Immune Reconstitution in the First Year of Potent Antiretroviral Therapy and Its Relationship to Virologic Response

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The effects of 1 year of zidovudine, lamivudine, and ritonavir treatment on immune reconstitution were evaluated in 34 human immunodeficiency virus (HIV)-infected individuals. After 48 weeks of therapy, 20 (59%) subjects had <100 copies HIV RNA/mL. CD4+ T cells increased from a median of 192/mm3 at baseline to 362/mm3 at week 48. Lymphocyte proliferative responses to Candida normalized within 12 weeks, but responses to HIV and tetanus remained depressed throughout therapy. Alloantigen responses increased within 12 weeks and then declined to baseline levels. Recovery of delayed-type hypersensitivity responses occurred after 12 weeks for Candida and after 48 weeks for mumps. The magnitude of virologic suppression was correlated with numeric increases in CD4+ T cells, but not with measures of functional immune reconstitution. Plasma virus suppression <100 copies/mL was not significantly correlated with increases in CD4+ T cells or functional immune reconstitution.

Infection with human immunodeficiency virus type 1 (HIV-1) results in the loss of CD4+ T cells, lymphocyte proliferative responses, and delayed-type hypersensitivity (DTH) responses, all of which are independently associated with an increased risk of disease progression and death [1]. The advent of potent combination antiviral therapy of HIV infection has resulted in profound suppression of HIV-1 replication, substantial increases in CD4+ T cells, and significant declines in morbidity and mortality from HIV [2]. The impact of such therapies on immune function in adults has been reported in a few studies [3–8], including our report of the immunologic effects of 12 weeks of zidovudine, lamivudine, and ritonavir therapy [9]. We now report the immunologic consequences of 1 year of potent antiretroviral therapy.

Methods

Study design and recruitment. This study, ACTG 315, was conducted at University Hospitals of Cleveland, Rush-Presbyterian-St. Luke’s Medical Center, and University of Colorado Health Sciences Center. Eligibility criteria included a CD4+ T cell count of 100–300 cells/mm3, a minimum of 3 months prior zidovudine therapy, and no prior treatment with either lamivudine or an HIV-1 protease inhibitor. All subjects were observed for 5 weeks in the absence of any antiretroviral therapy, and were then treated with zidovudine, lamivudine, and ritonavir, as described elsewhere [9].

Virologic and immunologic assessments. Plasma HIV-1 RNA levels were measured by Nucleic Acid Sequence-Based Amplification (NASBA, Organon Teknika, Durham, NC), with the lower
limit of sensitivity of the assay adjusted to 100 copies/mL. Lymphocyte subsets were enumerated on whole blood by use of fluorescent monoclonal antibodies (Pharmingen, San Diego, CA) and flow cytometry. Lymphocyte proliferation assays were performed at each site as described elsewhere [9]. In each laboratory, an HIV-1-seronegative laboratory worker was tested every week that a lymphocyte proliferation assay was performed. Skin tests for DTH were performed at day −7, day 0, week 12, and week 48, as described elsewhere [9]. A positive response was defined as ≥10 mm of induration.

Results

Thirty-four (64%) of 53 subjects enrolled into the study completed 48 weeks of antiviral therapy. Reasons for failure to complete the study included intolerance to the medications (37%), laboratory abnormalities (21%), or subjects’ request or failure to appear at study visits (21%). Of the subjects who completed 48 weeks of therapy, the majority were male (94%) and white (85%), with a median age of 38 years. At study entry, median prior zidovudine experience was 111 weeks, median CD4 T cell count was 192 cells/mm³, and median plasma HIV RNA level was 4.79 log₁₀ copies/mL. Baseline characteristics did not statistically differ between the groups completing and not completing 48 weeks of therapy.

Changes in plasma HIV-1 RNA concentrations. HIV-1 RNA was undetectable in 20 (59%) subjects after 48 weeks of therapy (figure 1A). The median change in HIV-1 RNA from baseline to week 48 was −1.88 log₁₀ copies/mL. There were no significant differences in CD4 T lymphocyte count or plasma HIV-1 RNA concentration at baseline between subjects who achieved undetectable virus and those who did not.

Changes in peripheral blood CD4 T lymphocyte populations. The median CD3+CD4+ cell count increased from a baseline value of 192 to 362 cells/mm³ at week 48 (P < .00001). As shown in figure 1B, median memory (RA RO) CD4+ T lymphocyte counts increased during the first 2 weeks of therapy and then plateaued. Median naive (RA’62L) CD4+ T cell counts continued to rise slowly, but significantly throughout therapy, and constituted the majority of the later CD4+ T cell increases.

The magnitude of the CD4+ T cell increase over 48 weeks was significantly correlated with baseline plasma virus concentration (r = −.506; P = .00365), as well as the magnitude of viral suppression (r = −.5472; P = .00167), but was not associated with achieving an undetectable plasma virus concentration. The magnitude of increase in naive CD4+ T cell numbers was also significantly correlated with the baseline plasma virus concentration (r = −.3867; P = .04084), but not with the magnitude of viral suppression or with achieving an undetectable plasma virus level. Although age was inversely correlated with the proportion of naive CD4+ lymphocytes at baseline (r = −.5177; P = .00340), there was no correlation between naive CD4+ T cell increases and age.

Discussion

We assessed immune reconstitution in 34 adults with moderately advanced HIV infection during 1 year of therapy with zidovudine, lamivudine, and ritonavir. Similar to other researchers in previous studies [3, 5, 11], we observed biphasic increases in CD4+ T lymphocytes, with memory lymphocytes constituting the majority of the initial increase over the first 8 weeks, and then phenotypically naive lymphocytes constituting the majority of the CD4+ T cell increases after that time. We failed to observe any correlation between age and naive cell increases over time, which is noteworthy, since it does not support the hypothesis that survival is enhanced in younger individuals with HIV infection because of a superior capacity to regenerate naive CD4+ T cells.

Similar to previous researchers [3–9], we observed selective
recovery of antigen-specific lymphocyte proliferative responses in the setting of potent antiretroviral therapy. The failure to recover HIV-specific lymphocyte proliferative responses remains an enigma. Incomplete viral suppression and ongoing destruction by HIV of virus-specific cells, anergy of these cells, or suppression of HIV antigens below threshold concentrations necessary to induce virus-specific responses are all possible explanations. The failure to recover lymphocyte proliferative responses to tetanus is probably related to the infrequency of exposure to tetanus. Our observation of recovery of lymphocyte proliferative responses to *Candida*, as well as previous reports [3, 6] of reconstitution of lymphocyte proliferative responses to cytomegalovirus (CMV) and *M. tuberculosis* (MTb) in bacille Calmette-Guérin (BCG)–vaccinated individuals, probably reflect newly expanded populations of antigen-specific cells in these subjects because of endogenous reexposure. The kinetics of the recovery of lymphocyte proliferative responses that we observed for *Candida*, that is, rapid recovery within 12 weeks, were similar to those reported previously for CMV and MTb, but distinct from that reported for *Candida* in 1 study that found late recovery of responses at week 48 [7]. Our study is the first to assess the extent of antigen-specific immune reconstitution with respect to responses in HIV-seronegative individuals and also suggests that the reconstitution of lymphocyte
Lymphocyte proliferative responses in subjects ($n = 27$) during 48 weeks of treatment with zidovudine, lamivudine, and ritonavir and in untreated human immunodeficiency virus (HIV)-seronegative subjects ($n = 36$). Total of $10^6$ peripheral blood mononuclear cells (PBMC) were cultured in quadruplicate replicates with (A) HIV gp120, (B) HIV p24, (C) tetanus toxoid, (D) Candida, (E) irradiated allogenic PBMC, or medium alone. On day 6, $^3$H-thymidine incorporation was determined and stimulation index (SI) was calculated by dividing the median counts per minute (cpm) of 4 antigen-stimulated wells with the median cpm of unstimulated wells or control-antigen wells. Baseline values were calculated as the median of 2 different assays. There was no significant difference in the median cpm of unstimulated wells in study subjects over time or between study subjects and seronegative subjects. An SI < 1 was assigned a value of 1. Nos. in parentheses at bottom of a graph indicate no. of subjects with an SI < 3. Nos. in parentheses at top of a graph indicate no. of subjects with an SI > 50. Horizontal bars indicate the median value at each time point. An asterisk (*) indicates that difference between study subjects and HIV-seronegative controls was statistically significant, that is, $P < .05$, by use of the Wilcoxon rank sum test.
proliferative responses to endogenous organisms in the setting of potent antiretroviral therapy is not only rapid, but similar to levels in HIV seronegatives.

The transient increase in alloantigen responses has not been described previously. The early increase in alloreactivity parallels reconstitution of Candida responses and may reflect alloreactivity of antigen-specific cells. The subsequent decline in alloantigen responses remains unexplained, but represents another persistent functional defect in HIV-infected individuals treated with potent antiretroviral therapy. The clinical significance of depressed alloantigen responses is unclear, although before potent combination antiretroviral therapy, they were, in combination with antigen and mitogen responses, a prognostic marker for disease progression [1].

Positive DTH responses developed for some, but not all antigens in subjects treated with potent antiretroviral therapy for 1 year. Lack of increased DTH responses to PPD may reflect the low incidence of exposure to MTb in the United States. The absence of increased DTH responses to Trichophyton may reflect a low prevalence of Trichophyton-specific memory cells in the study population or an inadequate antigen preparation. The asynchronous recovery of DTH skin test responses to Candida and mumps at 12 and 48 weeks, respectively, was intriguing and further bolsters the hypothesis that reexposure to antigen is critical for reconstitution of immune responses. One explanation for the late recovery of mumps responses is that potent antiviral therapy reconstituted the booster phenomenon [10], that is, application of mumps skin tests at week 12 boosted the number of preexisting mumps-specific memory T lymphocytes, such that they became detectable at the next time tested, which was week 48. These data are consistent with the prognostic information provided by DTH skin tests prior to the introduction of protease inhibitors [1] and the well-established survival advantage conferred by protease inhibitor therapy [2].

The magnitude of virologic suppression correlated with CD4+ T cell increases, but surprisingly not with measures of functional immune recovery. In addition, our studies did not confirm a previous observation that incomplete virologic suppression is associated with poorer immunologic reconstitution [6]. We found there was no significant difference between those who achieved an undetectable plasma virus concentration and those who did not in terms of the magnitude of CD4+ T cell increases, the augmentation of lymphocyte proliferative responses to Candida, or the development of new DTH responses. Because our study was not designed to address the question of the relative immune benefits of maximal versus partial viral
suppression, it is possible it had insufficient power to detect a true difference. Nevertheless, these data are consistent with observations [12, 13] that subjects with detectable plasma HIV RNA levels on potent antiviral therapy benefit clinically. It is conceivable that a relative decrease in plasma HIV RNA level is most relevant to immune reconstitution, whereas maximal viral suppression may be most relevant to the duration of virologic control.

Our studies indicate that potent antiretroviral therapy can restore immune responses, but that memory cell functions may not be reconstituted in the absence of reexposure to antigen. These data further reveal that there is a dysjunction between phenotypic and functional measures of immune reconstitution in that CD4 T cell numbers continue to increase after 12 weeks of therapy, whereas functional restoration peaks within the first 3 months of therapy. These data have a number of clinical implications. First, they may provide rationale for the continuation of *Pneumocystis carinii* prophylaxis for 3 months in the setting of CD4 T cell increases >200 cells/mm^3^ and support the observation that prophylaxis beyond that time point is not necessary [14]. Second, they suggest that individuals with HIV infection who are treated with potent antiviral therapy may benefit from reimmunization to ensure the presence of sufficient memory CD4 T cells. Last, these data suggest that institution of antiviral therapy before the development of profound losses in CD4 T lymphocytes may be prudent, because functional memory populations are not uniformly restored.

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References