Long-Term Evaluation of T-Cell Subset Changes After Effective Combination Antiretroviral Therapy During Asymptomatic HIV-Infection


*Division of Clinical Immunology, Department of Internal Medicine, University Hospital, Zurich; †Division of Infectious Diseases and Hospital Epidemiology, Department of Internal Medicine, University Hospital, Zurich; ‡Outpatient Department of Internal Medicine, University Hospital, Basel; and §Medical Clinic A, Department of Internal Medicine, Cantonal Hospital, St. Gallen, Switzerland

Summary: Demonstration of long-lived HIV-reservoirs resistant to the effects of combination antiretroviral therapy raises concern over the ability of treatment to maintain long-term beneficial alterations in T-cell subset composition. To address this issue, we have examined the effect of antiretroviral therapy on T-cell subset change during early HIV-infection in a 2-year prospective open-label trial composed of treatment-naive asymptomatic HIV-infected patients with CD4+ T-cell counts ≥400 cells/μl. Therapy consisted of double (zidovudine and lamivudine) or triple (zidovudine, lamivudine, and ritonavir) combination antiretroviral therapy. Retrospective analysis based on magnitude of viral suppression was used to characterize responder and nonresponder groups. Among responders, long-term antiretroviral therapy maintained a significant increase in numbers of total CD4+, naive CD4+/CD45RA+, and memory CD4+/CD45RO+ T cells. A concomitant significant decrease in numbers of memory CD8+/CD45RO+ and both activated CD8+/HLA-DR+ and CD8+/CD38+ T cells was also maintained. In contrast, long-term antiretroviral therapy among nonresponders led only to a significant increase in the numbers of CD4+ T cells and a significant reduction in numbers of activated CD8+/HLA-DR+ T cells. The long-term ability of antiretroviral therapy during early asymptomatic HIV-infection to maintain reversal of disease-induced T-cell activation and maturation abnormalities continues to support the concept that immunologic advantage is gained by commencing early aggressive antiretroviral therapy. Nevertheless, continued management of T-cell subset recovery is significantly more effective in the presence of completely suppressed viral replication. Key Words: HIV-1—Asymptomatic—Antiretroviral therapy—T-cell subsets.
likely to reflect factors including the relatively constant CD4+ and CD8+ T-cell values encountered, particularly of the naive CD4+/CD45RA+ phenotype (2). The influence of near-normal T-cell values on successful immune recovery has recently been emphasized by observation of HIV-specific CD4+ (3,4) and CD8+ (3) T-cell responses after treatment of early primary HIV-infection.

Nevertheless, effectiveness of antiretroviral therapy during asymptomatic HIV infection in maintaining any long-term beneficial change in T-cell values remains poorly understood. Potential complicating factors associated with long-term therapy include problematic patient adherence, occurrence of viral resistance, and continued presence of latent virus reservoirs. Some evidence has also implicated long-term antiretroviral therapy with suppression of HIV-specific CD4+ (5) and CD8+ (6) T-cell function, most likely reflecting the effectiveness of treatment in restricting sources of viral antigen.

Within the context of a prospective clinical study investigating the long-term effect of antiretroviral therapy during asymptomatic HIV infection (7), we have assessed the effectiveness of treatment over a 2-year period in maintaining beneficial changes in peripheral blood CD4+ and CD8+ T cells expressing cell-surface markers of activation (human leukocyte antigen HLA-DR, CD38) and maturation (CD45RA, CD45RO), to estimate the influence of treatment efficacy, we have further compared the efficacy of CD4+ and CD8+ T-cell subset reconstitution among responders who undergo full viral suppression and nonresponders who demonstrate only partial viral suppression.

### METHODS

In this study, experimental protocols were approved by the local ethics committee of the participating centers and all participants gave informed written consent.

Study participants included 23 asymptomatic HIV-infected patients enrolled in the Early Antiretroviral Therapy (EARTH) Study (7). HIV infection was diagnosed by the presence of anti-p24 antibodies; inclusion criteria were the absence of prior antiretroviral therapy and peripheral blood CD4+ T-cell counts ≥400 cells/µl at screening. Participants were randomized to either double (zidovudine and lamivudine) or triple (zidovudine, lamivudine, and ritonavir) therapy arms. Open-label antiretroviral therapy was administered at a dosage of 2 × 300 mg/d for zidovudine, 2 × 150 mg/d for lamivudine and 2 × 600 mg/d for ritonavir. After 102 weeks of antiretroviral therapy, a responder group was retrospectively defined as those HIV-infected patients whose plasma HIV-RNA had been fully suppressed to ≤50 copies/ml during the last 60 weeks of therapy (n = 17; 11 receiving ritonavir). In contrast, HIV-infected patients with ≥50 copies/ml of plasma HIV-RNA at least twice during the last 60 weeks of therapy were assigned to the nonresponder group (n = 6; 2 receiving ritonavir). A control population consisting of 17 healthy volunteer blood donors attending the Zurich branch of the Swiss Red Cross blood bank was also recruited. Baseline characteristics of the study population are outlined (Table 1).

Plasma HIV-RNA levels were determined from frozen plasma using the Amplicor HIV Monitor quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assay (Roche Diagnostic Systems, Branchburg, NJ, U.S.A.) with a lower detection limit of 200 to 400 copies/ml. Specimens demonstrating undetectable plasma HIV-RNA levels by this method were retested using ultrasensitive modifications of the Amplicor HIV Monitor assay (8). The absolute number and percentage of peripheral blood T-cell subsets were prospectively assessed using a whole-blood method of sample preparation followed by two-color flow cytometry (1). Absolute lymphocyte values were calculated using a particle concentration method based on precalibrated fluorescent microspheres and an initial fluorescence back-gating procedure was performed.

### TABLE 1. Baseline characteristics of study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Healthy controls</th>
<th>HIV-Infected patients</th>
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<tr>
<td></td>
<td>Responders</td>
<td>Nonresponders</td>
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<td>Sample size</td>
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<tr>
<td>Sex</td>
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<td>Female</td>
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<td>Male</td>
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<tr>
<td>T-cell counts (cell/µl)</td>
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<tr>
<td>CD4+ T-cells</td>
<td>716 (554–989)</td>
<td>526 (416–626)</td>
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<tr>
<td>CD8+ T-cells</td>
<td>394 (355–564)</td>
<td>655 (556–900)</td>
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<tr>
<td>Plasma HIV-RNA (log10 copies/ml)</td>
<td>—</td>
<td>4.1 (3.2–4.6)</td>
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<td>Risk factors</td>
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<tr>
<td>Homosexual men</td>
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<td>Injecting drug users</td>
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<td>Combination therapy</td>
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<td>NRTI + NRTI</td>
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<td>6</td>
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<tr>
<td>PI + NRTI + NRTI</td>
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<td>11</td>
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*Median (interquartile range).

PI, protease inhibitor; NRTI, nucleotide analog reverse transcriptase inhibitor.
Significance of differences between study parameters was estimated using Dunnett’s post-hoc test for multiple comparisons incorporated in the StatView 5.0 (Abacus Concepts, Berkeley, CA, U.S.A.) statistical-software package. A p value of ≤ .05 was regarded as significant. Results are expressed as median values with interquartile ranges.

RESULTS

Long-term antiretroviral therapy led to an increase in CD4+ T-cell levels among both responders (930 cells/µl; range, 769–1059) and nonresponders (735 cells/µl; range, 578–816), with absolute numbers at 102 weeks of treatment not differing significantly from those observed in healthy controls (716 cells/µl; range, 554–989) (Fig. 1). This change in CD4+ T-cell levels was reflected within the different CD4+ T-cell subsets primarily as an increase in naive CD4+/CD45RA+ T-cell levels, with the absolute number of naive CD4+/CD45RA+ T cells among responders (490 cells/µl; range, 327–678) actually reaching a level significantly higher than that observed for healthy controls (394 cells/µl; range, 355–564) (Fig. 1). In contrast, a limited increase in memory CD4+/CD45RO+ T-cell levels was observed, with only absolute numbers of memory CD4+/CD45RO+ T cells among responders (327 cells/µl; range, 274–402) reaching a level similar to that observed for healthy controls (368 cells/µl; range, 318–564) (Fig. 1).

The influence of long-term antiretroviral therapy on CD8+ T-cell levels was evident primarily as seen in change within CD8+ T-cell subsets. Reduction in activated CD8+/CD38+ T-cell levels was observed for both responders (278 cells/µl; range, 246–305) and nonresponders (381 cells/µl; range, 302–483), reaching absolute numbers not significantly different to that observed for healthy controls (150 cells/µl; range, 136–243) (Fig. 2). This trend was reflected in a similar decrease in activated CD8+/HLA-DR+ T-cell levels among both responders (80 cells/µl; range, 68–115) and nonresponders (191 cells/µl; range, 132–201), again reaching absolute numbers not significantly different to that observed for healthy controls (29 cells/µl; range, 16–46) (Fig. 2). A limited decrease in memory CD8+/CD45RO+ T-cell levels also resulted in a lack of significant difference between absolute numbers observed for responders (184 cells/µl; range, 150–240), nonresponders (294 cells/µl; range, 245–348) and healthy controls (154 cells/µl; range, 95–235).

DISCUSSION

We thus can report that long-term administration of antiretroviral therapy during asymptomatic HIV infection is able to sustain beneficial changes in CD4+ and CD8+ T-cell subset composition over a 2-year period. However, using viral load estimates to retrospectively generate responder and nonresponder groups, we also report that long-term management of T-cell subset recovery depends on sustained suppression of viral replication to <50 copies/ml.

Long-term administration of antiretroviral therapy during asymptomatic HIV-infection resulted in complete normalization of absolute CD4+ T-cell counts among both responders and nonresponders. The relative robustness of CD4+ T-cell recovery among nonresponders is similar to that previously described for symptomatic HIV-infected patients who continued to remain viremic despite treatment with antiretroviral therapy (9). This long-term normalization of CD4+ T-cell values after treatment of early HIV infection can now be contrasted with long-term treatment of late HIV infection in which, although initial CD4+ T-cell recovery is rapid, a plateau level is often reached from which normalization is not achieved (10).

Long-term influence of early antiretroviral therapy on CD4+ T-cell subset redistribution was most apparent as an increase in naive CD4+/CD45RA+ T-cell values. This observation confirms the long-term stability of increases in naive CD4+ T cells encountered during short-term treatment of asymptomatic HIV-infection (1) and continues to emphasize the success of this treatment approach in forestalling the development of disease-induced maturation defects. However, retrospective evaluation suggested that recovery was more pronounced in the absence of viral rebound, with absolute naive CD4+ T-cell counts among responders actually exceeding those encountered for healthy individuals. In addition to effective control of viral replication, this marked increase is likely to reflect factors including the relatively normal number of naive CD4+ T cells present during early HIV-infection (1) and the treatment-naive status of the patients enrolled in this study (10). In parallel, long-term increases in memory CD4+/CD45RO+ T-cell counts were apparent among both nonresponders and responders, although the importance of effective viral suppression was again highlighted by normalization of absolute memory CD4+ T-cell counts only among responders. Given that HIV replication is most effective in activated CD4+/HLA-DR+ T cells, particularly of the memory T-cell phenotype (2), long-term observation of inverse change in these CD4+ T-cell subsets continues to suggest a benefit in early antiretroviral therapy.

Despite a limited impact on total CD8+ T-cell values, the long-term effect of early antiretroviral therapy was apparent in the pattern of CD8+ T-cell subset redistribu-
tion. As observed during the first months of treated asymptomatic HIV infection (1), long-term therapy continued to reduce activated CD8+/HLA-DR+ and CD8+/CD38+ T-cell subset values. This clear-cut effect of antiretroviral therapy on levels of CD8+ T-cell activation confirms reports describing use of antiretroviral therapy at all stages of disease progression (11,12). High levels of activated CD8+ T cells have been associated with high plasma HIV-RNA levels and expression of CD38 on CD8+ T cells functions as a strong independent

FIG. 1. Percentage and absolute numbers of peripheral blood T-cell subsets in healthy controls (n = 17) and asymptomatic HIV-infected patients (n = 23) undergoing combination antiretroviral therapy. Responders (R) are treated HIV-infected patients demonstrating ≤ 50 copies/ml of HIV-RNA during the last 60 weeks of therapy (n = 17), whereas nonresponders (NR) are those demonstrating ≥ 50 copies/ml of HIV-RNA at least twice during the last 60 weeks of therapy (n = 6). Significant differences between healthy controls (C) and treated HIV-infected patients are indicated (p ≤ 0.05).
FIG. 2. Percentage and absolute numbers of peripheral blood T-cell subsets in healthy controls \((n = 17)\) and asymptomatic HIV-infected patients \((n = 23)\) undergoing combination antiretroviral therapy. Responders \((R)\) are treated HIV-infected patients demonstrating \(\leq 50\) copies/ml of HIV-RNA during the last 60 weeks of therapy \((n = 17)\), whereas nonresponders \((NR)\) are those demonstrating \(\geq 50\) copies/ml of HIV-RNA at least twice during the last 60 weeks of therapy \((n = 6)\). Significant differences between healthy controls \((C)\) and treated HIV-infected patients are indicated \((p \leq .05)\).
predictor of disease progression (13). In this study, observation of persisting decreases in activated CD8\(^+\) T-cell values, ultimately resulting in reduced levels of proinflammatory cytokines known to actively support viral replication, continues to provide support in favor of early antiretroviral therapy. Nevertheless, our observation of a failure to normalize activated CD8\(^+\) T-cell values completely also suggests the continuing presence of low-level viral replication, similar to that observed after long-term treatment of late infection (14).

In conclusion, our 2-year assessment of the effect of early antiretroviral therapy confirms the stability of beneficial CD4\(^+\) and CD8\(^+\) T-cell subset reconstitution previously observed in the short term (1). Nevertheless, the impaired T-cell subset reconstitution evident among patients experiencing intermittent viral rebound demonstrates that optimal benefit can only be expected in patients who achieve strict control of HIV replication.

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