32. When **P** is subjected to a jump from mildly alkaline pH to below pH 6.5, it converts within minutes to the **F′** species—a spectral form at the peroxy level in terms of number of oxidizing equivalents (*13*, *14*, *17*, *38*), but spectrally identical to **F** (*8*). Iodination of **P**, rather than **F′**, at acidic pH was possible by finding that exposure to moderate concentrations of urea (1 M) during formation of **P** at alkaline pH abolishes the **P**→**F′** conversion upon a subsequent acid jump [half-time ($t_{1/2}$) > 30 min)]. Stabilization was not observed when **P** was generated without urea and added to an acid buffer containing urea. Treatment with 1 M urea had no effect on the characteristic difference absorption spectra of **P**, **F**, or **F′** at pH 5 when compared with typical spectra of these forms, indicating that the structure of the binuclear center remains intact. When **P** and **F′** were iodinated under identical conditions, **F′** exhibited only a modest ~twofold increase in labeling of both the heme and subunit I. A much greater increase in labeling of **F′** is expected (>10 times) from the optical spectra of samples if the possibility of **F′** contamination as a source of peptide labeling in **P** is considered. Labeling of both the heme and subunit I in **F′** can be expected because its structure is likely to be similar to that of **P**. Location of the iodide label in **F′** is currently under investigation.

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39. We thank M. I. Verkhovsky for critical and helpful discussions. Supported by NIH grants GM25480 and GM57323.

12 April 2000; accepted 25 October 2000

Control of SIV Rebound Through Structured Treatment Interruptions During Early Infection

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In a randomized controlled trial with acute simian immunodeficiency virus (SIV)–infected macaques, both highly active antiretroviral therapy (HAART) and HAART with fixed-schedule structured treatment interruption (STI-HAART; alternating 3 weeks on and 3 weeks off therapy) suppressed viral load. In the STI-HAART group, T cell virus-specific immune response (VIR) and control of viral rebound increased concurrently during subsequent interruptions. In contrast, VIR did not increase and SIV rebounded after permanent treatment withdrawal in all animals on continuous HAART. Fixed-schedule STI-HAART appears to be an effective alternative to continuous HAART for the early treatment of retroviral infection.

The introduction of HAART represented a milestone in the treatment of HIV infection, and has been associated with a 70 to 80% decline in mortality among AIDS patients. However, virus suppression by HAART is not associated with the appearance of HIV-specific immune responses, and withdrawal of HAART is usually followed by a rapid increase in the number of viral particles in the blood, or viral rebound, and loss of CD4 T lymphocytes (*1*–*4*). Further, the long-term use of HAART is prohibitively expensive for many patients, and has been associated with toxicity and adherence problems (*5*).

STI-HAART, involving repetitive on-and-off cycles of HAART, is an attractive alternative to continuous treatment (*6*), because it might be used to enhance the utility of HAART. The initial excitement began with the description of the Berlin patient, who was able to control HIV after cycling on and off therapy twice (*7*). There is some evidence that STI-HAART can be used shortly after infection to induce immune control of viral replication (*6*–*9*), or during established infection (*10*) to reduce drug-related toxicity or to favor the reappearance of the wild-type virus (*11*). However, no well-controlled study has yet demonstrated a clear advantage of STI-HAART over HAART. Two potential strategies might be followed with STI-HAART: cycle HAART according to a fixed schedule or resume drug treatment after the virus reappears in the plasma. We evaluated a fixed-schedule STI-HAART, because it can be translated into a simple method of managing patients. We selected a symmetrical schedule, with 3 weeks on, then 3 weeks off drug treatment.

We chose to use infection of rhesus macaques by simian immunodeficiency virus (SIVmac251) as a model to compare continuous HAART with a fixed-schedule STI-HAART, because the course of that disease is analogous to that of HIV infection in humans. Seventeen rhesus macaques were infected via mucosal (intrarectal) inoculation with SIVmac251. All animals had seroconverted before treatment was initiated (6 weeks after challenge). The animals were randomized into three groups. One group (five animals) served as an untreated control. The other two groups, (six animals each) were treated for 21 weeks. One of these groups, ("continuous HAART"), was treated with (*R*)-9-(2-phosphonylmethoxypropyl) adenine (PMPA) (*12*) (20 mg/kg body weight, once daily subcutaneously), didanosine (ddI) (10 mg/kg, once daily intravenously), and hydroxyurea (HU) (*13*–*15*) (15 mg/kg, once daily intravenously). The other group ("STI-HAART") was treated with the same drugs according to a fixed schedule consisting of 3 weeks on and 3 weeks off therapy.

Plasma viremia in all three groups had reached a plateau, with an average of 200,000 to 300,000 copies/ml before treatment was started. As expected, viremia continued to increase in the untreated animals (Fig. 1). All 12 treated animals responded to therapy with a rapid decrease in plasma viremia. In the continuous HAART group, viremia became undetectable in all animals by 8 weeks of therapy. In the STI-HAART group, viremia became undetectable in four of six (4/6) animals at week 8 (during the second cycle of treatment) and in 6/6 animals at weeks 14 and 20 (during the third and fourth cycle of treatment, respectively). In both groups, viremia was significantly lower than their baseline values ($P < 0.05$) (*16*) and also significantly lower ($P < 0.01$) than that in the untreated group at all times during therapy. From week 14 of therapy until permanent withdrawal of treatment, the viremia level of the STI-HAART group was not significantly different from that of the continuous HAART group ($P = 0.8$, 0.2, and 0.8, at weeks 14, 17, and 20, respectively). In the STI-HAART group, the rate of plasma viral load rebound during the first interruption was 0.17 log/day, a statistically significant figure ($P < 0.05$), comparable to the rate of

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HIV rebound of patients interrupting HAART (*3*). The rate of viral rebound decreased during the subsequent interruptions, and the changes in viral load became statistically insignificant (log/day rebound = 0.05, 0.03, and 0.00, and P = 0.8, 1.0, and 1.0 at second, third, and fourth interruption, respectively). Viremia was detectable in 6/6, 3/6, 1/6, and 0/6 animals after the first, second, third, and fourth interruptions, respectively. No significant changes in CD4 count were observed during any of the interruptions (P = 0.8, 0.6, 0.3, and 0.3 during first, second, third, and fourth interruption, respectively).

Treatment was permanently withdrawn in both groups 21 weeks after therapy began. All of the continuous HAART group experienced a rapid rebound of viremia, beginning about 14 days after withdrawal. At 41 days after withdrawal, the average viremia in this group had almost returned to baseline values (Fig. 2A). The average rate of viral rebound was 0.2 log/day, a figure comparable to the increase in viral load found in patients interrupting HAART (*3*) and also found after the first treatment interruption in the STI-HAART group. In contrast, all animals in the STI-HAART group controlled virus replication. There was no virus detectable in the blood 41 days after treatment withdrawal. Viremia in the STI-HAART group was significantly lower than in the continuous HAART group ($P < 0.05$, both at 14 and 41 days after withdrawal). The continuous HAART group also had a significant ($P < 0.05$) decrease in CD4 count (Fig. 2B), but the STI-HAART group did not.

During a 6-month follow-up period, the STI-HAART group had minor changes in viremia (Fig. 1). Six months after permanent treatment discontinuation, viremia was still undetectable and CD4 counts were unchanged in all animals. One animal in the continuous HAART group died of AIDS about 7 weeks after withdrawal. Viremia continued to increase in another animal, and a third animal could only transiently control viremia. The remaining three animals spontaneously controlled SIV, and their CD4 count normalized. These results are consistent with previous observations of occasional suppression of SIV in animals after withdrawal of early antiretroviral treatment (*17*). Two animals died of AIDS in the untreated control group.

T cell–mediated immune responses were followed throughout the study. SIV-specific CD8 T lymphocytes were quantified by flow cytometer measuring intracellular interferon-γ (IFN-γ). We called the percentage of $CD8^+$ lymphocytes that expressed IFN-γ in response to SIV stimulation CD8VIR (CD8 virus-specific immune response). Intracellular IFN-γ is an early response marker and is produced after antigen-specific T cell activation in cytotoxic T cell and Th1 type of responses. These cells can respond in case of viral rebound and eliminate infected cells. Zinc finger–inactivated SIV was added to mimic the viral rebound in vitro because, unlike single recombinant proteins or peptides, the whole SIV has the advantage of activating most SIV-specific T cells. Figure 3A illustrates an example of the VIR assay. Unlike animal #19197 (continuous HAART group), animal #774 (STI-HAART group) had

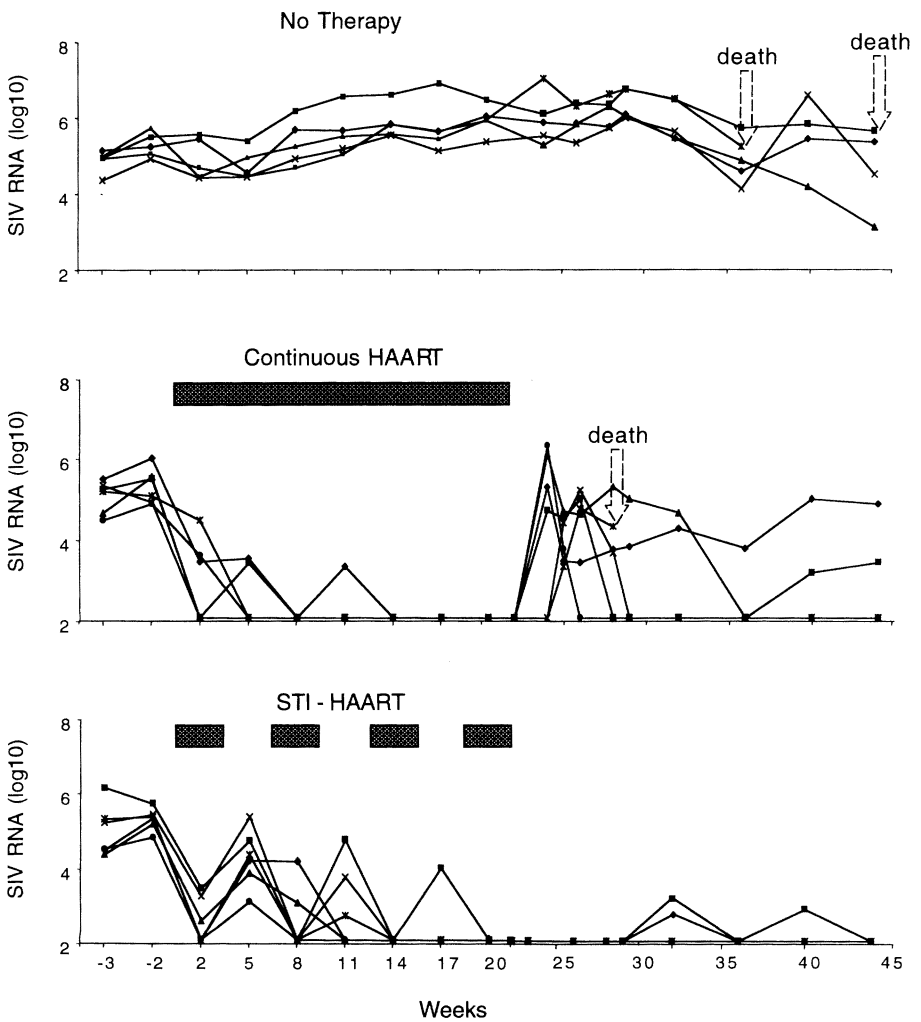


Fig. 1. Plasma viremia (SIV RNA) (*22*) in untreated (**top**), continuously treated (**middle**), and STI-HAART–treated animals (**bottom**). Shaded bars represent time of therapy. Death = animal has been sacrificed because of AIDS.

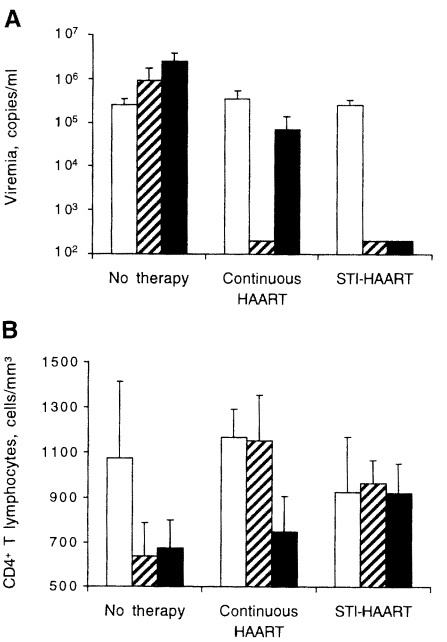
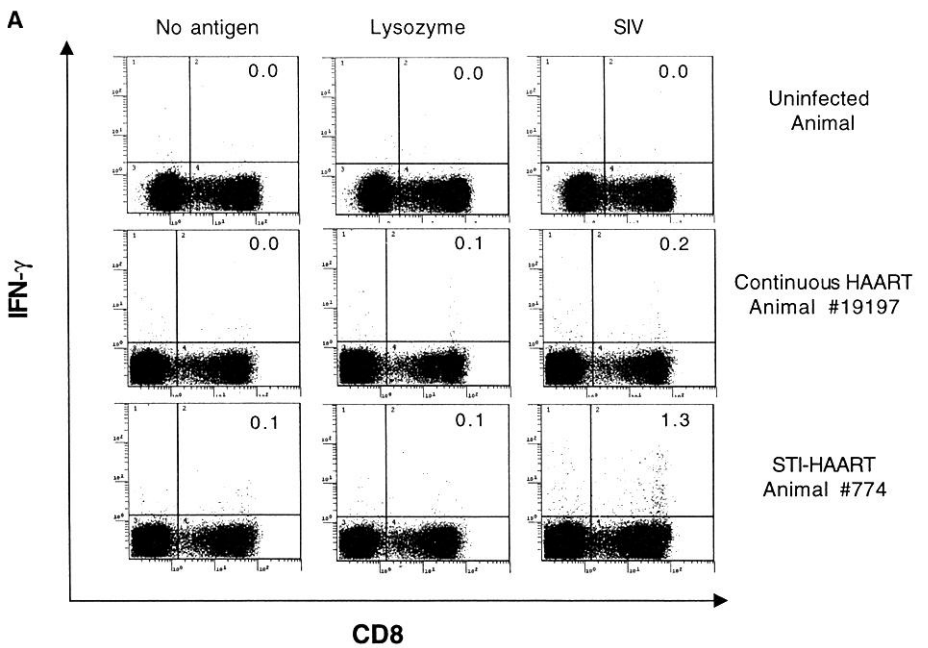


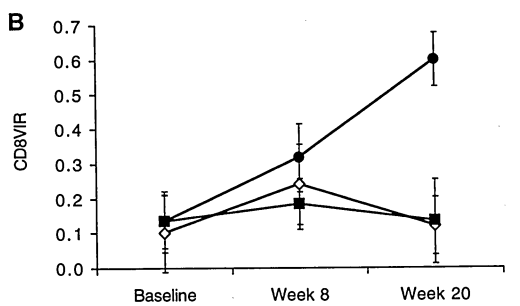
Fig. 2. (**A**) Plasma viremia and (**B**) CD4 T lymphocyte counts in the three randomized groups of SIV-infected rhesus monkeys: untreated controls, continuous HAART, and fixed-schedule STI-HAART. Shown are baselines before therapy (white columns), during therapy at 21 weeks treatment (striped columns), and 41 days after withdrawal (black columns). Standard deviations are indicated as error bars. The threshold of detection of the polymerase chain reaction assay was 200 copies/ml.

1.3% SIV-specific CD8 T lymphocytes. STI-HAART–treated animals mounted a vigorous CD8VIR during the course of the treatment, whereas untreated and continuous HAART–treated animals did not (Fig. 3B). At the end of the fourth treatment cycle (before permanent treatment withdrawal), CD8VIR in the STI-HAART group was significantly higher than in the continuous HAART group ($P < 0.05$). CD8VIR was still high in the STI-HAART group 6 months after withdrawal (Fig. 3C, left panel). The T cell proliferative responses to SIV were vigorous in all the animals treated with STI-HAART (stimulation index > 3), but these responses were not significantly different ($P = 0.9$) between the two groups (Fig. 3C, right panel).

This is the first randomized, controlled study demonstrating that STI-HAART can control plasma viremia and maintain the number of CD4 T lymphocytes as efficiently as continuous HAART during therapy. Subsequent treatment interruptions gradually decreased the rate of viral rebound, and, in contrast to continuous HAART, neither viral rebound nor decrease of CD4 was observed after permanent withdrawal of STI-HAART. Because all animals started the same drug treatment simultaneously, we propose that the STI component was largely responsible for inducing T cell–mediated immunity and achieving viral control after therapy had stopped. Vigorous T cell immunity is typical for individuals with long-term resistance to the virus and is associated with control of HIV viremia (*18–21*).

Early administration of drugs might be crucial for STI-HAART. If the results described here apply to patients infected with HIV, as originally suggested by our description of the case of the Berlin patient (*7*), early treatment with STI-HAART might help to induce immune control of HIV. Because STI-HAART has the potential to improve the tolerance and the affordability of HAART, clinical trials are urgently needed to test whether STI-HAART can become part of the clinical practice.





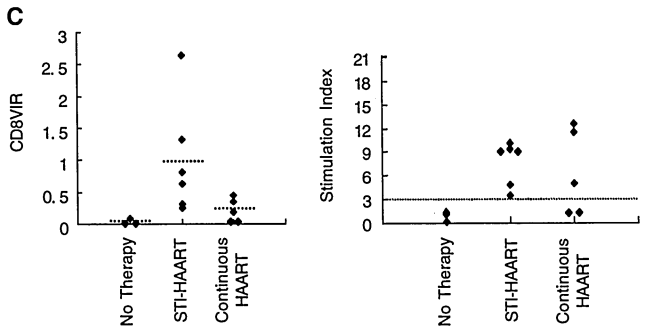


Fig. 3. Analysis of SIV-specific T cell responses. (**A**) Histograms illustrate the detection of VIR, measured as IFN-γ expression by $CD8^+$, CD3-gated T lymphocytes (CD8VIR). Left panels: percentage of $CD8^+$, IFN-γ^+ T lymphocytes, CD3-gated, in the absence of SIV stimulation (background); central panels: after stimulation with an unspecific antigen (Lysozyme); right panels: after SIV stimulation. Upper, middle, and lower panels illustrate representative results obtained in one uninfected animal, one continuous HAART animal, and one STI-HAART animal, respectively. (**B**) CD8VIR (average and standard deviation) in untreated, continuous HAART, and STI-HAART groups at baseline and at the end of the second (week 8) and the fourth (week 20) treatment cycles. Solid squares, no therapy; open diamonds, continuous HAART; solid circles, STI-HAART. (**C**) (left panel) CD8VIR in the untreated control, STI-HAART, and continuous HAART groups 6 months after therapy withdrawal. Dotted lines represent averages. (right panel) Proliferative responses to SIV antigen in the untreated control, STI-HAART, and continuous HAART groups 6 months after therapy withdrawal. Dotted line represents the threshold (stimulation index = 3).

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23. We thank R. Black and N. Miller for their invaluable support, and the National Institute of Allergy and Infectious Diseases for providing the animals. We also thank J. Lifson for his generous gift of Zn finger–inactivated SIV, GILEAD for providing PMPA, and Bristol-Myers Squibb for providing ddI and HU. We gratefully acknowledge technical help from L. Whitman, J. Trocio, C. Hazel, L. Beteta, J. Miller, S. Heatherly, G. Donnelly, L. Wetzel, A. Foli, R. Johnson, and E. Poggio.

6 October 2000; accepted 12 October 2000