Highly active antiretroviral therapy (HAART) can suppress HIV replication to undetectable levels in plasma, but it is unlikely to eradicate cellular reservoirs of virus. Immunotherapies that are cytolytic may be useful adjuncts to drug therapies that target HIV replication. We have generated HIV-specific CD4+ and CD8+ T cells bearing a chimeric T-cell receptor (CD4/9256) composed of the extracellular and transmembrane domain of human CD4 (which binds HIVgp120) linked to the intracellular−/9256 signaling chain of the CD3 T-cell receptor. CD4/9256-modified T cells can inhibit viral replication, kill HIV-infected cells in vitro, and survive for prolonged periods in vivo. We report the results of a phase II randomized trial of CD4/9256 gene–modified versus unmodified T cells in 40 HIV-infected subjects on HAART with plasma viral loads < 50 copies/ml. Serial analyses of residual blood and tissue HIV reservoirs were done for 6 months postinfusion. No significant between-group differences were noted in viral reservoirs following therapy. However, infusion of gene-modified, but not unmodified, T cells was associated with a decrease from baseline in HIV burden in two of four reservoir assays and a trend toward fewer patients with recurrent viremia. Both groups experienced a treatment-related increase in CD4+ T-cell counts.

Key Words: gene therapy, adoptive immunotherapy, HIV infection, HIV reservoirs, highly active antiretroviral therapy

INTRODUCTION

Combination antiretroviral therapy can durably suppress HIV-1 replication [1]. However, despite undetectable levels of virus in plasma, replication-competent virus persists in chronically infected, long-lived cellular reservoirs in all patients [2–5]. Furthermore, drug-mediated suppression of virus replication may be incomplete [5,6], and drug-resistance mutations have been shown to develop in the absence of sustained rebound of plasma viremia [7]. These observations indicate that antiretroviral therapy alone is unlikely to eradicate HIV. Immune-based therapies that are cytolytic and have the potential to eradicate reservoirs of chronically HIV-infected cells may be a useful adjunct to current drug therapies that simply inhibit viral replication.

An effective immune response to chronic viral infections requires the expansion and maintenance of virus-specific CD4+ and CD8+ T lymphocytes. These cells are present during early HIV infection and persist in some patients who remain clinically stable for years (“long-term non-progressors”) [8–10]. In contrast, HIV-specific immune responses are absent in most patients with evidence of disease progression and fail to recover after the initiation of...
effective antiretroviral therapy [11,12]. These data suggest that virus-mediated destruction of the anti-HIV cellular immune response leads to disease progression and that antiretroviral therapy alone may be insufficient for reconstitution of an effective immune response to HIV.

Adoptive transfer of virus-specific expanded CD8+ T cells has shown promise for the treatment of both cytomegalovirus (CMV) [13] and Epstein–Barr virus (EBV) [14,15] infection. However, results in HIV infection have been largely unsuccessful [16–18]. This failure may be due, in part, to difficulties in isolating and expanding major histocompatibility complex (MHC)–restricted HIV-specific T cells from chronically infected individuals. We therefore developed a technique for rapid generation of large numbers of HIV-specific T cells using gene transfer techniques to insert a chimeric T-cell receptor, allowing for T-cell activation and signaling in an MHC-unrestricted manner through the chimeric receptor rather than the native MHC-restricted CD3 T-cell receptor complex. As described elsewhere [19], we constructed an HIV-targeting receptor using the transmembrane and extracellular domains of human CD4 (which targets the gp120 component of HIV expressed on the surface of HIV-infected cells) and the \( \zeta \)-subunit of the CD3 T-cell receptor (the cytoplasmic domain involved in signal transduction and T-cell activation).

\textit{In vitro}, CD4\( \zeta \)-modified T cells specifically mediate the killing of HIV-infected T cells [19] and suppress viral replication in HIV-infected T-cell and macrophage cultures [20]. During early phase I/II clinical studies, infusions of CD4\( \zeta \)-modified T cells from HIV-infected subjects (autologous cells) or identical twins of HIV-infected subjects (syngeneic cells) were well tolerated [21,22]. Gene-modified CD4+ and CD8+ T cells were detected at relatively stable levels in the circulation for as long as 42 weeks post infusion, and trafficking to mucosal reservoirs of HIV was detected in a subset of patients [21].

On the basis of these results, we undertook a randomized phase II study to determine the antiviral activity of CD4\( \zeta \)-modified CD4+ and CD8+ T cells. We selected TABLE 1: Baseline characteristics of patients in HIV-specific T-cell gene therapy study groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Gene modified</th>
<th>Unmodified</th>
<th>( \rho )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>( n = 20 )</td>
<td>( N = 20 )</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>Mean (28, 54)</td>
<td>43 (28, 59)</td>
<td>0.18</td>
</tr>
<tr>
<td>HIV infection (Years)</td>
<td>Mean (1.2, 15)</td>
<td>6.8 (1.5,18)</td>
<td>0.76</td>
</tr>
<tr>
<td>HAART therapy (Years)</td>
<td>Mean (0.4, 3.0)</td>
<td>1.7 (0.6, 3.0)</td>
<td>0.27</td>
</tr>
<tr>
<td>CD4+ count (cells/mm(^3))</td>
<td>Mean (345, 474)</td>
<td>435 (366,505)</td>
<td>0.60</td>
</tr>
<tr>
<td>HIV coculture (log(_{10}) IUPM(^c))</td>
<td>Mean (1.44, 1.90)</td>
<td>1.65 (1.49, 1.82)</td>
<td>0.91</td>
</tr>
<tr>
<td>HIV DNA blood (log(_{10}) copies/( \mu )g DNA)</td>
<td>Mean (1.10, 1.72)</td>
<td>1.40 (1.14, 1.66)</td>
<td>0.97</td>
</tr>
<tr>
<td>HIV DNA rectal (log(_{10}) copies/10(^6) cells)</td>
<td>Mean (2.21, 2.75)</td>
<td>2.36 (2.03, 2.69)</td>
<td>0.59</td>
</tr>
<tr>
<td>HIV RNA rectal (log(_{10}) copies/( \mu )g mRNA)</td>
<td>Mean (1.10, 1.72)</td>
<td>1.40 (1.14, 1.66)</td>
<td>0.97</td>
</tr>
</tbody>
</table>

\( \rho \), test of significance (Wilcoxon).
\( \zeta \)IUPM, infectious units per million.
through monitoring of quantitative changes in residual HIV reservoirs in blood and tissue as well as monitoring for virologic failure on HAART.

**RESULTS**

**Patients**

Of 42 patients who were enrolled and underwent lymphapheresis, 40 patients (20 gene-modified and 20 unmodified) proceeded to study treatment. All 40 subjects completed the three scheduled T-cell infusions, and 38 completed the 24-week followup. All treated subjects were male (mean age 41 years; Table 1). There were 33 Caucasians, 3 African Americans, 3 Hispanics, and 1 Native American. The dominant HIV risk factor was homosexual contact, in 39 patients. The mean duration of taking any HIV medication was 3.6 years, and of HAART, 1.6 years. Of 42 enrolled subjects, 28 had received treatment with antiretroviral regimen other than their current regimen. The mean CD4+ cell count was 422 cells/mm$^3$. In 37 subjects, both screening viral-load measurements were undetectable (< 50 copies/ml). In three subjects, one of three assays yielded low-level positive results (53, 57, and 65 copies/ml). There were no significant differences between treatment groups in any of the baseline characteristics (Table 1).

We evaluated six quantitative assays in the efficacy analysis (plasma viral load, HIV coculture on peripheral blood mononuclear cells (PBMCs), blood HIV DNA, rectal biopsy HIV DNA, rectal biopsy HIV RNA, and blood CD4$^+$ cell count). There were no significant differences between groups in any parameter at baseline (Table 1).

**T-Cell Products**

*Ex vivo* T-cell expansion with or without CD4$^+$ gene modification was successful in all 42 enrolled subjects. Cells were expanded for a median of 14 days (range 12–16), yielding a mean of $4.5 \times 10^{16}$ cells in both the gene-modified and unmodified groups. Average transduction efficiency as measured by DNA PCR for CD4$^+$ was 24 ± 9%. The final T-cell products yielded an average ratio of CD8$^+$ to CD4$^+$ T cells of 1.8:1 overall (2.5:1 gene-modified; 1.5:1 unmodified). Phenotypic analysis revealed a high proportion of cells expressing CD62L (91%) and CD28 (90%), with intermediate expression of CD25 (50% of CD4$^+$ cells and 20% of CD8$^+$ cells). Expression of the gut-homing receptor, $\alpha$4$\beta$7 [25], was also seen in 80% of the cells. We detected no differences between cohorts with respect to any of these phenotypic markers.

**Plasma HIV RNA Levels**

During the 8- to 12-week preinfusion period, 8 of 40 subjects (5 gene-modified; 3 unmodified) experienced a viral-load “blip” (single HIV RNA determination > 40 copies/ml; Fig. 1). During the initial 12 weeks of postinfusion followup, 1 of 20 gene-modified versus 5 of 20 unmodified subjects experienced a viral-load “blip” ($P = 0.18$); however, this difference diminished by week 24 (4/20 gene-modified versus 6/20 unmodified, $P = 0.72$). In addition, one gene-modified and four unmodified subjects experienced viral relapse ($\geq 2$ consecutive measurements > 40 copies/ml or a single determination > 40,000 copies/ml) during the 24-week observation period ($P = 0.34$). Through 20 weeks of observation we noted a trend toward fewer patients with any viral-load rebound (blip and relapse combined) in the gene-modified than the unmodified group (2/20 versus 8/20, respectively; $P = 0.07$). Overall, this trend toward less viral rebound in the subjects receiving gene-modified cells decreased over time, suggesting that repeated CD4$^+$ T-cell infusion might be required to maintain this suppressive effect.

**Quantitative HIV Coculture**

Of the 191 successful quantitative microcultures that we conducted, 147 (77%) were positive for HIV detection. At study entry, 35 of 40 (88%) were positive (all 5 HIV-undetectable patients were in the gene-modified arm). Of the 5 subjects with negative HIV cocultures at baseline, 2 of 5 remained undetectable at all 3 postinfusion time points and 3 were transiently positive at a low level at 1 of 3 time points. There were more patients with a $> 0.5$ log decrease from baseline in cultured virus in the gene-modified than the unmodified group at all postinfusion time points (60 versus 35%, week 8; 46 versus 30%, week 12; and 46 versus 35%, week 24); however, the differences between
groups did not reach significance. Similarly, the mean decrease from baseline was greater in the gene-modified group at all time points, but again the difference did not reach significance (Fig. 2A). The mean change from baseline was statistically different from zero in the gene-modified group at weeks 8 (−0.34 log, \( P = 0.02 \)), 12 (−0.32 log, \( P = 0.02 \)), and 24 (−0.36 log, \( P = 0.02 \)), whereas no significant decrease was measured in the unmodified group at any time point. Finally, we noted a trend toward more rapid decay of infectious virus in the gene-modified group with a shorter half-life (33 months) than the unmodified group (60 months; \( P = 0.14 \)).

Blood HIV DNA
We detected HIV DNA in PBMCs at baseline in 38 of 40 (95%) patients. The two subjects in whom baseline blood HIV DNA was undetectable also had undetectable HIV in the coculture assay, and the results remained undetectable in both assays throughout the study. Interestingly, despite having undetectable virus in blood, both had detectable HIV DNA in rectal biopsies at all study time points, and one showed a > 1 log decrease by 6 months postinfusion. There was no significant overall difference in HIV DNA values between groups at any time point, and no significant overall decay in this reservoir in either group over the 6-month observation period (Fig. 2B).

Rectal Biopsy HIV DNA
Of 40 subjects, 37 completed all 5 planned sigmoidoscopies and rectal biopsies (195/200 planned procedures) and there were no significant procedure-related adverse events. We detected HIV DNA in rectal biopsies of 38 of 40 (95%) subjects at baseline. The two subjects with undetectable HIV DNA in the gut at baseline remained undetectable in this assay through 12 weeks of followup; however, both had positive detection of HIV in the blood HIV DNA and coculture assays throughout the study. At week 24, there was a significant decrease from baseline in gut HIV DNA in the gene-modified group (−0.50 log; \( P = .007 \); Fig. 2C), and the difference between groups approached significance (\( P = 0.06 \)).

Rectal Biopsy HIV RNA
Despite undetectable levels of plasma HIV RNA in all subjects, we detected HIV mRNA in rectal biopsy samples...
of 26 of 40 (65%) patients at baseline, suggesting ongoing viral replication in this mucosal reservoir. The mean gut HIV mRNA levels and change from baseline showed no consistent differences between groups (Fig. 2D). However, we were limited in our ability to measure a change from baseline in this assay by the fact that most measurements were < 0.5 log above the lower limit of assay detection. In 4 of 13 gene-modified and 5 of 13 unmodified subjects, there was conversion from detectable gut HIV mRNA at baseline to undetectable at week 24.

Longitudinal Changes in Multiple HIV Reservoir Assays

Among the four HIV reservoir assays, blood HIV DNA showed the slowest overall decay (slope = -0.00025; half-life = 6060 days) and the rectal biopsy HIV DNA showed the fastest decay (slope = -0.00691; half-life = 169 days), whereas rectal biopsy HIV RNA (slope = -0.00168; half-life = 948 days) and HIV coculture (slope = -0.0017; half-life = 1390 days) showed intermediate decay rates. Only the HIV coculture and rectal biopsy HIV DNA assays yielded decay rates for which the 95% confidence intervals did not include zero.

To analyze the relationship among blood and tissue HIV reservoir assays, we calculated a correlation matrix using pooled data from both cohorts. All four assays were significantly correlated with each other. Blood HIV DNA and HIV coculture showed the strongest correlation at baseline (P = 0.003) and week 24 (P = 0.006). Rectal biopsy HIV RNA and DNA correlated most strongly with HIV coculture at baseline (rectal RNA versus coculture: P = 0.004; rectal DNA versus coculture: P = 0.002), whereas postinfusion they showed a stronger correlation with blood HIV DNA (rectal RNA versus blood DNA: P = 0.02; rectal DNA versus blood DNA: P = 0.008). We also analyzed the four reservoir assays for the correlation between their change from baseline values over time. At week 24, the changes from baseline in all four assays were significantly correlated with each other. HIV coculture showed the strongest correlation with the other three assays (coculture versus blood DNA, P = 0.05; versus rectal DNA, P = 0.02; versus rectal RNA, P = 0.0002).

CD4+ Cell Counts

We noted a consistent increase from baseline in CD4+ cell counts over time in both cohorts. At week 24 the mean increase was 41 cells/mm³ (10%) in the gene-modified and 37 cells/mm³ (8%) in the unmodified group (Fig. 3). There were no significant differences between groups in mean CD4+ T-cell counts or changes from baseline. To determine whether T-cell infusion affected the rate of change in CD4+ cell counts, we calculated slopes of CD4+ cell counts over time pre- and posttreatment. There was a negative mean slope (decreasing CD4+ counts) in both groups before treatment, but a positive mean slope (increasing CD4+ counts) in both groups after treatment. The change between preinfusion and postinfusion slopes approached significance in both the gene-modified (P = 0.04) and unmodified arms (P = 0.09). These data suggest that T-cell infusion rather than 6 additional months of HAART was likely responsible for the increasing CD4+ cell counts after treatment.

Impact of Viral Rebound on HIV Reservoirs and CD4+ Cell Counts

Recent data suggest that HIV reservoirs decay more rapidly in subjects who remain fully suppressed on HAART compared with those with intermittent viremia [6]. We therefore conducted a subset analysis on the four HIV reservoir assays, separating patients into those who maintained an undetectable plasma viral load throughout the study (n = 25) and those who experienced either a viral blip or sustained relapse in plasma viral load (n = 15). Because of the small numbers of patients, we pooled data from both treatment arms for this analysis. For HIV coculture, consistent decreases in culturable virus over time were only noted in the “never blip” subgroup (Fig. 4A). Similarly, a significant decrease in rectal HIV DNA was limited to the “never blip” subgroup from week 12 through week 24 (Fig. 4B). Decay half-life analysis suggested a somewhat more rapid decay in the “never blip” compared with the “blip or relapse” subgroup in the HIV coculture assay (40 versus 58 weeks, respectively). There was no consistent change in the level of blood HIV DNA or rectal HIV RNA in either subgroup. Finally, we compared changes in CD4+ cell counts over time. Somewhat surprisingly, a significant increase from baseline in CD4+ cells was only seen in the group with intermittent or sustained recurrence of viremia, whereas there was no change in the group with consistently undetectable viral loads (81 versus –3 cells/mm³ at week 24 respectively, P = 0.02; Fig. 4C).
TRIAL

CD4ζ-Modified T-Cell Survival, Trafficking, and Function
We documented sustained, high-level persistence of gene-modified T cells in the blood of all patients for at least 24 weeks (0.1–10% of PBMCs; Fig. 5A). A subset analysis done on six patients confirmed persistence of both gene-modified CD4+ and CD8+ T cells (Fig. 5B) as well as relatively stable levels of CD4ζ RNA expression (Fig. 5C). Furthermore, we detected CD4ζ-modified T cells by flow-cytometric analysis of PBMCs, confirming persistent surface expression of the chimeric receptor (data not shown). Finally, we documented tissue trafficking of gene-modified T cells, with 76% of patients showing detectable levels of CD4ζ-modified T cells in rectal mucosal biopsies at one or more time points (60% at week 8, 63% at week 12, and 50% at week 24). We analyzed in vivo T-cell function by isolating T cells from peripheral blood before and after T-cell infusion followed by stimulation with anti-CD3 and anti-CD28 (to assess nonspecific T-cell activation) and anti-CD4 and anti-CD28 (to measure CD4ζ chimeric receptor–specific stimulation). T-cell activation was assessed by measurement of γ-interferon release in an ELISPOT assay. Although CD4ζ-specific stimulation was greater in the gene-modified group at all postinfusion time points, the differences between groups were not significant (data not shown).

Safety
We administered a total of 120 infusions of gene-modified or unmodified T cells. There were no serious product-related adverse events. The majority of adverse events related to T cells were mild in severity and included fever (19%), chills (17%), asthenia (10%), headache (10%), and nausea (10%). There were no differences in toxicities between groups.

DISCUSSION
In this study we have confirmed the safety and feasibility of adoptive therapy with CD4ζ gene–modified T cells. We observed prolonged, stable persistence of gene-modified T cells in blood and trafficking to gut mucosa, thus confirming our previous observations following CD4ζ T-cell infusion in HIV-infected subjects with uncontrolled viremia [21]. These data suggest that the presence of actively replicating virus is not required for in vivo maintenance of CD4ζ-modified CD4+ or CD8+ T cells. The prolonged in vivo survival of CD4ζ-modified CD4+ and CD8+ T cells is in sharp contrast to other studies in which gene-modified HIV-specific CD8+ T cells were cleared rapidly from the circulation [22,26]. The improved T-cell survival seen in our studies with CD4ζ T cells may be due to coinfusion of HIV-specific CD4+ “helper” T cells, ex vivo stimulation through CD3 and CD28, short duration of ex vivo cell culture, and/or the lack of immune responses directed against the CD4ζ chimeric receptor.

Although we observed no significant between-group differences in levels of residual blood and gut HIV rese-
voirs following gene-modified versus unmodified T-cell infusion, there were some interesting trends suggesting the possibility of antiviral activity of CD4\textsuperscript{+}/H9256-modified T cells. These included a sustained decrease from baseline over the 6-month study period in the amount of replication-competent virus cultured from blood, a decrease from baseline in HIV DNA isolated from rectal mucosa, and a trend toward less transient or persistent viral rebound in plasma in the gene-modified group. Taken as a whole, these data suggest that CD4\textsuperscript{+}/H9256-modified T cells may have antiviral activity in vivo. Although a previous study suggested that addition of interleukin-2 to HAART was associated with a smaller pool of resting CD4\textsuperscript{+} cells containing replication-competent HIV [27], to our knowledge, this is the first prospective trial evaluating the impact of adoptive T-cell immunotherapy on the clearance of residual virus in long-lived reservoirs.

The decay half-life of latent HIV reservoirs in subjects on HAART, as measured by quantitative HIV coculture from blood, has been reported by other groups with disparate results. The overall decay half-life we saw in this study for both treatment groups combined (46 months) was similar to that previously reported in chronically infected adults (44 months) [3], but much longer than that reported in acutely infected individuals (6 months) [5]. This probably reflects the chronicity of HIV infection in our study population, in which the median duration of infection was 7 years with a range up to 18 years. Although the decay half-life was shorter in the gene-modified group than the unmodified group (33 versus 60 months, respectively), the difference was not statistically significant. Technical issues limited the utility of the blood HIV DNA and rectal HIV RNA assays as tools for monitoring changes in reservoirs of infectious virus. The minimal change in blood HIV DNA noted over the 24-week study period may have been due to the lack of selectivity for replication-competent provirus in the assay utilized. Assays designed to measure HIV cDNA episomal circles may be more sensitive for picking up changes in DNA that reflect recent HIV replication [28,29].

Similarly, the ability to detect between-group differences in rectal mucosa–associated HIV RNA following study treatment was limited by the sensitivity of the RNA assay; 35% of subjects had undetectable levels of mucosal HIV RNA at baseline, and > 30% of the remaining patients in both treatment groups converted from detectable at baseline to undetectable at week 24. On the other hand, a significant decrease in gut-associated HIV DNA was noted in the gene-modified group, suggesting a possible impact of gene-modified T cells on this tissue reservoir of virus.

Our data provide suggestive evidence that CD4\textsuperscript{+}-modified T-cell infusions may suppress virologic rebound (viral blip or relapse) in plasma for up to 4 months following infusion, and it is possible that repeated T-cell infusions may prolong this effect. In a large randomized trial, virologic failure occurred in 4% of subjects over a 16-week...
period maintained on optimal therapy who initially maintained undetectable viral loads on HAART for 6 months. This failure rate was higher in subjects maintained on suboptimal regimens, those who were not antiretrovirally “naive” at the time of protease inhibitor initiation, and those with higher pretreatment viral loads or longer time to initial viral suppression [30]. The overall virologic failure rate in our study (12% at 6 months) was higher than previously reported and probably reflects the less rigid entry criteria in our trial. The kinetics of initial viral decay and pre-HAART viral loads were not available on subjects in our study, and the majority of patients had received previous antiretroviral therapy before initiation of HAART. Although the long-term clinical significance of transient viral blips is unclear [31], they are a marker of active viral replication and have been associated with a slower decay rate in latent HIV-infected T-cell reservoirs than that seen in subjects with consistent maintenance of plasma HIV RNA levels of < 50 copies/ml [6]. Immune-mediated cytolysis of HIV-infected cells or secretion of soluble HIV-suppressive factors [32] may lead to more complete suppression of viral replication than that which can be achieved with HAART alone and, therefore less selective pressure to generate antiretroviral resistance mutations. If this is true, addition of HIV-specific T-cell therapy to HAART may lead to enhanced virologic failure–free survival in patients. This pilot study was small and underpowered due to the complexity and expense of the extensive analyses performed; assessment of the true impact of CD4+–modified T cells on virologic rebound will require further exploration in larger studies with longer patient followup.

A subset analysis comparing patients with complete and sustained viral-load suppression (consistent plasma HIV RNA < 50 copies/ml throughout the study) to those with transient or persistent virologic failure showed that significant decay in two of the four reservoir assays (HIV coculture and rectal HIV DNA) was only seen in the consistently aviremic subset. This supports the data previously reported in acutely infected individuals, which demonstrated significant decay of latently infected cellular reservoirs only in subjects with durable and complete suppression of plasma viremia [6]. However, the differences we saw in this study were much less pronounced than previously reported, again perhaps reflecting the chronicity of HIV infection in our study population. Interestingly, we noted significant increases in CD4+ cells only in the subgroup with intermittent or sustained recurrence of viremia. This observation supports previously published data in patients on HAART, where subjects with the greatest increases in CD4+ cells were at highest risk for return of plasma viremia [30]. One potential explanation is that subjects who experienced a greater treatment-mediated expansion of their CD4+ cell population may have had more virus-susceptible target cells available to support viral replication.

Finally, our data support the feasibility of quantifying reservoirs of residual HIV infection in subjects with undetectable plasma HIV RNA as feasible surrogate endpoints of antiviral activity in clinical trials. We detected HIV at baseline in blood and tissue cellular reservoirs in 65–95% of patients, depending on the assay, and results from all four reservoir assays correlated with each other both before and after T-cell infusions. Our data suggest that these blood and tissue viral reservoirs are closely linked. Of the four assays (quantitative HIV coculture from PBMCs, HIV DNA in PBMCs, and HIV DNA and RNA in rectal mucosa), the coculture assay and the rectal biopsy DNA assay were the most useful for monitoring changes in blood and tissue HIV reservoirs over time. The quantitative HIV coculture assay is too labor-intensive and expensive to be considered as a routine monitoring tool in large trials. Analysis of rectal mucosal biopsy samples, however, is a promising tool. Compliance with the multiple sigmoidoscopic procedures conducted in this study was extremely high, the procedures were well tolerated, and the assays rapid and reproducible.

The prolonged survival of chimeric receptor–modified T cells seen in our studies with CD4+–modified CD4+ and CD8+ T cells represents a major step forward in the clinical application of adoptive T-cell gene therapy. However, the in vivo function of chimeric receptor–modified T cells remains in question. Measurement of in vivo T-cell function is technically difficult, and results can be difficult to interpret because of the requirement for additional ex vivo stimulation in most cases. In this trial we attempted to measure in vivo T-cell function by analysis of CD4+–specific T-cell activation in vivo before and after treatment, but no significant between-group differences were noted. Strategies to enhance in vivo expansion of chimeric receptor–modified T cells and optimize in vivo function may be necessary. Such strategies might include infusion of immunostimulatory cytokines (for example, interleukin-2) with the T cells, or genetic modification of T cells with chimeric costimulatory receptors to provide a second signal for T-cell activation [33]. An additional strategy reported recently is modification of EBV-specific T cells with a tumor antigen–specific chimeric receptor to capitalize on the known ability of EBV-specific T cells to persist and remain functional for prolonged periods in vivo, potentially allowing more potent and sustained in vivo T-cell function following stimulation through the chimeric receptor [34].

In conclusion, CD4+ gene–modified T-cell infusions were well tolerated and demonstrated prolonged in vivo survival in subjects with undetectable viremia, confirming the results seen in our previous trial in subjects with active viral replication. Trends suggesting a potential impact of gene-modified T cells on blood and tissue reservoirs of HIV as well as on the frequency of virologic rebound in plasma will require further exploration in larger studies.
MATERIALS AND METHODS

Study design. This was an investigator-blinded, randomized trial of CD4+ gene-modified versus unmodified T-cell infusions in HIV-infected adults conducted at five clinical sites. Eligibility included 24 weeks of stable treatment with at least three antiretroviral drugs, including at least one protease or nonnucleoside reverse transcriptase inhibitor ("highly active antiretroviral therapy" or HAART), two biweekly (or two of three) plasma HIV RNA levels below the 50 copies/ml detection limit of the AMPLICOR HIV-1 MONITOR Test (Roche Molecular Systems, Pleasanton, CA), and CD4+ cells at the time of study entry > 200 cells/mm3. We excluded patients with a history of an AIDS-defining complication within 2 years. We randomly assigned patients to one of two treatment groups. The first group received a total of 3 x 10^6 CD4+ modified CD4+ and CD8+ T cells divided over three intravenous infusions (treatment weeks 0, 2, and 4). The second group received the same dose and schedule of genetically unmodified T cells. All patients continued their stable HAART regimen. We followed patients for 24 weeks following the infusion, and those who received gene-modified cells subsequently entered lifelong followup for monitoring of replication-competent retrovirus (RCR). The patients and clinical sites, but not the sponsor, were blinded as to the treatment assignment.

T-cell processing. We conducted lymphapheresis and T-cell processing as described [21], with the exception that unmodified T cells were not subjected to retroviral transduction. Briefly, PBMCs were isolated by density gradient separation, stimulated using magnetic beads coated with anti-CD3 and anti-CD28 [35], transduced with CD4+ retroviral vector supernatant on days 5 and 7 (gene-modified only), and expanded. Cryopreserved T-cell products were subsequently shipped frozen to the clinical sites, thawed, and immediately infused.

Quantitative HIV coculture. This assay was a modification of an earlier described assay [4]. Briefly, patient PBMCs were isolated from 60 ml blood, CD8-depleted using immunomagnetic beads, stimulated for 24 hours with plate-bound anti-CD3/CD28, and then plated in twofold limiting dilution in duplicate beginning at 5–20 x 10^6 cells/well in six-well plates. Cells were cultured for 4 weeks with addition of 5–10 x 10^6 phytohemagglutinin-stimulated, CD8-depleted pooled donor PBMCs on days 1, 7, and 14. Supernatant from each well was then harvested and analyzed for HIV p24 by ELISA (Couler Immunotech, S. San Francisco, CA) and scored as positive or negative.

Blood HIV DNA. This analysis was done with a prototype quantitative HIV DNA assay as described [36]. Briefly, DNA was extracted from PBMCs with a buffer that contains proteinase K and detergent. A DNA quantitation standard was comphlied with each sample. Quantitation was conducted on microwell plates with colorimetric detection as currently used in the AMPLICOR HIV-1 Monitor Test. The level of HIV DNA in PBMCs was then normalized to the amount of genomic DNA as determined by Hoechst dye–based quantitation.

Rectal biopsy HIV burden. Rectal onobi galleries were acquired using flexible sigmoidoscopy and analyzed as described [21]. Briefly, the frozen tissue was pulverized with a steel mortar and pestle that had been precooled on dry ice. mRNA was extracted using the QuickPrep Micro mRNA Purification Kit (AmershamPharmacia Biotech, Piscataway, NJ) according to the manufacturer's instructions. HIV-1 mRNA was quantified by RT-PCR as described [21]. DNA was isolated in duplicate from pul- verized biopsy samples using urea lysis buffer and standard phenol-chloroform extraction. HIV-1 DNA was quantified by PCR as described [21] and the results normalized to 2 x 10^6 copies of β-globulin. The yield was determined spectrophotometrically, and an average biopsy sample yielded 1 μg of mRNA and 20–40 μg of DNA.

Quantitation of CD4+ RNA and DNA. CD4+ DNA was quantified by Taqman PCR on PBMCs and isolated rectal biopsy DNA at Specialty Labs (Los Angeles, CA) as described. In six patients we quantified CD4+ RNA on PBMCs by Taqman RT-PCR at Cell Genesys (Foster City, CA) as described [21].

Statistical analysis. All HIV reservoir assay data was log_{10} transformed before analysis. For undetectable values, we assigned a value equal to 50% of the detection limit for all assays except HIV coculture, for which the lower limit of detection was used. We used a limiting-dilution analysis fortan program to analyze the raw HIV coculture data to calculate the infectious units per million input cells (IUPM) [37]. Between-group differences were analyzed parametrically using the analysis-of-variance and nonparametrically using the Wilcoxon test and the analysis-of-variance of ranks. We used the SAS system to do all analyses. We calculated the decay half-life of HIV in cellular reservoirs using a random-effects stepwise regression model [38]. Data were calculated on the natural log scale. We replaced data with negative estimates with the largest positive decay half-life value in the group. We calculated the slopes of the CD4+ cell counts versus time for pretreatment (four time points over 8–12 weeks) and posttreatment (seven time points over 24 weeks) periods using ordinary linear regression models. Given the exploratory nature of this study and the small sample size, we made no adjustments for the multiple analyses done.

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