Immunologic Responses Associated with 12 Weeks of Combination Antiretroviral Therapy Consisting of Zidovudine, Lamivudine, and Ritonavir: Results of AIDS Clinical Trials Group Protocol 315

Michael M. Lederman, Elizabeth Connick, Alan Landay, Daniel R. Kuritzkes, John Spritzler, Marty St. Clair, Brian L. Kotzin, Lawrence Fox, Margo Heath Chiozzi, John M. Leonard, Franck Rousseau,* Michael Wade, Joana D'Arc Roe, Ana Martinez, and Harold Kessler

Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, Ohio; University of Colorado Health Sciences Center and VA Medical Center, Denver, Colorado; Rush Medical College, Rush-Presbyterian-St. Luke's Medical Center, Chicago, and Abbott Laboratories, Abbott Park, Illinois; Harvard School of Public Health, Boston, Massachusetts; Glaxo-Wellcome Inc., Research Triangle Park, North Carolina; Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, and Social and Scientific Systems, Inc., Rockville, Maryland

Human immunodeficiency virus (HIV)-1 infection is associated with progressive cell-mediated immune deficiency and abnormal immune activation. Although highly active antiretroviral therapy regimens can increase circulating CD4 T lymphocyte counts and decrease the risk of opportunistic complications, the effects of these treatments on immune reconstitution are not well understood. In 44 persons with moderately advanced HIV-1 infection, after 12 weeks of treatment with zidovudine, lamivudine, and ritonavir, plasma HIV-1 RNA fell a median of 2.3 logs ($P < .0001$). Circulating numbers of naive and memory CD4 T lymphocytes ($P < .001$), naive CD8 T lymphocytes ($P < .004$), and B lymphocytes ($P < .001$) increased. Improved lymphocyte proliferation to certain antigens and a tendency to improvement in delayed-type hypersensitivity also were seen. Dysregulated immune activation was partially corrected by this regimen; however, the perturbed expression of T cell receptor V regions in the CD4 and CD8 T lymphocyte populations was not significantly affected. Ongoing studies will ascertain if longer durations of virus suppression will permit more complete immune restoration.

Infection with the human immunodeficiency virus type 1 (HIV-1) is associated with progressive and profound loss of immune function that places infected persons at risk for opportunistic infections, malignancies, and death. HIV-1–related immune deficiency is characterized by decreases in the numbers of circulating CD4 T helper lymphocytes. Decreases in the numbers of other circulating lymphocyte populations that also play important roles in host defenses, such as CD8 T lymphocytes and B lymphocytes, eventually occur as well [1, 2]. T lymphocyte dysfunction in HIV-1 infection is characterized in vitro by diminished proliferative and cytokine responses [3, 4]. In vivo, this dysfunction is reflected in diminished delayed-type hypersensitivity (DTH) responses to skin test antigens, which is associated with an accelerated risk of disease progression [5, 6]. Perturbation of the T lymphocyte receptor repertoire has been recognized, with an apparent expansion of some families and relative depletion of others [7, 8]. It is widely held that these impairments in cell-mediated immune responses underlie the enhanced risk for opportunistic infections that characterizes HIV-1 infection and AIDS.

Paradoxically, HIV-1 infection also is associated with evidence of immune activation. For example, heightened expression of lymphocyte activation markers CD38 and HLA-DR is seen in HIV-1 infection, and increased CD38 expression on CD8 T lymphocytes is associated with a poorer outcome in HIV-1 disease [9]. Immune activation may contribute to HIV-1 disease pathogenesis through activation of HIV-1 expression [10] and perhaps through activation of cytotoxic mechanisms that contribute to cell depletion [11, 12]. Heightened expression of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), also is seen in HIV-1 disease [13], and plasma levels of TNF-α correlate with plasma HIV-1 RNA levels [14, 15]. Whether TNF-α expression drives HIV-1 propagation, whether HIV production drives TNF-α expression, or whether the relationship is bidirectional remains to be determined.

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Reprints or correspondence: Dr. Michael M. Lederman, Division of Infectious Diseases, University Hospitals of Cleveland, AIDS Clinical Trials Unit, 11100 Euclid Ave., Cleveland, OH 44106 (MXL6@po.cwru.edu).

* Present affiliation: Triangle Pharmaceuticals, Research Triangle Park, North Carolina.

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Potent antiretroviral agents and combinations of antiretroviral agents have produced dramatic decreases in circulating and tissue virus levels. These therapies have been associated with decreases in the occurrence of opportunistic infections and in short-term mortality [16]. It is not clear whether these clinical benefits are related simply to a halting of the progressive immune deterioration that is seen in HIV-1 disease or whether immune responses are actually restored as a result of therapy. AIDS Clinical Trials Group (ACTG) protocol 315 was designed to ascertain if administration of highly active antiretroviral therapy (HAART) to persons with moderately advanced HIV-1 infection was associated with evidence of immunologic restoration.

Materials and Methods

Eligibility criteria included laboratory documentation of HIV-1 infection, a circulating CD4 lymphocyte count between 100 and 300 cells/μL, and history of zidovudine therapy for at least 3 months. Patients were excluded from participation if they had ever received lamivudine or any HIV-1 protease inhibitor or if they required therapy with medications known to or thought to have clinically significant interactions with ritonavir. Laboratory exclusions included a hemoglobin concentration $<$9.0 g/dL for women or $<$9.5 g/dL for men; an absolute neutrophil count $<$1000/μL; a platelet count $<$20,000/μL; serum alanine aminotransferase, aspartate aminotransferase, or alkaline phosphatase levels more than three times the upper limit of normal; an elevated serum bilirubin level; serum creatinine higher than one and a half times the upper limit of normal; or a fasting serum triglyceride level $>$400 mg/dL.

Before initiating the HAART regimen, all patients discontinued antiretroviral treatment for 5 weeks. Patients were required to receive prophylaxis for *Pneumocystis carinii* pneumonia during the antiviral washout period and thereafter at the discretion of the treating physician. On day 0, patients began therapy with ritonavir, 300 mg orally twice a day, and increased the dosage to 600 mg twice daily by day 7. On day 10, lamivudine, 150 mg orally twice daily, and zidovudine, 200 mg thrice daily, were begun. Patients intolerant of full doses of ritonavir were permitted to decrease dosing to 500 mg twice daily or 300 mg thrice daily.

Immunologic evaluations included skin testing for DTH responses to purified protein derivative of *Mycobacterium tuberculosis* (Connaught, Swiftwater PA) and *Candida*, trichophyton (ALK Laboratories, Berkeley, CA), and mumps (Connaught) antigens, which was done on day -7, day 0, and week 12 of therapy. Skin tests were obtained twice at baseline to ensure that positive tests were not the result of a booster phenomenon [17]. The day 0 skin test was considered the baseline. Skin test responses were read by use of the ball-pen technique [18] after 48–72 h. At least 10 mm of induration was required for a positive response, and to minimize variability in measurement of DTH [19], the development of a positive response from a negative response required an increase in the magnitude of induration of at least 3 mm.

Lymphocyte subsets were enumerated on freshly obtained whole blood with directly labeled murine monoclonal antibodies (PharMingen, San Diego) and dual-color and three-color flow cytometry by use of ACTG consensus immunology protocols [20].

Peripheral blood mononuclear cells were prepared by ficoll-hypaque density sedimentation, and these freshly prepared cells were used for assays of proliferation, NK cell activity against K562 tumor targets, and spontaneous apoptosis [20]. Lymphocyte proliferation was assayed in response to antigens of *Candida albicans* (50 μg/mL; Greer Laboratories, Lenoir, NC), tetanus toxoid (0.5 Lfu/mL; Connaught), streptokinase (200 μg/mL; Sigma, St. Louis), and HIV-1 p24 (1 μg/mL), p66 (1 μg/mL; MicroGeneSys, Meriden CT), and gp120 (derived from HIV-$1_{SF\text{-}2}$; 0.5 μg/mL; Chiron, Emeryville, CA) antigens. Alloantigen responses were measured in response to thawed peripheral blood mononuclear cells pooled from 15 unrelated donors that had been gamma irradiated and frozen in liquid nitrogen.

Peripheral blood mononuclear cells also were analyzed by three-color immunofluorescence for T cell receptor (TCR) V region expression using monoclonal antibodies directed to 13 different TCR Vβ and 2 TCR Vα regions. Separate aliquots of cells were stained with biotinylated monoclonal antibodies directed to Vβ2, Vβ3.1, Vβ5.1, Vβ5.2/5.3, Vβ6.7, Vβ8.1/8.2, Vβ9, Vβ12, Vβ13.1, Vβ13.2, Vβ14, Vβ17, Vβ23, Vα2.3, and Vα12.1 as previously described [21]. Streptavidin-cytochrome (PharMingen) was used as a second-step reagent for TCR staining, and cells were also triple-stained with fluorescein-labeled anti-CD4 and phycoerythrin-labeled anti-CD8 (Becton Dickinson, Mountain View, CA). Fluorescent cells were analyzed by flow cytometry (FACSCalibur; Becton Dickinson) as previously described [21], and data for 10^4 cells were collected for each antibody combination. The results are presented as the percentage of CD4 or CD8 cells that express a particular TCR V region.

Plasma was prepared from blood obtained in EDTA-containing tubes and was frozen at -70°C. Levels of TNF-α were measured by ELISA (Medgenix; INCSTAR, Stillwater, MN).

HIV-1 RNA levels were measured in batch by nucleic acid sequence-based amplification (NASBA; Organon Teknika, Durham, NC) [22]. The lower limit of sensitivity of this assay was 100 viral copies/mL.

The primary statistical analyses are of three responses to treatment: lymphocyte proliferation in response to tetanus toxoid antigen, lymphocyte proliferation in response to streptokinase, and overall DTH to *M. tuberculosis* purified protein derivative, trichophyton, *C. albicans*, and mumps antigens.

Lymphocyte proliferation was recorded as the stimulation index (SI), defined as the ratio of counts per minute with antigen to the counts per minute without antigen, where the SI was never assigned a value $<$1. Response to treatment was defined as the dichotomous indicator of whether a subject's SI increased at least 3-fold from baseline to week 12.

Overall DTH response to treatment was defined as the dichotomous indicator of whether a patient had a positive skin test response to more antigens at week 12 than at baseline.

To adjust for multiple testing of three correlated primary responses, and for the fact that this is the second analysis of the data (an interim analysis was reported earlier [23]), we used a Bonferroni correction ($k = 6$) to the type I error set for the primary analysis [24]. The lymphocyte proliferation and DTH hypotheses were tested with the two-sided McNemar test of significant changes [25].

The secondary analyses are of changes from baseline to week 12 in all other virologic and immunologic indices. Null hypotheses of no change in continuous outcomes were tested with two-sided

Table 1. Baseline characteristics of 53 patients who enrolled in AIDS Clinical Trials Group protocol 315 and were eligible for inclusion in 12-week analysis or who were not eligible for analysis.

	Eligible for analysis (n = 44)	Ineligible for analysis (n = 9)
Sex, no. male/ no. female	41/3	8/1
Median age, years (range)	37 (26–63)	33 (23–63)
Race		
White non-Hispanic	36	6
Black non-Hispanic	4	1
Hispanic	2	2
Other	2	0
Injecting drug use		
Never	37	8
Currently	2	0
Previously	5	1
Median CD4 cell count/ μL (range)	187 (16–498)	226 (115–394)
Median HIV RNA (range)		
Copies/mL	87,096 (323–2,884,031)	58,884 (8128–151,356)
Log_{10}	4.9 (2.5–6.5)	4.8 (3.9–5.2)

Wilcoxon signed rank [25] tests on the difference between week 12 and baseline values. Independence of continuous outcomes in different pairs of subgroups of the study population were tested with the two-sided Wilcoxon rank sum test [25]. These secondary analysis results do not have a well-defined type I error and should be considered exploratory.

Results

Fifty-three patients were enrolled in the trial. Of these, 44 received study treatment for at least 9 of the first 12 weeks and are therefore eligible for analysis. Intolerance of the treatment regimen was responsible for almost all treatment discontinuations. The baseline characteristics of these patients are shown in table 1. The baseline enumerations were obtained after the enumerations used to determine eligibility. The 9 patients who did not complete at least 9 weeks of treatment during the first 12 weeks of the study and were therefore ineligible for analysis were comparable to the group as a whole except that the median HIV-1 RNA level in this group was slightly but not significantly lower.

Plasma HIV-1 RNA measurements. After initiation of antiretroviral therapy, plasma HIV-1 RNA levels fell rapidly, to a median of 10,000 copies/mL at 1 week, 2138 copies/mL at 2 weeks, 1000 copies/mL at 4 weeks, 288 copies/mL at 8 weeks, and 166 copies/mL (median 2.3-log decrease; range, 0.32–3.7) by 12 weeks (figure 1). It should be noted that patients were treated initially with ritonavir alone; zidovudine and lamivudine were added on day 10. By 12 weeks, 17 of 40 patients achieved a plasma HIV-1 RNA level that was below the 100-copies/mL limit of detection of the assay.

Assays of lymphocyte proliferation in response to antigenic stimulation. Lymphocyte proliferation was examined in response to HIV antigens, other microbial antigens, and alloantigens (table 2). At baseline, only a minority of patients exhibited a proliferative response to the soluble antigens of tetanus (5/42), streptokinase (6/42), or the HIV antigens p24 (3/42), p66 (3/42), or env (1/42). In contrast, 26 of 42 patients (62%) exhibited a proliferative response to *Candida* antigen at entry and 29 (69%) had a baseline proliferative response to alloantigens. Enhancement was defined as a $\geqslant$3-fold increase in the SI and a decrease was defined as a $\geqslant$3-fold decrease in the SI. By these criteria, although there tended to be more enhanced responses to tetanus (the primary end point) and HIV antigens at week 12, the frequency of these increases was not significantly increased. There was also no significant change in the response to streptokinase (the second primary end point). In contrast, at weeks 4 and 12, 17 of 40 patients and 15 of 42 patients, respectively, had an increase in lymphocyte proliferative responses to *Candida* antigen, as opposed to 6 and 4 patients whose responses fell at these times (P = .038). Likewise, at 4 and 12 weeks, 16 of 40 and 17 of 42 patients, respectively, had enhanced responses to alloantigens, as opposed to 3 and 5 patients, respectively, whose responses fell (P = .034).

DTH skin test responses. The results of DTH skin testing at baseline and week 12 are shown in table 3. Of the 4 patients who were not anergic at baseline, 2 became anergic at 12 weeks, and of the 36 patients who were anergic at study entry, 11 (31%) developed a new skin test response at 12 weeks (P = .0225). After adjusting for multiple testing and multiple looks at the data (Bonferroni k = 6), the test for an increased likelihood of having a DTH reaction after 12 weeks of HAART was not significant at the $P < .05$ level, but the evidence is suggestive of an increase.

Changes in circulating lymphocyte populations. Lymphocyte subpopulations were enumerated by two- and three-color flow cytometry at baseline, week 4, and week 12. Treatment was associated with substantial increases in the numbers of circulating lymphocytes and all lymphocyte subpopulations except cells with NK cell phenotype (figure 2). Total lymphocytes increased from a median of 1255/μL at baseline to 1521/μL at week 4 and 1537/μL at week 12 ($P < .0001$). The median absolute CD4 T lymphocyte count rose from 189/μL at baseline to 271/μL at week 4 and 297/μL at week 12 (range, 97/μL decrease to 315/μL increase at week 12; $P < .0001$). CD8 T lymphocyte counts also increased from a median of 726/μL at baseline to 806/μL at week 4 and 922/μL at week 12 (range, 549/μL decrease to 1274/μL increase at week 12; $P < .0001$). There was also an increase in the numbers of circulating B ($CD3^-$, $CD19^+$) lymphocytes, from a median of 118/μL at baseline to 154/μL at week 4 and 161/μL at week 12 ($P < .0001$). In contrast, the numbers of circulating NK cells, as defined by expression of either CD16, CD56, or both, remained unchanged.

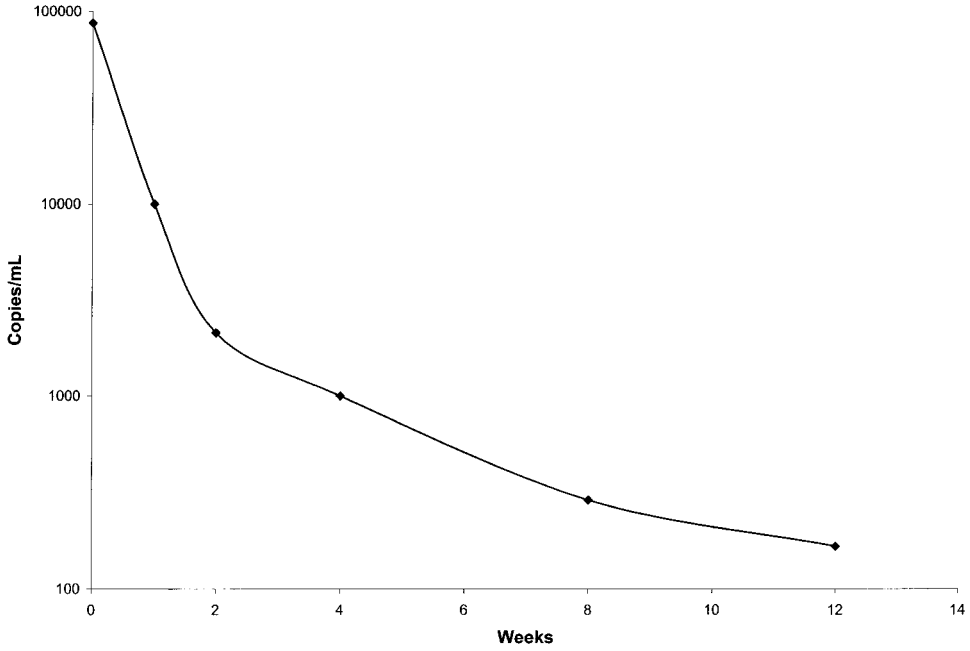

Figure 1. Plasma levels of HIV RNA before initiation of and during highly active antiretroviral therapy. Data represent median levels as measured by nucleic acid sequence-based amplification. Lower limit of detection = 100 copies/mL. Baseline and week 12 values were significantly different, $P < .0001$, Wilcoxon signed rank test.

CD4 and CD8 T lymphocytes were categorized as naive if they coexpressed CD45RA and CD62L; cells were defined as memory cells by the presence of CD45RO and the absence of CD45RA expression [26]. At baseline, the median CD4 naive cell count was 48/μL. This increased to 66 cells/μL at week 4 and 73 cells/μL at week 12 (figure 3; $P < .0001$). CD4 lymphocytes with memory phenotype also increased, from a median of 88 cells/μL at baseline to 144 cells/μL at 4 weeks and 164 cells/μL at 12 weeks ($P < .0001$). Among the CD8 T cells, the naive subpopulations increased, having a median of 78 cells/μL at baseline that rose to 97 cells/μL at 4 weeks and 110 cells/μL at 12 weeks ($P < .0004$). In contrast, CD8 memory cell numbers increased within the first 2 weeks of treatment, but by week 12, the numbers of these cells in peripheral blood were not greater than at baseline. It should be noted that with these definitions, some cells in each subset are not identified as naive or memory. When naive CD4 cells and CD8 cells were identified by expression of the CD45RA isoform and the absence of the CD45RO isoform (irrespective of CD62L expression), comparable significant increases in each population also were seen (not shown).

The distribution of T cell receptor V regions among circulating CD4 and CD8 lymphocytes was examined by flow cytometry among 16 patients enrolled at the University of Colorado. Shown in figure 4 are two enumerations of Vβ

Table 2. Lymphocyte proliferative responses to antigens before and after administration of highly active antiretroviral therapy.

Antigen	No. positive at baseline (n = 42)	No. with increase/decrease at Week 4 (n = 40)	No. with increase/decrease at Week 12 (n = 42)	P
Tetanus	5	1/2	3/1	NS
Streptokinase	6	1/4	2/5	NS
Candida	26	17/6	15/4	.038
Alloantigen	29	16/3	17/5	.034
HIV p24	3	3/0	3/1	NS
HIV p66	3	3/1	2/2	NS
HIV env	1	1/0	1/0	NS

NOTE. Lymphocyte proliferative responses are defined as positive at baseline if SI (stimulation index: cpm stimulated/cpm unstimulated) $\geq$3. Baseline values represent means of day −7 and day 0 stimulation indices. Week 4 and week 12 increases are defined as increase in SI $\geq$3-fold from baseline when baseline SI is at least 1.0. Week 4 and 12 decreases are defined as decrease in SI >3-fold from baseline when week 4 or week 12 SI is at least 1.0. NS, not significant.

Table 3. Skin test responses to intradermal administration of antigens of *Candida*, mumps, and trychophyton and purified protein derivative of *Mycobacterium tuberculosis*.

No. of positive skin tests at week 0	No. of positive skin tests at week 12: 0	1	Total
0	25	11	36
1	2	2	4
Total	27	13	40

NOTE. Delayed-type hypersensitivity skin testing was done at day −7 and day 0. Skin test results at day 0 were considered baseline for subsequent analyses. Positive response required greatest diameter of induration of $\geq$10 mm. Development of new positive skin test required reaction of $\geq$10 mm in greatest diameter and represented $\geq$3-mm increase above reaction at baseline.

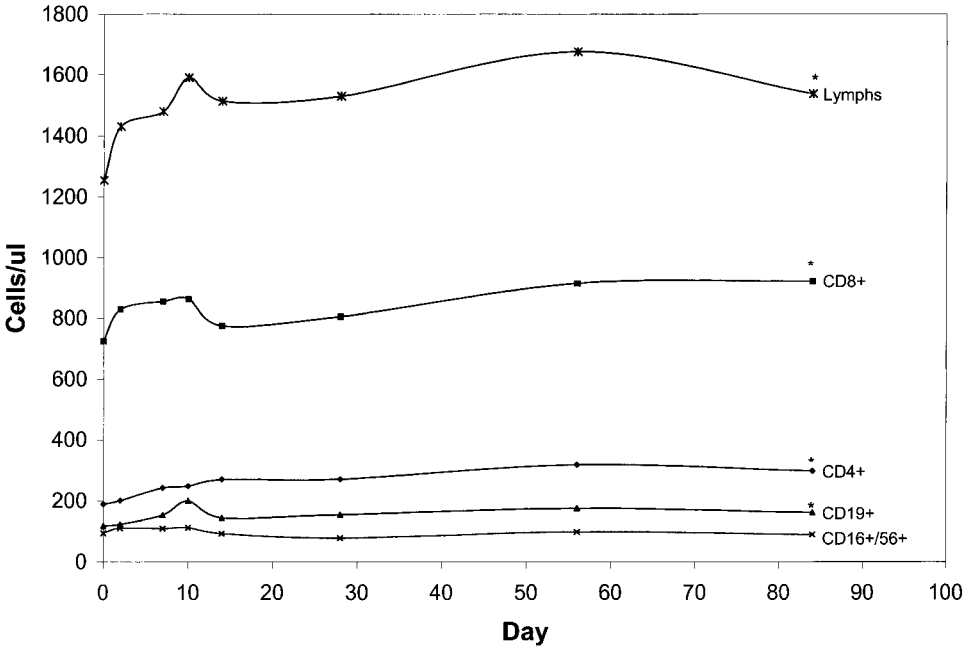

Figure 2. Numbers of circulating lymphocytes before and during administration of highly active antiretroviral therapy. Data represent median nos. of total lymphocytes, CD4 and CD8 T lymphocytes, B lymphocytes ($CD3^{-}CD19^{+}$) and NK cells ($CD16^{+}$ or $CD56^{+}$) in peripheral blood as enumerated by two-color flow cytometry. * $P < .0001$, baseline vs. week 12, Wilcoxon signed rank test.

and Vα subset distributions over a 12-week period in a representative HIV-1–seronegative control and in 1 patient with an abnormal CD4 T cell receptor repertoire at baseline. We concentrated this preliminary analysis among the patients with the most abnormal Vβ and/or Vα expression levels by defining as abnormal a patient who had at least one V region expressed on >12% of the CD4 or CD8 lymphocyte subsets and also representing >3 SD above the mean value for that V region among 24 healthy infants. By these criteria, 5 of the 16 ACTG 315 participants studied had an abnormality at baseline. Although 2 of these 5 patients experienced a normalization of the abnormal family, the magnitude of the changes was modest and exceeded 2% in only 1. In 1 of these who normalized one Vβ family and in 1 other patient who had normal Vβ family distributions at baseline, at least one Vβ family became abnormal after 12–14 weeks of ther-

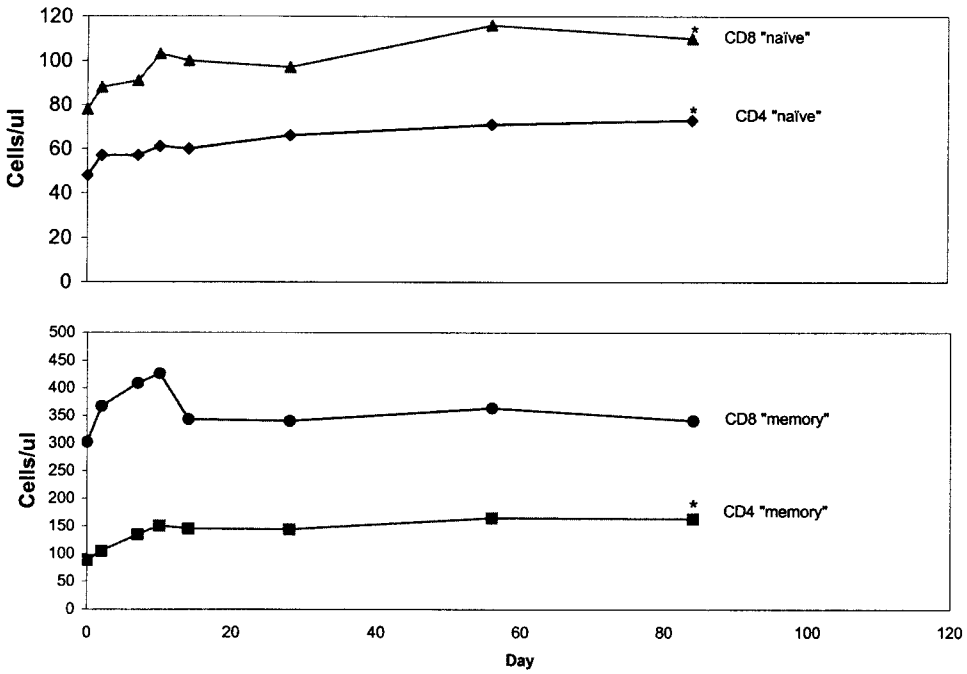

Figure 3. Nos. of circulating naive and memory T cells before and during administration of highly active antiretroviral therapy. Naive subsets of CD4 and CD8 T lymphocytes were identified by coexpression of CD45RA and CD62L. Memory subsets of CD4 and CD8 T lymphocytes were identified by expression of CD45RO and absence of surface CD45RA. All subsets were enumerated by 3-color flow cytometry. * $P < .0005$, baseline vs. week 12, Wilcoxon signed rank test.

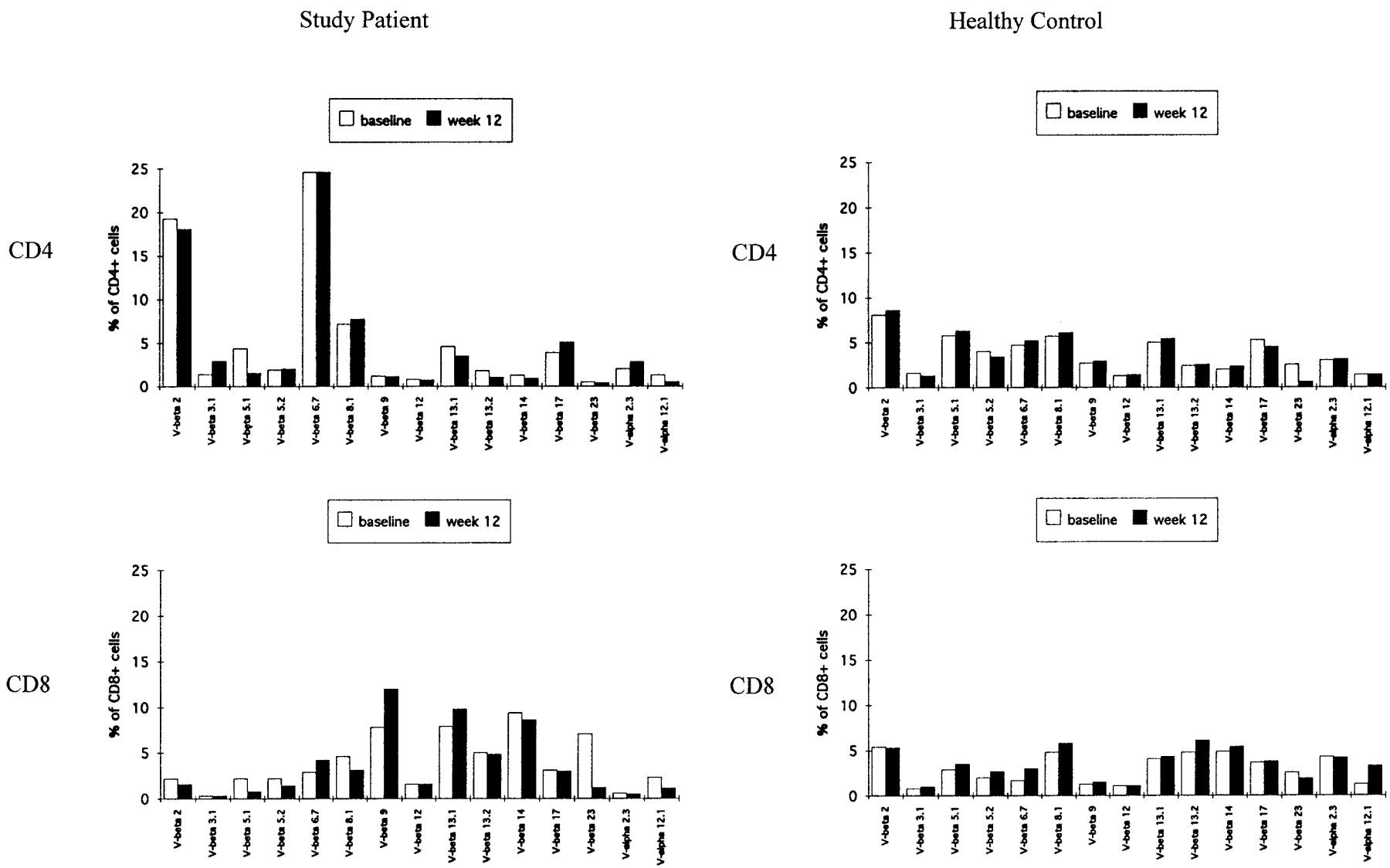

Figure 4. Percentages of CD4 (top) and CD8 lymphocytes (bottom) expressing T cell receptor V regions in patient before and during administration of highly active antiretroviral therapy (left) and in healthy control (right).

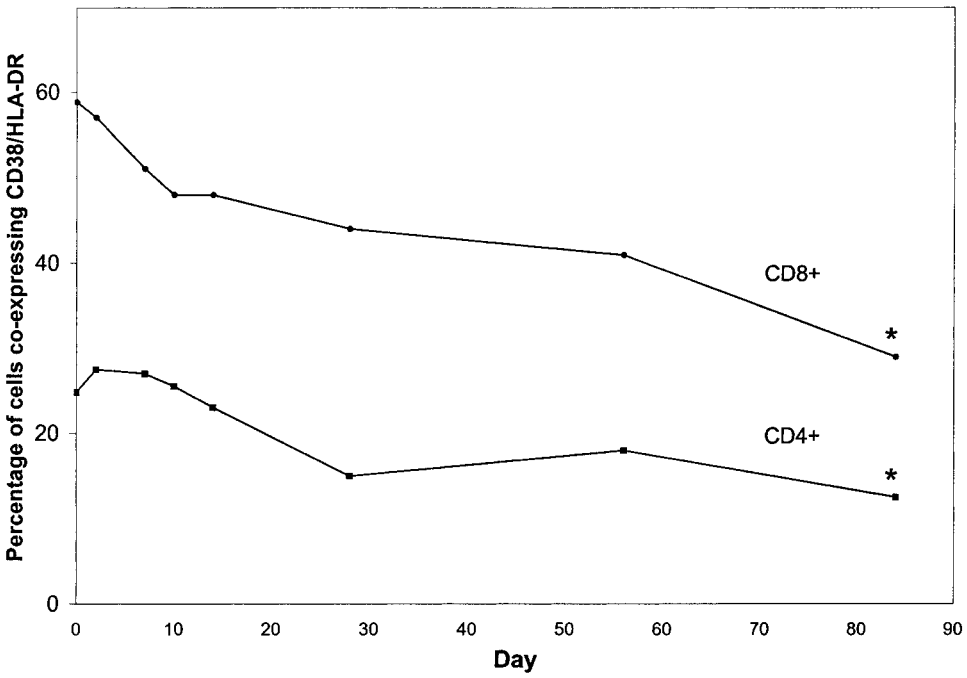

Figure 5. Percentages of CD4 and CD8 lymphocytes coexpressing activation antigens CD38 and HLA-DR before and during administration of highly active antiretroviral therapy. Data represent median percentages as enumerated by 3-color flow cytometry. * $P < .0001$, baseline vs. week 12 values, Wilcoxon signed rank test.

apy. Thus, we have no evidence that disturbances in the T cell receptor V region repertoire are corrected after 12–14 weeks of HAART.

Changes in indices of immune activation after HAART. To evaluate the effects of HAART on indices of immune activation in HIV-1 infection, CD4 and CD8 T lymphocytes expressing the activation antigens CD38 and HLA-DR and plasma levels of TNF-α were measured. The percentages of CD4 T lymphocytes that coexpressed CD38 and HLA-DR antigens fell from a median of 25% at day 0 to 15.0% at week 4 and 12.5% at week 12 (figure 5; $P < .0001$). Likewise, the percentage of CD8 T lymphocytes that coexpressed CD38 and HLA-DR fell from a median of 59% at week 0 to 44.0% at week 4 and 29% at week 12 ($P < .0001$).

Plasma levels of the proinflammatory cytokine TNF-α fell from a median of 45.3 pg/mL at week 0 to 31.8 pg/mL at week 4 and 28.2 pg/mL at week 12 ($P < .0001$). Though the decreases in plasma TNF-α levels are highly significant, plasma TNF-α levels in healthy HIV-1–seronegative subjects are generally <20 pg/mL by this assay (not shown).

Discussion

Our results suggest that persons with moderately advanced HIV-1 infection demonstrated evidence of partial immunologic restoration after receiving 12 weeks of treatment with a HAART regimen consisting of zidovudine, lamivudine, and ritonavir. In an earlier study, Kelleher et al. [27] reported increases in circulating CD4 and CD8 lymphocytes among 21 persons enrolled in a phase I/II trial of ritonavir. Increases in $CD45RA^+$ and $CD45RO^+$ CD4 cells, diminished CD38 expression, and increases in lymphocyte proliferation were observed in a small subset of these patients. More recently, in studies among 8 previously untreated patients who received zidovudine, zalcitabine, and ritonavir for 12 months, Autran et al. [28] also found sustained increases in CD4 lymphocytes, transient increases in CD8 lymphocytes, increases in lymphocyte proliferation, and decreased lymphocyte activation marker expression. Increases in circulating naive and memory CD4 cells were also observed in a subset of these patients.

In the present study, we report that 44 patients with moderately advanced HIV-1 infection receiving treatment for 12 weeks with a HAART regimen consisting of zidovudine, lamivudine, and ritonavir experienced substantial increases in the numbers of circulating CD4 and CD8 lymphocytes. Both naive and memory CD4 and CD8 lymphocyte subpopulations increased dramatically over the first 2 weeks of therapy; over the next 10 weeks of therapy, there was a decrease to levels not different from baseline in circulating memory CD8 cells, but a significant increase was seen in the other three cell populations. In the present study, frequent monitoring of a larger number of patients permitted a more detailed characterization of the earlier cellular increases seen in patients receiving HAART. Thus, the dynamics of early cellular restoration in our studies are more detailed than the increases over this period described by Kelleher et al. [27] and Autran et al. [28]. In addition, Kelleher et al. [27] identified naive cells by CD45RA expression only, and this may overestimate the frequency of naive cells, which may be more accurately defined by coexpression of CD45RA and CD62L.

Increases in CD4 T lymphocytes are expected in HIV-1–infected persons receiving active antiretroviral therapies. In-

creases in circulating CD8 T lymphocytes have not been seen, however, in HIV-1–infected patients receiving treatment with nucleoside analogues alone but have been seen after treatment with regimens that include protease inhibitors [27–29]. Why HAART regimens that include protease inhibitors such as ritonavir lead to increases in circulating CD8 lymphocyte numbers that are not seen in patients treated with nucleoside regimens alone is not clear. This may be related to the greater magnitude of the antiviral effect seen in recipients of the combination regimens, as we observed a correlation between the magnitude of the change in plasma HIV-1 RNA and the increase in circulating CD8 lymphocyte numbers ($r = -.482$, $P = .004$). Alternatively, nucleoside analogue inhibitors of DNA polymerase may block T cell proliferation directly [30, 31], and this effect may be overcome by the greater antiviral effect of protease inhibitors, which results in restoration of CD8 T cell numbers. Interestingly, the numbers of circulating B lymphocytes also increased during administration of HAART. To our knowledge, this response has not been recognized in other HIV treatment trials and may be a consequence of enhanced T helper function facilitating B lymphocyte maturation. The failure of this HAART regimen to correct the perturbations in CD4 and CD8 T lymphocyte Vβ repertoires despite increases in naive subpopulations suggests that the naive cell increase represents either a redistribution or peripheral replication of already matured cells or an interruption of premature cell death as a consequence of HAART. These findings also could be explained if the process responsible for perturbations in the T cell receptor repertoire occurs within the thymus or at extrathymic sites of T cell maturation. Alternatively, some cells identified as phenotypically naive ($CD45RA^+/CD62L^+$ and $CD45RA^+/CD45RO^-$) after HAART may have reverted to this phenotype despite prior antigenic exposure.

While, as defined, both naive and memory CD4 cell counts rose, only the naive CD8 cell counts increased after 12 weeks of HAART. This suggests that the mechanisms underlying the repopulation of these cell types may differ. If a substantial proportion of CD8 T lymphocytes in these patients was HIV-reactive, the decrease in HIV-expressing cells may underlie the failure of these cells to expand.

These studies also indicate that HAART results in only modest improvements in overall lymphocyte function in the short term. Enhancement of DTH responses as defined by an increase in skin test induration was seen in about one-fourth of the treated patients. Similar modest enhancements in DTH responses have been seen in earlier antiretroviral trials using only reverse transcriptase inhibitors, such as zidovudine [32, 33].

As had been observed in earlier studies among patients receiving nucleoside analogue antiviral therapies [34] and among patients receiving protease inhibitor–containing regimens [27, 28, 35], we observed modest increases in lymphocyte proliferative responses. In this study, we observed increases in the magnitude of lymphocyte proliferative responses to *Candida* antigens and to pooled alloantigens. Importantly, there were no increases in proliferative responses to any of three HIV-derived antigens or to the soluble bacterial antigens streptokinase and tetanus toxoid. It should be noted that proliferative responses to *Candida* and alloantigens were relatively preserved at baseline in the majority of patients, whereas responses to the other antigens were infrequent at baseline. Conceivably, the failure to respond to HIV antigens reflects a deletion of HIV-reactive clones at the site of HIV replication through either viral infection and lysis or induction of apoptosis or a failure to develop HIV responses through the immunosuppressive effects of HIV envelope and tat proteins [36–39]. Alloresponses are often preserved later in HIV disease [4], perhaps because alloreactive cells are generally more frequent even in health. The preservation of *Candida* responses (and the failure to enhance responses to streptococcal and tetanus antigens) may be related to constant exposure of HIV-1–infected persons to *Candida* antigens or to other characteristics of the *Candida* preparation. If exposure is sufficient to restore responses, then immunization strategies may prove effective in restoring immune responses in persons with moderately advanced HIV-1 infection after administration of HAART. Studies to test this hypothesis are ongoing.

Administration of HAART also resulted in a diminution in the heightened state of immune activation that characterizes HIV-1 infection. As has been reported earlier, the increased plasma levels of TNF-α decreased toward normal in patients receiving antiretroviral therapies [40]. In other studies, plasma TNF-α levels and plasma levels of HIV-1 RNA were correlated [14, 15], and it was not clear whether TNF levels drove HIV-1 replication or vice versa. The results of these studies suggest that viral replication directly or indirectly results in heightened plasma levels of TNF-α. Conceivably, high-level TNF-α expression also may enhance HIV-1 replication in a positive feedback loop. Interestingly, the magnitude of the decrease in plasma TNF levels was associated with the restoration of DTH responses, as the median decrease in plasma TNF-α levels among 26 patients who experienced no increase in DTH at week 12 was 10 pg/mL compared with a median 27-pg/mL decrease among the 10 patients who developed a new positive skin test response ($P < .004$). Whether enhancement of DTH is a direct consequence of decreased circulating TNF levels is not entirely clear. Conceivably, high levels of circulating TNF impair T lymphocyte thymic maturation [41] or enhance lymphocyte apoptosis [42, 43]. Alternatively, these events represent independent responses to antiviral therapies and are not causally related.

Lymphocyte activation marker expression was also diminished among recipients of HAART. Earlier studies had indicated that the intensity of CD38 expression on the surface of CD8 lymphocytes was a predictor of an accelerated disease course [9]. In the present studies, CD38 density was not measured; nonetheless, the frequency of cells with demonstrable surface expression of CD38 and HLA-DR antigens was diminished among both CD4 and CD8 lymphocytes after administra-

tion of HAART. The precise nature of the relationship between lymphocyte activation and acceleration of HIV-1 disease progression is uncertain. Lymphocyte activation may be a consequence of HIV-1 replication. On the other hand, activated CD4 lymphocytes are more permissive for completion of the HIV-1 life cycle [43, 44]; in addition, nonspecific activation of cytotoxic lymphocyte mechanisms has been postulated as contributing to cell-mediated mechanisms for lymphocyte loss in HIV-1 infection [11, 12]. The results of the present studies indicate that HIV-1 replication drives activation marker expression on CD8 T lymphocytes and also on CD4 T lymphocytes. Conceivably, CD4 T cell activation resulting from continued HIV-1 replication potentiates HIV-1 propagation within a pool of CD4 cells that are more supportive for HIV-1 replication than are unactivated cells.

In summary, 12 weeks of HAART resulted in partial immunologic restoration in persons with moderately advanced HIV-1 infection. This restoration is characterized by increases in circulating naive and memory CD4 lymphocytes, increases in naive CD8 lymphocytes, and an attenuation of the heightened immune activation state that characterizes HIV disease, but only a modest enhancement in DTH skin test responses and increases in lymphocyte proliferative responses to certain antigens. An ongoing evaluation of patients who participated in ACTG 315 will ascertain if immune responses continue to improve with sustained suppression of viral replication.

What are the clinical ramifications of these findings? On the one hand, partial immune restoration seen in this study provides some evidence that HAART improves functional host defenses in persons with moderately advanced HIV-1 infection. Several preliminary reports indicate that opportunistic complications of HIV-1 infection can occur despite dramatic increases in circulating CD4 lymphocyte counts in persons who have within the past 2 months initiated HAART regimens [45, 46]. On the other hand, sustained administration of protease inhibitor–containing regimens has been associated with a diminished frequency of major opportunistic infections and death [16, 47]. Although our observations indicate that HAART is associated with partial immune reconstitution, it is unclear if the magnitude of reconstitution is sufficient to ensure that regimens used for prophylaxis of opportunistic infections can be withdrawn as CD4 cell counts rise above levels used to begin prophylaxis. Therefore, until clinical studies clarify the timing and settings wherein prophylaxis can be safely withdrawn, patients who had met criteria for initiating prophylaxis for opportunistic infections should continue to receive these treatments after initiation of HAART.

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