, or 500 000 copies/mL once. The drugs used for each individual patient remained identical between weeks 0 and 52, unless a change was

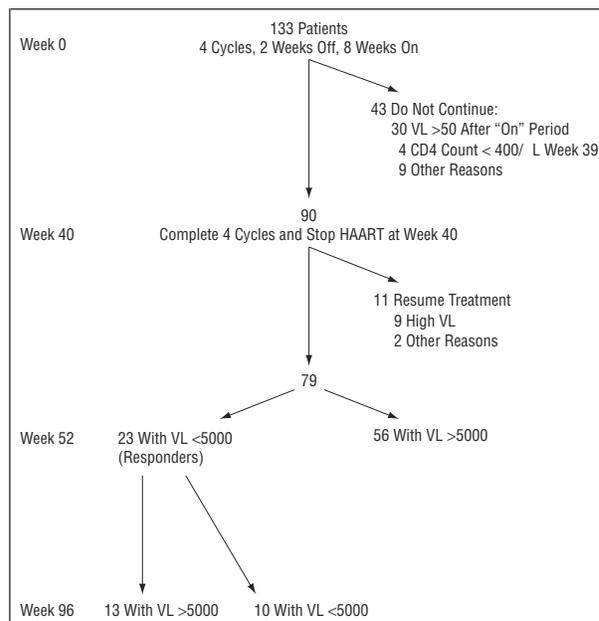


Figure 1. Trial profile. VL indicates viral load (given in copies per milliliter); HAART, highly active antiretroviral treatment.

indicated because of viral escape (n = 1, see below) or drug intolerance.

At week 52, patients who had undergone four 2-week and one 12-week treatment interruption were classified as “responders” if their viral load was below 5000 copies/mL. If their viral load was higher, or if they had stopped treatment interruptions for any reason before week 52 they were considered “nonresponders.” The protocol recommended HAART for nonresponders, and for responders whose viral load rebounded above 5000 copies/mL after week 52. From week 52 to week 64, viral load and CD4 cell counts were measured every 4 weeks, and every 8 weeks from week 64 to week 96.

MEASUREMENT OF HIV-SPECIFIC CD8 CYTOTOXIC T-LYMPHOCYTE RESPONSE, HLA TYPING, AND LYMPHOCYTE TYPING

The HIV-specific CD8⁺ T-cell frequencies were determined on frozen peripheral blood mononuclear cells, by direct ex vivo interferon γ enzyme-linked immunospot analysis.^{11,12} Synthetic peptides corresponding to previously described optimal HLA class I–restricted cytotoxic T-lymphocyte epitopes were used at a concentration of 2mM. According to the HLA genotype, each patient was screened at each time point for responses to a median of 16 (range, 2–32) different cytotoxic T-lymphocyte epitopes (the list of peptide epitopes is available from the authors). Results were expressed as spot-forming cells per million peripheral blood lymphocytes (SFC/10⁶ PBL). A positive response to a given peptide epitope was defined as SFC/10⁶ PBL greater than 3 SEs above background and equal to or above 50 SFC/10⁶ PBL. HLA type was determined by gene amplification.¹³ CD3, CD4, and CD8 lymphocyte counts were determined by flow cytometry (Coulter EPICS IV, Basel, Switzerland) using fluoresceinated DAKO-T3, DAKO-T8, and R-Phycoerythrin DAKO-CD4 (Dako Corp, Glostrup, Denmark).

RESISTANCE TESTING

Resistance was defined as the occurrence of mutations associated with resistance in the reverse transcriptase or protease genes,^{14,15} whereas “virologic escape” was defined as a viral load

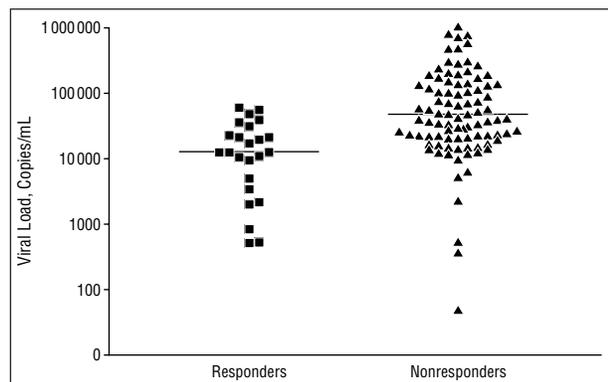


Figure 2. Viral load before highly active antiretroviral treatment in responders and nonresponders. $P = .001$.

above 500 copies/mL in patients who were compliant with HAART, after at least 4 months of continuous treatment. Compliance was assessed by the number of prescriptions filled and by patient interview.

STATISTICAL ANALYSIS

Participants were described using simple statistics (proportions with 95% confidence intervals, means, medians, and ranges). Viral loads and CD4 cell counts were compared using nonparametric tests for matched data (Friedman test to compare 4 cycles, Wilcoxon test for paired comparisons); proportions of participants with a viral load greater than 200 copies/mL were compared using the Cochran Q test (to compare 4 cycles) and McNemar tests (paired comparisons). Baseline characteristics of responders were compared with those of nonresponders using Mann-Whitney tests (for continuous variables) and χ^2 tests (for discrete variables). We also compared the HIV-specific cytotoxic response (ie, the number of HIV-specific interferon γ -producing CD8⁺ T lymphocytes) in responders and nonresponders at week 52, and examined the Spearman correlation between cytotoxic response and the cumulated exposure to the HIV antigen (estimated by the sum of the heights of rebounds in viral load at weeks 2 to 42). All calculations were done with SPSS version 9.0 statistical software (SPSS Inc, Chicago, Ill).

RESULTS

PATIENT CHARACTERISTICS

Ninety-two men and 41 women were recruited. Thirty-six (27%) were from several hospitals in Spain, mostly in Barcelona, and 97 (73%) were from Switzerland (Zurich, 29; Geneva, 26; Bern, 11; Basel, 11; St Gall, 8; Lugano, 7; and Lausanne, 5). Ninety-two (69%) were men. The probable route of acquisition of HIV was homosexual intercourse in 38%, heterosexual intercourse in 45%, and intravenous drug abuse in 17%. Most (83%) had always been asymptomatic, with pretreatment CD4 cell counts ranging from 1/ μ L to 1892/ μ L (median, 398/ μ L), and the log₁₀ of the pretreatment viral load ranging from 2.2 to 6.2 copies/mL (median, 4.5 copies/mL).

The median duration of HAART at study entry was 26 months (range, 8.5-44.5 months) with undetectable viral load for a median of 21 months (range, 6-43 months). The treatment before SSITT consisted of nelfinavir, indinavir, or zidovudine plus 2 NRTIs in 88.5% of patients;

of zidovudine plus zalcitabine plus 2 NRTIs in 4%; and of 2 NRTIs without protease inhibitors in 7.5%. At the start of SSITT the median CD4 cell count was 740/ μ L (range, 318-1909/ μ L).

VIRAL LOAD THROUGH 4 CYCLES OF TREATMENT INTERRUPTION

Of the 133 patients who started SSITT, 43 (32%) left the study protocol before week 40: 30 because their viral load did not decrease to less than 50 copies/mL 8 weeks after restarting treatment; 4 because their CD4 cell counts were less than 400/ μ L at week 39 (Figure 1); 8 withdrew consent; and 1 died of chronic hepatitis C. Hence, 90 (68%) patients completed all 4 cycles.

We compared viral loads during the 5 treatment interruptions at weeks 2, 12, 22, 32, and 42, restricting analysis to 86 patients for whom all 5 values were available. There were no significant differences, either in the proportion of patients having a detectable rebound (>200 copies/mL) (ranging from 60% to 66%; Cochran test, $P = .7$) or in the height of the median rebound (2.7 to 3 log₁₀; Friedman test, $P = .25$).

After 4 cycles of stopping and starting therapy again, HAART was definitively suspended at week 40. The percentage of patients who experienced viral load rebound (>200 copies/mL) was 86% at week 44, and 97% at week 46. In 65% of patients, viral load peaked and then fell spontaneously by greater than 0.5 logs before week 52; in the other patients viral load rose and then remained stable (variation of <0.5 logs between peak measured viral load, and viral load at week 52).

ANALYSIS OF RESPONDERS VS NONRESPONDERS

Results were analyzed at the protocol-defined time point, ie, week 52 (after 12 weeks off treatment). Twenty-three (17%) of the 133 patients (95% confidence interval, 11%-25%) were responders at week 52. Among the 115 patients with a pretreatment viral load of greater than 5000 copies/mL, the response rate was 14% (95% confidence interval, 8%-22%).

Among the 110 nonresponders, 43 did not continue the study protocol before week 40. An additional 11 restarted treatment between weeks 40 and 52, 9 because of an excessive rebound of viremia and 2 for other reasons. Fifty-six additional patients were nonresponders because their viral load at week 52 exceeded 5000 copies/mL.

Responders differed from nonresponders with regard to viral load before HAART (median, 4.09 logs vs 4.57 logs in nonresponders; Mann-Whitney test, $P = .001$). None of the 44 patients with a pre-HAART viral load of more than 60000 copies/mL was a responder (Figure 2).

The proportion of patients experiencing rebounds during weeks 0 to 42 also differed between responders and nonresponders (Table).

There was a tendency for responders to have started HAART earlier than nonresponders, with 10 of (44%) 23 responders starting within 2 years of the probable date of infection, compared with 24 (23%) of 103 nonre-

Presence/Absence of Rebound and Probability of Response

Rebound Status	Total No.	Responders, No. (%)	Nonresponders, No. (%)	P Value
With rebound at week 2	88	8 (9)	80 (91)	<.001
With no rebound at week 2	45	15 (33)	30 (67)	
With at least 1 rebound at weeks 2, 12, 22, 32, or 42	71	12 (17)	59 (83)	<.001
With no rebound at week 2, 12, 22, 32, and 42	15	10 (67)	5 (33)	

sponders ($P = .10$). Six patients (2 responders and 4 nonresponders) had started HAART within 3 months of seroconversion. No correlation could be established between response and pre-HAART CD4 cell counts (responders: median, 441/ μL ; range, 131-745/ μL ; nonresponders: median, 392/ μL ; range, 1-1892/ μL [$P = .7$]), and CD4 cell counts at start of SSITT (responders: median, 752/ μL ; nonresponders: median, 744/ μL [$P = .8$]).

We compared viral loads after 12 weeks' treatment interruption (at week 52) with the last viral load before starting HAART. Eighteen (13.5%) of 133 had a viral load less than 5000 copies/mL before HAART, and 25 (19%) at week 52 (McNemar test, $P = .2$).

LONG-TERM FOLLOW-UP

All 23 responders continued to be followed up without HAART. At the second protocol-specified time point (week 96), 10 (8%) of the original collective of 133 patients still had a viral load below 5000 copies/mL, without antiretroviral treatment.

For nonresponders, the protocol (written in 1999) specified reintroduction of HAART at week 52. However, because official guidelines for antiviral therapy had changed in the meantime,¹⁶ many nonresponders elected not to start treatment again. Among all 133 patients starting SSITT, the percentage of those without treatment was 64% at week 52 and 41% at week 96.

SAFETY OF TREATMENT INTERRUPTION

No major (Centers for Disease Control and Prevention [CDC] class C¹⁷) or minor (CDC class B¹⁷) HIV-related opportunistic diseases were observed during SSITT.

CD4 Cell Counts in the Absence of HAART

There was a slight rise of CD4 cell counts after the 4 cycles of short treatment interruptions (medians, 759/ μL at week 0 and 792/ μL at week 40; paired Wilcoxon test, $P = .02$). After suspension of HAART at week 40, median CD4 cell counts decreased from 792/ μL to 615/ μL at week 52 ($P < .001$). The decrease was observed in both responders (from 699 to 549/ μL ; $P = .002$) and nonresponders (from 860 to 618/ μL ; $P < .001$), and was greater in nonresponders ($P = .01$, Mann-Whitney test).

After week 52, the fall in median CD4 cell counts was slower: from 625/ μL at week 52 to 569/ μL at last follow-up after a median of 56 weeks without treatment ($P = .01$, paired Wilcoxon test). Nonresponders went from a median of 643/ μL at week 52 to 564/ μL ($P < .005$), whereas responders showed a nonsignificant increase from

549/ μL to 574/ μL . The difference in CD4 cell count changes between responders and nonresponders did not reach statistical significance ($P = .13$, Mann-Whitney test).

Viral Escape and Resistance

One (0.7%) of 133 patients developed virologic escape. He had a viral load of 141 copies/mL at week 9 and had no further treatment interruptions. He continued his initial treatment (a combination of lamivudine, zidovudine, and nelfinavir); his viral load rose to 2730 copies/mL after a further 23 weeks. His treatment was changed to a regimen of stavudine, efavirenz, abacavir, and saquinavir boosted with ritonavir, with a decrease of the viral load to less than 10 copies/mL after a further 6 weeks.

The protocol specified viral genotyping for all patients with virologic escape, and for all patients from Geneva. The 1 patient with virologic escape had the 184V mutation in the reverse transcriptase (RT) gene, and multiple mutations in the protease gene before he was switched to salvage treatment. Of the 24 Geneva patients, 8 did not continue treatment interruptions between weeks 0 and 40 because viral load remained above 50 copies/mL after retreatment. In these patients, we analyzed the viral genotype during the last viral rebound before discontinuing treatment interruptions. Regarding the RT gene, viruses from 3 patients were wild type, and 5 had the 184 mutation. When the earliest available sample (week 2) was analyzed in these 5 patients, the 184 mutation was already present in 3. There were no relevant mutations in the protease gene.

In the remaining 16 patients from Geneva, who suspended treatment at week 40, the HIV genotype was determined when the viremia first exceeded 1000 copies/mL (weeks 42 to 46). Regarding the protease gene, viruses from 16 patients had wild-type sequences, or minor variants corresponding to polymorphism. Regarding the RT gene, in 1 patient the virus had multiple RT mutations (41L, 67N, 210W, 215Y). He had been treated with zidovudine for several months, 7 years before SSITT. Had this been known before, he would not have been included in SSITT.

Occurrence of the Acute Retroviral Syndrome

Symptoms resembling those of the acute retroviral syndrome may occur during treatment interruptions.¹⁸ Two patients developed fever, skin lesions, and pharyngitis in association with viremia above 500 000 copies/mL. Symptoms resolved promptly upon retreatment, and these 2 patients did not continue the protocol.

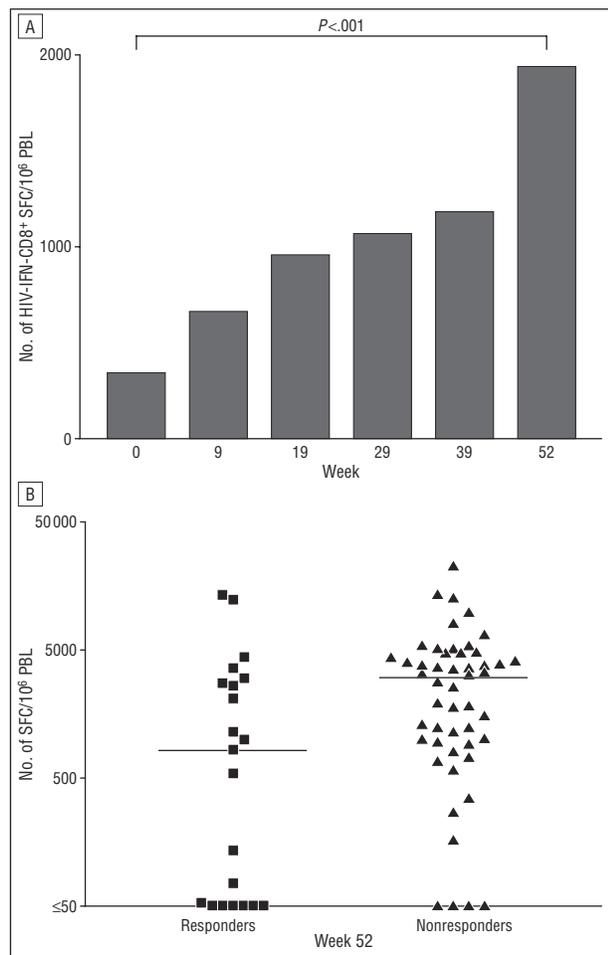


Figure 3. Results of enzyme-linked immunospot analysis in all 71 patients for whom results from weeks 0 and 52 were available. A, Numbers of human immunodeficiency virus-specific, interferon γ -producing, CD8⁺ spot-forming cells per million peripheral blood lymphocytes (HIV-IFN- γ -CD8⁺ SFC/10⁶ PBL) from weeks 0 to 52. B, Numbers of SFC/10⁶ PBL (and median) at week 52 in responders and nonresponders. All patients stayed off therapy between weeks 40 and 52. Responders have statistically lower number of SFC than nonresponders ($P=.01$, Mann-Whitney test).

Although patients were warned about the importance of using condoms, there was one instance of probable transmission of HIV.¹⁹

Results of Retreatment After SSITT

Eighty-two patients restarted treatment after SSITT. When last checked 3 to 6 months after starting treatment again, 68 had an “undetectable” viral load less than 200 copies/mL, 12 had a detectable viral load above 200 copies/mL but were either off drugs or noncompliant, 1 patient (mentioned above) was receiving salvage therapy after viral escape, and 1 was lost to follow-up.

HIV-SPECIFIC CYTOTOXIC T-CELL RESPONSES

The HIV-specific CD8⁺ T-cell responses are shown in **Figure 3A**. The median number of HIV-specific, interferon γ -producing CD8⁺ T lymphocytes increased from 343 per million at week 0 to 1930 per million at week 52 (paired Wilcoxon test, $P<.001$ compared with week 0).

At week 52, responders had a median of 813 SFC/10⁶ PBL, whereas nonresponders had a median of 2999 (Mann-Whitney test, $P=.01$; Figure 3B). The number of SFC at week 52 correlated with the degree of antigen exposure, as measured by the mean of the rebounds in viral load at weeks 2 to 42 (Spearman coefficient $r=0.38$, $P=.002$).

COMMENT

Results of the SSITT do not favor the autovaccination hypothesis. Viral loads after SSITT were similar to those before HAART. Response was more frequent in patients who showed few, or no rebounds during on-off cycling (Table 1). Increases in HIV-specific CD8⁺ T-cell frequencies did not correlate with control of viral replication (low viral load) at week 52. Indeed, there was a statistically significant trend in the other direction: nonresponders tended to have more SFCs at week 52 than responders. Direct correlation of CD8⁺ HIV-specific T-cell response and viral load has also been shown in untreated chronic HIV infection and most probably reflect the extent of antigen exposure.²⁰ Our findings do not exclude the possibility that changes in other components of the immune response, not measured here, might predict viremia during treatment interruptions.

The patients who were eligible for SSITT are representative of a population commonly found in clinical practice. Ninety-five percent had started HAART after the acute retroviral syndrome had passed, usually years after infection. Half had been immunosuppressed (<400 CD4 cells/ μ L) before HAART. Treatment before SSITT had been effective, as evidenced by a viral load that had remained undetectable for a median of 21 months, and a CD4 cell count that had risen from a median of 398/ μ L to a median of 740/ μ L. Among 5248 patients receiving HAART in the Swiss HIV Cohort Study in August 2001, 1405 (27%) had been antiretroviral-naïve before HAART, had had a viral load below the limits of detection for at least 6 months, and had never changed therapy because of a rise in viral load. It is to these patients that the results of SSITT are potentially applicable.

About 1 in 6 participants was a “responder,” with a viral load less than 5000 copies/mL at week 52, after 12 weeks off treatment. Viremia was significantly lower before initiation of HAART in responders than in nonresponders ($P<.001$). Contrary to expectations, the presence or absence of immunodeficiency before HAART did not seem to make a difference, as the pre-HAART CD4 cell counts were similar among responders and nonresponders.

Many additional nonresponding patients stayed off therapy for varying periods, up to more than 1 year. In the absence of HAART, CD4 cell counts fell rapidly during the first 12 weeks, then stabilized. Patients in SSITT stopped therapy when the CD4 cell count was relatively high (median of 740/ μ L), so that the fall during the first 12 weeks did not have clinical consequences. These results suggest that a substantial minority of patients who are presently treated with HAART can safely discontinue the drugs for several months without undue rise in viremia or a dangerous fall in CD4 cell counts. How-

ever, whether such discontinuation is beneficial or harmful can only be determined in future studies, with randomized comparison of continuous with intermittent treatment.

Indeed, one important question may arise regarding the study design: Why did the SSITT not include control groups? Two types of controls were considered: (1) A "simple-stop" control group would have stopped treatment without on-off cycling. Preliminary inquiries indicated that a simple-stop group would not be approved by institutional review boards in 1999, because of then current guidelines that mandated continued treatment for almost all HIV-infected patients.²¹

(2) Another possible control group would have received continuous therapy, the current standard. With a total of 133 patients, such a comparison between continued and intermittent therapy would have lacked power and could only have detected improbably large differences between groups. It would have halved the number of patients in the intermittent treatment group and would have endangered attainment of our primary goal, which was to explore the correlation between HIV-specific immune response and control of viremia without therapy. We therefore believe that the study design was well adapted to the goals of the study.

Were the 2-week interruptions optimal to stimulate HIV-specific immunity, or would longer interruptions have been more productive? We decided on 2 weeks because previous experience indicated that most patients would show a rebound²²⁻²⁵; and because longer interruptions might be associated with an increased risk of the acute retroviral syndrome²⁶ and might depress, rather than enhance, HIV-specific immune responses.²⁷ Rebounds after 2 weeks were measurable in 88 of our patients (66%), and 2 instances of the acute retroviral syndrome occurred. The HIV-specific CD8⁺ T-cell frequencies, as measured by interferon γ enzyme-linked immunospot analysis, were enhanced. It seems therefore unlikely that changing the duration of interruptions would have produced substantially different results.

Treatment interruption was quite safe in this population in that no opportunistic events and little viral resistance were observed. It should be noted, however, that SSITT did not enroll patients who had been exposed to partially effective treatment before HAART, thus eliminating a group at high risk for development of resistance.²⁸

The SSITT lends perspective to some previously reported studies with smaller numbers of patients. In comparison to patients who started their treatment during acute HIV infection,¹⁰ the frequency of response appears lower in the SSITT patients. In contrast to the study by Lori et al,²⁵ we found no evidence that the time until detection of rebound increased. Lori et al observed an increase in time to rebound, during successive treatment interruptions in 3 patients, to above 20 days. In our study, such an increase would have produced an increasing proportion of patients without rebound during the four 2-week treatment interruptions from week 0 to week 40. However, the proportion of patients with rebound remained similar.

Results of SSITT show that iterative treatment interruptions by themselves are rarely sufficient to attain the goal of low, or even undetectable, viremia without antiretroviral therapy. Additional measures such as non-specific immune stimulation using cytokines (in analogy to cancer vaccinology)²⁹ or specific immune stimulation through therapeutic vaccination^{30,31} should be explored.

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