Intensification and Stimulation Therapy for Human Immunodeficiency Virus Type 1 Reservoirs in Infected Persons Receiving Virally Suppressive Highly Active Antiretroviral Therapy

Joseph Kulkosky, Giuseppe Nunnari, Miguel Otero, Sandra Calarota, Geetha Dornadula, Hui Zhang, Anne Malin, Julie Sullivan, Yan Xu, Joseph DeSimone, Timothy Babinchak, John Stern, Winston Cavert, Ashley Haase, and Roger J. Pomerantz

Highly active antiretroviral therapy (HAART) has led to significant changes in mortality and morbidity in the human immunodeficiency virus type 1 (HIV-1) epidemic. Nevertheless, because of molecular mechanisms of viral persistence, HAART does not eradicate HIV-1. Didanosine and hydroxyurea were added to the antiretroviral regimens of 3 HIV-1–infected men who were receiving stable HAART and who had HIV-1 RNA levels <50 copies/mL at the initiation of the study protocol, as a novel intensification to attack cryptic viral replication; low-dose OKT3 was then administered, followed by a course of interleukin-2, to stimulate latent provirus. Replication-competent virus was undetectable after treatment, and plasma viral RNA was either undetectable or <5 copies/mL. In trial periods during which no antiretroviral therapy was administered, the patients developed plasma viral rebound. This translational approach combines novel intensification and stimulation therapy to deplete residual HIV-1 reservoirs. Additional experimental approaches must be developed if HIV-1 eradication is to become possible in patients receiving virally suppressive HAART.

Effective combination therapy, called “highly active antiretroviral therapy” (HAART), has changed the human immunodeficiency virus type 1 (HIV-1) epidemic, at least in developed countries [1, 2]. In a significant percentage of infected persons, HIV-1 is inhibited to undetectable levels of viral RNA in the peripheral blood by HAART. Nevertheless, data from several groups indicate that a proviral reservoir exists in resting CD4+ T lymphocytes of patients treated with HAART who do not have clinically detectable levels of viral RNA in peripheral blood plasma [3–7]. Although significant levels of defective proviruses accumulate in CD4+ T lymphocytes in vivo (i.e., viral graveyard sequences) [8], the relatively small quantity of replication-competent provirus in CD4+ T lymphocytes may hinder attempts at reducing the viral reservoirs, and the body may be reseeded with virus if HAART is discontinued. Recent studies have also found replication-competent HIV-1 in peripheral blood monocytes from patients receiving virally suppressive HAART [9, 10]. Thus, there now exists an increasingly large group of patients who must maintain a daily regimen of several antiretroviral agents for indefinite periods of time.

Viral replication can also occur in patients in whom HAART is suppressing virus to such low levels that the virus is not detectable in peripheral body fluids by standard clinical assays. Studies show that during what appears to be full suppression of HIV-1 in the plasma (determined by clinical RNA assays), there is ongoing viral replication in most, but possibly not all, patients, as demonstrated by evolution of viral sequences in cellular reservoirs [10–12]. Sensitive measures of the “footprints” of persistent viral replication also have been used to evaluate HIV-1 mRNA species in infected cells and by HIV-1 long terminal repeat (LTR) DNA circles, which are formed by self-ligation of proviral DNA by cellular nuclear ligases after transport of the viral preintegration complex to the nucleus. Cell-associated HIV-1 RNA and LTR DNA circles have been shown in the CD4+ T lymphocytes of most patients receiving suppressive HAART [9, 10, 13–18]. Although it has been suggested that HIV-1 2-LTR DNA circles have a short in vivo half-life [14], other studies point to a relatively long half-life in vitro [19, 20].

Finally, in a study in which a laboratory-based reverse-transcriptase polymerase chain reaction (RT-PCR) assay that can

Received 29 May 2002; revised 16 July 2002; electronically published 29 October 2002.

All patients in this study signed an institutional review board–approved consent form. The human experimentation guidelines of the US Department of Health and Human Services were followed in conducting this research. The study was also reviewed and approved by the US Food and Drug Administration (investigational new drug 8301).

Financial support: National Institutes of Health (grant AI-46289); Ortho Biotech (clinical grants); Bristol-Myers Squibb (to R.J.P.).

Reprints or correspondence: Dr. Roger J. Pomerantz, Thomas Jefferson University, Jefferson Alumni Hall, 1020 Locust St., Ste. 329, Philadelphia, PA 19107 (roger.j.pomerantz@mail.tju.edu).

The Journal of Infectious Diseases 2002; 186:1403–11
© 2002 by the Infectious Diseases Society of America. All rights reserved.
quantify <5 copies/mL of plasma viral RNA was used, low but detectable levels of virus were demonstrated in all HIV-1–infected patients who had clinically undetectable levels of plasma HIV-1 RNA (i.e., <50 copies/mL) [21]. This has been confirmed by other groups [10, 22]. Of importance, this ongoing viral replication may infect cells in local sites and at a distance within the body.

On the basis of our understanding of molecular lentiviral pathogenesis, approaches can be developed to activate persistently infected cells, which might lead to virus-induced cell death and purging of the viral reservoirs. This approach was attempted using interleukin (IL)–2 alone [23, 24]. Antiretroviral therapy was interrupted in 18 patients who had undetectable plasma virus levels for >1 year [24]. All patients relapsed to relatively high levels of plasma HIV-1 RNA within 2–3 weeks after cessation of HAART. The use of relatively long-term suppressive HAART, alone and in combination with IL-2, did not lead to the elimination of HIV-1 infection.

An initial study was unsuccessful in an attempt to eliminate HIV-1 reservoirs in patients during suppressive HAART by use of anti-CD3 murine monoclonal antibodies (OKT3) and IL-2. Patients had increased viral replication with recrudescence of viremia and experienced extremely serious clinical side effects from high doses of these drugs [25, 26]. No intensification therapy (i.e., additional antiretroviral drugs) was added in this clinical trial in an attempt to halt low-level viral replication [21]. Therefore, persistently infected cells were stimulated, but the ongoing cryptic viral replication probably was not affected in a substantial manner. Because low levels of ongoing viral replication occur in most HIV-1–infected persons who are receiving effective HAART [27], intensification therapy must be added to initial combination therapy to abate this viral replication, if eradication or long-term remission is a goal.

Hydroxyurea has been used, especially in combination with didanosine, with which it has synergistic effects, to inhibit HIV-1 replication. Hydroxyurea selectively inhibits cellular ribonucleotide reductase, a scavenger for tyrosyl free radicals that are essential for enzyme activity, leading to a decrease in intracellular dNTP pools. Thus, hydroxyurea inhibits HIV-1 replication, notably in nonactivated CD4+ T lymphocytes, by indirectly blocking RT, which is dependent on intracellular dNTPs as substrates [28].

It was hypothesized that combining novel intensification therapy with didanosine/hydroxyurea and stimulation of latently infected cells with low-dose OKT3 [29, 30] and IL-2 might lead to remission or eradication of HIV-1 in vivo in selected patients. This would amount to a 2-pronged approach, in which “cryptic” replication was inhibited and latently infected cells were stimulated. In addition, continuation of intensification therapy may be critical during the stimulation phase, to inhibit HIV-1 replication and spread to initially uninfected cells. Three HIV-1–infected men who were receiving stable virally suppressive HAART were included in this initial pathophysiologically based protocol.

### Subjects, Materials, and Methods

**Clinical subjects and protocol.** Three HIV-1–infected men receiving virally suppressive HAART who had plasma HIV-1 RNA levels <500 copies/mL for >1 year (determined by clinical RT-PCR assays) and ≥2 prestudy plasma viral RNA levels <50 copies/mL (determined by clinical ultrasensitive RT-PCR; Roche) were recruited for this protocol (the Residual HIV-1 Disease Eradication/Remission study). All had stable baseline CD4+ T lymphocyte counts (>500 cells/mm3; table 1) and no history of opportunistic infections or other significant medical conditions. Patients 1 and 2 were initially treated with antiretroviral therapy during the chronic stage of HIV-1 infection. Of importance, patient 3 was initially treated with HAART within 2 months of symptomatic primary HIV-1 seroconversion (table 1). These patients were men who had sex with men and were not intravenous drug users. Each was receiving stable HAART and had had no changes in the antiretroviral regimen for ≥1 year before the start of this study.

The patients were treated with novel intensification therapy (didanosine and hydroxyurea) and stimulation therapy (OKT3 and IL-2) (figure 1). OKT3 is an IgG2a murine monoclonal...
antibody that binds to the CD3 molecular complex of T lymphocytes. Hydroxyurea and didanosine were added to the HAART regimen (i.e., intensification) for \( \geq 1 \) month before treatment with OKT3 and IL-2. Hydroxyurea was started at 500 mg orally twice daily (500-mg capsules) with didanosine, 400 mg/day orally (two 200-mg tablets). The patients were monitored while receiving this intensification therapy for \( \geq 1 \) month before treatment with OKT3 and IL-2 was initiated.

Although the term “intensification therapy” usually refers to the addition of antiretroviral drugs to baseline HAART regimens in patients with still-detectable plasma HIV-1 RNA levels, in this eradication study, as described earlier, didanosine and hydroxyurea were added to baseline HAART regimens in patients with plasma virus loads \(< 50\) copies/mL. Of note, recent data suggest that toxicity may limit the use of didanosine/hydroxyurea at daily doses higher than those used in the present study [31].

The patients were admitted to the Thomas Jefferson University Hospital Bone Marrow Transplant (BMT) Unit for intensive monitoring and were treated with a combination of OKT3 and IL-2 to stimulate latently infected cells. Didanosine/hydroxyurea and HAART were continued during OKT3/IL-2 stimulation therapy. OKT3 was administered at 400 \( \mu \)g intravenously on inpatient day 1 (infused over the course of 15 min. This is a lower dose than that used for T lymphocyte depletion in treating solid-organ transplant rejection and in bone marrow transplantation, but it has T lymphocyte activation effects, as shown in data from selected oncology patients in whom OKT3 with or without IL-2 was used for immunotherapy [32, 33]. This dose was chosen in hopes of decreasing the risk of clinical side effects caused by the combination of OKT3 and IL-2 and to limit T lymphocyte depletion that might occur during therapy [29, 30, 32].

No preparative treatment with corticosteroids was used, because corticosteroids decrease the T cell activation induced by OKT3 when it is administered at milligram levels [34]. In addition, subcutaneous IL-2, \( 1.2 \times 10^8 \) IU/m2/day, was administered on days 2–15. The dose of IL-2 was selected to decrease the potential for occurrence of adverse cytokine release effects secondary to use of IL-2 in combination with OKT3. After treatment with OKT3/IL-2, the patients continued to receive didanosine/hydroxyurea and HAART.

The course of OKT3/IL-2 mimics in vitro activation of peripheral blood mononuclear cells (PBMC) for growth and expression of HIV-1 [6]. OKT3 binds to the T cell receptor, leading to initial cellular activation. Because IL-2 most potently stimulates proliferation of T lymphocytes, which express high-affinity IL-2 receptor on their surfaces, OKT3 therapy was begun 24 h before initiation of IL-2.

If replication-competent virus from CD8+ cell-depleted PBMC was not obtainable in these patients from any site after the course of OKT3/IL-2, then, with the patient’s agreement, we discontinued all anti-HIV-1 medications. In such cases, the patient was closely monitored to evaluate virus rebound. Before use of antiretroviral agents was interrupted, a tonsil biopsy specimen was obtained for evaluation of HIV-1 replication and latency in lymphoid tissues. The samples were analyzed by in situ hybridization for unspliced HIV-1-specific RNA and cultured for replication-competent virus [6, 35].

**HIV-1 RNA isolation and quantitative RT-PCR.** Blood plasma and seminal fluid samples were concentrated via ultracentrifugation at 35,000 rpm for 1 h (T90 rotor; Beckman), and RNA was isolated and analyzed by laboratory-based, supersensitive RT-PCR, as described elsewhere [21]. Comparison of the test samples with this serially diluted standard curve of the amplified in vitro transcribed standard was used to quantitate viral unspliced RNA levels to 5 copies, which is within the linear amplification range of this assay. Of note, although it was outside the linear amplification range and therefore was not amenable to precise quantitation, a viral transcript level \(< 5 \) copies could nonetheless be detected with this assay system [21].

**Coculture assays.** CD8+ T lymphocyte-depleted PBMC and seminal cells from the patients were cocultured with phytohemagglutinin-stimulated CD8+ T lymphocyte-depleted PBMC from HIV-1–seronegative donors and monitored for HIV-1 growth for 6 weeks, as described elsewhere [6].

**In situ hybridization for unspliced HIV-1 RNA.** Tonsil biopsy samples were fixed for 4 h in 4% neutral buffered paraformaldehyde and maintained briefly in 70% ethanol until they were paraffin embedded. At least forty 8-mm sections distributed throughout were cut from each biopsy specimen for exhaustive HIV-1 RNA in situ hybridization, using 35S-labeled antisense riboprobes complementary to \( \sim 90\% \) of the full-length viral genome. The slide autoradiographs were exposed for 10–
Figure 2. Virologic and immunologic effects of intensification and stimulation therapy in patient 1 over time, including changes in peripheral blood CD4⁺ T lymphocytes, in vitro outgrowth in CD8⁺ cell–depleted peripheral blood mononuclear cell (PBMC) coculture assays, human immunodeficiency virus type 1 (HIV-1) 2-long terminal repeat (LTR) DNA episomal circle analyses, and plasma HIV-1 RNA levels. *, Seminal fluid and cells were always negative for HIV-1 RNA and viral outgrowth. ddI, didanosine; d4T, stavudine; HU, hydroxyurea; IL, interleukin; ND, not determined; 3TC, lamivudine.

12 days and read against background radiation, as described elsewhere [35], to maximize sensitivity, so that an estimated 625 productively infected cells or 10,000 HIV-1–specific RNA virion copies per gram of lymphoid tissue could be detected.

Results

Clinical effects secondary to intensification and stimulation therapy. Each patient tolerated the addition of didanosine and hydroxyurea with no significant adverse side effects. Of note, no pancreatitis, neuropathy, significant CD4⁺ T lymphocyte depletion (all decreases were <10%), or severe neutropenia occurred in any patient. During their stay in the BMT unit for treatment with OKT3 and then initiation of subcutaneous IL-2 therapy, each patient developed relatively modest clinical side effects from the low-dose OKT3. These consisted of fevers, chills, and myalgia, which occurred ~1 h after administration of the intravenous dose of OKT3, followed by generalized headache and initial stiff neck. These findings, which are consistent with aseptic meningitis, have been described with use of low-dose OKT3 to treat patients with malignancies [29, 30, 34]. The etiology of this syndrome is likely proinflammatory cytokine release in the central nervous system [29, 34]. The chills and fever abated within 24 h, but headaches continued for ~5–7 days. No focal neurologic symptoms were demonstrable during this period, and all patients recovered fully.

Each patient had significant but transient lymphopenia (i.e., total peripheral blood lymphocyte count nadirs of 125, 912, and 284 cells/mm³) secondary to OKT3 administration that fully resolved in 7–14 days without other hematologic side effects. This likely represents rapid redistribution of activated T lymphocytes to lymphoid tissues [29, 30]. Of note, in patient 3, the decrease in CD4⁺ T lymphocytes to 37 cells/mm³ occurred 24 h after OKT3 infusion. Patient 3 developed transient and asymptomatic hyponatremia (blood sodium level ≤122 mEq/dL) after treatment with OKT3; it resolved rapidly and spontaneously without therapy and without clinical side effects. Administration of didanosine and hydroxyurea was stopped for 1 week in patient 1 because of modest neutropenia, which resolved when these drugs were withheld, at which point didanosine and hydroxyurea were reinitiated. No other change in didanosine or hydroxyurea therapy was required for any patient. Thus, our findings show that intensification and stimulation therapy can be given safely to certain patients with HIV-1 infection without subsequent serious adverse side effects.

Virologic and immunologic effects and outcomes. No patient
developed plasma viremia >50 copies/mL during therapy, likely as a result of the intensification of HAART. Figure 2 shows the detailed time-course relationship of therapy with virologic and immunologic parameters in patient 1. We also evaluated 2-LTR DNA circles in PBMC and tonsil tissue from this patient, using semiquantitative DNA PCR, as a potential measure of past in vivo viral infection of cells [18]. The laboratory-based supersensitive RT-PCR showed that plasma HIV-1 RNA levels in patient 1 became undetectable after stimulation and intensification therapy was initiated. At several time points after intensification and stimulation therapy, as well as during therapy with IL-2, plasma viral RNA was completely undetectable with this assay. Two-LTR DNA circles were present at certain time points during the therapy, but these moieties were undetectable at multiple time points before antiretroviral therapy was discontinued, including the final time point. Two-LTR DNA circles also were not detectable in tonsil tissue (data not shown).

Very low levels of plasma HIV-1 RNA were detectable in patient 2 at certain times after OKT3 and IL-2 therapy was initiated, although plasma viral RNA was not clearly demonstrated during IL-2 therapy (table 2). In patient 3, no viral outgrowth was demonstrable, although plasma viral RNA was detected several times during the study, albeit at extremely low levels (table 3). Of interest, in peripheral blood samples from patient 3, extremely low levels of virus (0.2 infectious units/10^6 cells) was detected using a resting CD4+ T lymphocyte assay (described in [4]; data from R. Siliciano, personal communication). As noted in table 2, in patient 2, unlike patient 1, no 2-LTR DNA circles were detectable in PBMC before the trial began.

To evaluate in vivo stimulation of peripheral blood CD4^+ T lymphocytes after in vivo OKT3 treatment, the percentage of these cells that expressed CD69, a late T lymphocyte activation antigen [30, 32], was analyzed by fluorescence-activated cell sorting. CD25^+CD4^+ T lymphocyte counts increased by only minimal amounts (1.2-fold). CD69^+ T lymphocyte counts increased by ~2.5-fold, and double-positive CD69^+CD25^+ T lymphocyte counts increased ~14-fold. These findings are in general agreement with the T lymphocyte activation parameters found in oncology patients treated with low-dose OKT3 [30, 32]. It may be useful to evaluate somewhat higher OKT3 doses in future studies, but toxicity remains a major concern [25].

Each patient’s tonsil biopsy specimens, which were evaluated for unspliced viral RNA by use of robust in situ hybridization techniques [35], were completely negative for HIV-1-specific RNA in mononuclear cells and attached to follicular dendritic cells (data not shown).

**Outcomes of discontinuation of antiretroviral therapy.**

Each
Table 3. Laboratory values and treatment regimens for patient 3 in a study of the use of intensification and stimulation therapy to eliminate human immunodeficiency virus type 1 (HIV-1) reservoirs.

<table>
<thead>
<tr>
<th>Date of culture</th>
<th>Viral outgrowth*</th>
<th>CD4+ cell count, cells/mm³</th>
<th>Blood plasma HIV-1 RNA level, copies/mL</th>
<th>Seminal fluid HIV-1 RNA level, copies/mL</th>
<th>Antiretroviral therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>By clinical RT-PCR</td>
<td>By laboratory-based RT-PCR</td>
<td></td>
</tr>
<tr>
<td>15 Jun 1999</td>
<td>–</td>
<td>1273</td>
<td>&lt;50</td>
<td>42</td>
<td>0</td>
</tr>
<tr>
<td>28 Jul 1999</td>
<td>–</td>
<td>1180</td>
<td>&lt;50</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>2 Dec 1999</td>
<td>ND</td>
<td>1134</td>
<td>&lt;50</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>21 Dec 1999</td>
<td>ND</td>
<td>1382</td>
<td>&lt;50</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10 Jan 2000</td>
<td>ND</td>
<td>1340</td>
<td>&lt;50</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3 Feb 2000</td>
<td>–</td>
<td>952</td>
<td>&lt;50</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>2 Mar 2000</td>
<td>ND</td>
<td>1389</td>
<td>&lt;50</td>
<td>6</td>
<td>ND</td>
</tr>
<tr>
<td>21 Apr 2000</td>
<td>–</td>
<td>1150</td>
<td>&lt;50</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>11 May 2000</td>
<td>–</td>
<td>1114</td>
<td>&lt;50</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>22 Jun 2000</td>
<td>ND</td>
<td>474</td>
<td>&lt;50</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>11 Aug 2000</td>
<td>–</td>
<td>1592</td>
<td>&lt;50</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>5 Oct 2000</td>
<td>ND</td>
<td>1477</td>
<td>&lt;50</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7 Dec 2000</td>
<td>–</td>
<td>1254</td>
<td>&lt;50</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>15 Mar 2001</td>
<td>–</td>
<td>985</td>
<td>&lt;50</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>29 Mar 2001 (day 0)</td>
<td>–</td>
<td>911</td>
<td>&lt;50</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>12 Apr 2001</td>
<td>–</td>
<td>1121</td>
<td>&lt;50</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>2 May 2001 (administration of OTK3)</td>
<td>–</td>
<td>37</td>
<td>&lt;50</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>4 May 2001</td>
<td>–</td>
<td>225</td>
<td>&lt;50</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>8 May 2001</td>
<td>–</td>
<td>355</td>
<td>&lt;50</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>15 May 2001</td>
<td>–</td>
<td>1023</td>
<td>&lt;50</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>30 May 2001</td>
<td>ND</td>
<td>1113</td>
<td>&lt;50</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>26 Jul 2001</td>
<td>–</td>
<td>1114</td>
<td>&lt;50</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>20 Sep 2001</td>
<td>–</td>
<td>1271</td>
<td>&lt;50</td>
<td>&lt;5</td>
<td>11</td>
</tr>
<tr>
<td>8 Oct 2001</td>
<td>– d</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>15 Nov 2001</td>
<td>ND</td>
<td>992</td>
<td>&lt;50</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>26 Nov 2001</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
<td>All antiretroviral therapy stopped</td>
</tr>
<tr>
<td>20 Dec 2001</td>
<td>ND</td>
<td>1047</td>
<td>&lt;50</td>
<td>ND</td>
<td>None</td>
</tr>
<tr>
<td>24 Jan 2002</td>
<td>–</td>
<td>889</td>
<td>22,000</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>6 Feb 2002</td>
<td>ND</td>
<td>1381</td>
<td>&lt;50</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

NOTE. ddI, didanosine; HAART, highly active antiretroviral therapy; HU, hydroxyurea; IL, interleukin; ND, not determined; RT-PCR, reverse-transcriptase polymerase chain reaction.

* From CD8+ cell-depleted peripheral blood mononuclear cell coculture.

b Manufactured by Roche.

c The initial HAART regimen consisted of zidovudine, lamivudine, and efavirenz; on 7 December 2000, nelfinavir was substituted for zidovudine, because zidovudine could not be used with HU in the study protocol.

d Results from an in situ hybridization assay and a viral outgrowth assay performed on a tonsil biopsy sample were negative.

Patient 2 had highly detectable plasma viral RNA rebound after all antiretroviral drugs were discontinued. After HAART was restarted, the patient’s plasma viral RNA levels eventually became undetectable by clinical RT-PCR (table 2). Patient 3 also had a rebound of viremia after discontinuation of all antiretroviral drugs (table 3); after reinitiation of HAART, the plasma HIV-1 RNA levels decreased to <50 copies/mL. Patient 3 was initially treated with HAART within 2 months of primary HIV-1 seroconversion, which, it has been suggested, may lead to maintenance of a more robust anti–HIV-1–based immunity [36]. However, intensification and stimulation therapy did not eradicate HIV-1. Of note, the rebound viruses of all 3 patients had no significant new resistance mutations to antiretroviral drugs in the RT or protease genes by genotypic analyses (data not shown), which demonstrates that antiretroviral resistance did not develop during this protocol. These data suggest that, although plasma viral RNA was decreased to extraordinarily low levels with the intensification and stimulation approach, in
no patient was virus fully eradicated, and each patient experienced rebounds in plasma virus levels after all antiretroviral therapy was discontinued.

Discussion

This initial study of intensification combined with stimulation therapy for HIV-1–infected subjects demonstrates that such techniques can be used relatively safely for patients receiving virally suppressive HAART. These findings contrast with those of a previous study that used only stimulation therapy with OKT3 and IL-2 [25, 26, 33]; the difference between the results of that and the present study likely result from our use of a single dose (400 mg) of OKT3 that is low but still sufficient to stimulate T lymphocytes, rather than the multiple lymphodepletive doses (i.e., 5 mg) used in the previous study [25, 26, 33].

One small study examined the effects of stimulation therapy without intensification therapy but with IL-2 and interferon-γ in HIV-1–infected persons receiving HAART [37]. In that study, no patient had viral eradication, and all rebounded quickly, with high plasma HIV-1 RNA levels.

In the present study, even when intensification therapy with didanosine and hydroxyurea was added to baseline suppressive HAART, patients for whom all antiretroviral therapy was stopped at the end of the protocol developed plasma virus rebound. Most HIV-1–infected patients quickly develop plasma virus rebound a mean of 10–16 days after HAART is discontinued [38]. Patient 1 had a very interesting series of plasma viral RNA spikes, with subsequent transient undetectability that was not a response to reinitiation of antiretroviral therapy. The cause of this pattern is unclear but may be augmentation of anti–HIV-1 immunologic function in this patient, which is now being evaluated. Preliminary data suggest that HIV-1–specific cytotoxic T lymphocyte activity, measured by ELISPOT, was highly augmented during this patient’s stimulation therapy (data not shown). Nonetheless, a potential problem with use of hydroxyurea is the immunosuppression induced by this agent.

In the present study, stimulation of HIV-1 by OKT3/IL-2 leading to plasma viral RNA levels >50 copies/mL was not considered desirable, because this might yield increased infections of cells at a distance within the body [21]. Nonetheless, data showed that OKT3/IL-2 stimulated residual HIV-1 replication. In patient 1, findings included the transient ability to recover replication-competent HIV-1 after OKT3 treatment and a dramatic spike in 2-LTR DNA circles (figure 2). Recent data suggest that HIV-1 2-LTR DNA circles may have a long half-life in vitro [19, 20]. In patient 1, the level of 2-LTR DNA circles in vivo was quite dynamic, with a peak during stimulation and, importantly, a precipitous drop after the end of stimulation. In patient 2, a modest increase in plasma viral RNA after stimulation was detected by the supersensitive RT-PCR assay. Patient 3 also had a modest increase in plasma viral RNA during OKT3/IL-2 therapy (tables 2 and 3).

Viral sequence data that compared rebound plasma virus in the 3 patients after all antiretroviral agents were stopped are
now being analyzed. Preliminary data from patient 1 suggest that the in vitro outgrowth virus from CD8+ cell–depleted PBMC differed from the plasma virus strains isolated during rebound, when all antiretroviral agents had been discontinued (data not shown). Relatively little is known about rebound virus strains that are found after antiretroviral agents are stopped in patients who were receiving virally suppressive HAART. A previous small study suggested that rebound virus which appears after discontinuation of HAART may be present in PBMC and lymphoid tissue [39]. Another study suggested that rebound virus may come from a nonblood source in as-yet-undetermined reservoir sites [40], and a third study found that only actively replicating virus in PBMC (i.e., cell-associated viral RNA) is the source of rebound viral species [41]. We hypothesize that tissue-bound macrophages, which may be relatively resistant to certain intensification and stimulation therapeutic approaches, should be considered as a site of residual HIV-1 disease [27].

It is highly instructive that the use of rationally designed intensification and stimulation therapy did not lead to viral eradication or sustained remission in our study patients. There are a number of potential reasons for this finding, including the lack of stimulation of all latently infected cells or virus species, the lack of ablation of all residual viral replication with the intensification protocol used in this study, and the potential that HIV-1 sanctuary sites exist that were not affected by this regimen (e.g., the central nervous system, the genital tract, potentially the kidney [42], and possibly other hematopoietic cell types). Whether repeated courses of stimulation therapy would decrease the residual HIV-1 reservoirs remains an open question. Nonetheless, a recent study of HIV-1 latency in a severe combined immunodeficient (SCID)-hu mouse model suggested that even minimal stimulation will yield expression of latent provirus [43].

The design of the present study was based on an oncologic paradigm for treatment of HIV-1 infection [7]. This includes use of effective HAART as induction therapy, followed by therapeutic approaches directed against HIV-1 latency, cryptic replication, and sanctuary sites, for the removal of residual disease. Of note, more-potent baseline HAART regimens may be used in future eradication protocols. Nonetheless, other novel therapies appear to be necessary in attempting viral eradication in chronically HIV-1–infected persons; it appears that this may not be possible with the current US Food and Drug Administration–approved armamentarium.

In summary, intensification and stimulation therapy in this study of patients chronically infected with HIV-1 was safe, with relatively minimal adverse side effects. These findings are useful as a prototype for further design of stimulation and intensification therapy to combat HIV-1 infection that persists in patients receiving HAART. Understanding, in detail, the pathophysiology of residual HIV-1 disease, including latency, residual replication, and potential sanctuary sites [28, 44], will be critical in the rational design of future studies aimed at sustained HIV-1 remission and/or viral eradication.

Acknowledgments

We thank Carol Coates and Colleen Dascenzo, for assistance with the patients in this treatment protocol; Didier Trono and Steven Wolinsky, for critical discussions; Robert and Janet Siliciano, for performing resting T cell assays on certain patient samples and for helpful discussions; Rita M. Victor and Brenda O. Gordon, for excellent secretarial assistance; the staff of the Thomas Jefferson University Hospital Bone Marrow Transplant Unit; Marc Rosen and William Keane (Department of Otolaryngology, Thomas Jefferson University), for performing tonsil biopsies; and the patients who volunteered for this study.

References


