BRIEF REPORT

Interleukin-2 Increases CD4+ Lymphocyte Numbers but Does Not Enhance Responses to Immunization: Results of A5046s

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To ascertain whether CD4+ lymphocyte increases induced by interleukin (IL)–2 enhanced in vivo immune responses, 38 human immunodeficiency virus (HIV)–infected patients who had received highly active antiretroviral therapy (HAART) or HAART and IL-2 for at least 60 weeks were immunized with tetanus toxoid, inactivated glycoprotein 120–depleted HIV-1, and hepatitis A and B vaccines. Despite dramatic increases in CD4+ lymphocyte counts, IL-2 did not enhance immunization responses.

Despite the dramatic clinical and laboratory benefits of highly active antiretroviral therapy (HAART) [1, 2], immune restoration is often incomplete in subjects who have moderately advanced human immunodeficiency virus type 1 (HIV-1) infection when HAART is initiated [3]. In some, CD4+ lymphocyte numbers remain low, levels of activation markers remain high, and CD28 expression on CD4+ lymphocytes is decreased [3]. Moreover, responses to immunization are subnormal [4]. Poor immune restoration predicts poor clinical prognosis [5]; however, the long-term outcome for patients with modest but incomplete immune restoration is unknown. Strategies to normalize immune restoration may provide both clinical benefit and a better understanding of the mechanisms of immune restoration and its failure.

A regimen of intermittent high-dose interleukin (IL)–2 increases naive and memory CD4+ lymphocyte numbers [6]. Only limited information is available about the effects of IL-2 on functional immune enhancement [7, 8]. To ascertain whether IL-2–induced increases in CD4+ lymphocytes enhance immune competence in vivo, we immunized patients who had been receiving HAART or HAART and IL-2 and compared their immune responses. Although IL-2 recipients had substantially higher CD4+ lymphocyte counts, immune responses after immunization were not enhanced.

Subjects and methods. In the AIDS Clinical Trials Group (ACTG) 328 study, HIV-1–infected subjects with CD4+ lymphocyte counts of 50–350 cells/μL who were naive to protease inhibitors and to at least 1 nucleoside reverse-transcriptase inhibitor (NRTI) were treated with 2 NRTIs and indinavir. Patients who had plasma HIV-1 RNA levels <5000 copies/mL after 11 weeks were randomly assigned on a 1:1:1 ratio to also receive either IL-2, 9 MIU/day by intravenous infusion for 5 days every 8 weeks, or IL-2, 7.5 MIU/day subcutaneously twice daily for 5 days every 8 weeks, or to continue to receive only HAART. After at least 60 weeks, patients with HIV-1 RNA levels $\leq$2000 copies/mL could enroll in the A5046s substudy; these patients continued to receive the assigned treatment.

The A5046s substudy included 38 patients. Thirty-six of the 38 patients were male; the median age was 38 years. Twenty-five patients were receiving HAART and IL-2, and 13 were receiving only HAART; the median duration of treatment on substudy entry was 98 weeks (interquartile range [IQR], 89–107 weeks); patients in the IL-2 arm received a median of 10 IL-2 cycles before enrollment into A5046s.

Subjects were immunized intramuscularly with tetanus toxoid (TT) adsorbed (5 flocculation units/0.5 mL dose; Lederle), Remune (inactivated gp120-depleted HIV-1 antigen in incomplete Freund's adjuvant, 10 μg of p24/mL, Immune Response),

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Written informed consent was obtained from all patients, and the human experimentation guidelines of the US Department of Health and Human Services were followed.

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and hepatitis A and B vaccines (Havrix, 1440 elutriation units/mL, and Engerix, 20 μg/mL; SmithKline Beecham).

ACTG 328 subjects who were receiving IL-2 entered the A5046s substudy 4 weeks after their last IL-2 cycle; they continued to receive IL-2 every 8 weeks. At baseline (entry into A5046s), serum antibody levels were determined, and skin tests for delayed-type hypersensitivity (DTH) reactions and T lymphocyte proliferation assays (LPA) were performed. After 48–72 h, patients returned to the study center to have DTH responses read and were then immunized with TT, inactivated gp120-depleted HIV-1, hepatitis B (if the patient was seronegative and uninfected) and hepatitis A (if the patient was seronegative). Additional evaluations and immunizations were performed at weeks 8, 16, and 24, using ACTG consensus methods. Patients received a second dose of TT, inactivated gp120-depleted HIV-1, and hepatitis A and B vaccines at week 8, and a third dose of inactivated gp120-depleted HIV-1 and hepatitis B vaccine at week 16. All immunizations and immunologic evaluations for patients receiving IL-2 were performed 4 weeks after the patient's last IL-2 cycle.

Skin testing for DTH responses to TT (0.1 mL; Connaught) and inactivated HIV-1 (0.1 mL) were performed using intradermal injection and read, using the ballpoint-pen technique, at 48–72 h. Lymphocyte subsets were counted using 2- and 3-color flow cytometry. Lymphocyte proliferation in response to TT (0.5 flocculation units/mL), inactivated gp120-depleted HIV-1 (HZ321) (Immune Response), purified native p24 obtained from HZ321 (Immune Response), and recombinant p24 (Chiron) was assayed.

HIV-1–specific $CD8^+$ lymphocyte responses were quantified using ELISPOT on viably cryopreserved peripheral blood mononuclear cells (PBMC). Peptides representing optimal HIV-1 epitopes were selected on the basis of the HLA type of each subject. Cells producing interferon (IFN)–γ were counted and expressed as spot-forming cells (sfc) per 10^6 PBMC. The number of specific IFN-γ–secreting T cells was calculated by subtracting this from the negative control value. Results were considered to be positive when $\geq$50 sfc/10^6 PBMC were detected.

Tetanus antibodies were measured by EIA (LabCorp). Antibodies against hepatitis B virus surface antigen were measured by radioimmunoassay; hepatitis A virus and HIV-1 p24 antibodies were measured using a modified EIA. Plasma HIV-1 RNA levels (virus loads) were measured by bDNA assay (version 3.0; Bayer).

For the primary study end point, a "response" was a stimulation index (SI) $\geq$10 to native HIV p24 at week 24 that represented a $\geq$3-fold increase from baseline. For all other LPA analyses, a "response" was an SI $\geq$3 that represented a $\geq$3-fold increase from baseline. A DTH reaction that represented a $\geq$5-mm increase in induration from baseline was a "response." Antibody concentrations that were at least 4-fold above baseline (for p24 and TT) or were newly detectable (for hepatitis A or hepatitis B virus) were considered to be "responses."

Of the 38 participants in the A5046s substudy, 3 were lost to follow-up. The remaining patient received 2 of the 3 scheduled immunizations, was evaluated at week 24, and was included in the analysis. Equality of 2 proportions and identity of 2 continuous end-point distributions were tested using Fisher's exact test and the Wilcoxon signed rank test, respectively. Secondary analyses were exploratory, and $P < .05$ was considered to be significant.

Results. The median $CD4^+$ lymphocyte count before initiation of HAART for subjects participating in the substudy was 238 cells/μL (IQR, 132–275 cells/μL); at immunization (A5046s baseline), the median $CD4^+$ lymphocyte count was higher among IL-2 recipients than among subjects who received only HAART (865 vs. 445 $CD4^+$ lymphocytes/μL; $P < .03$; table 1). Before HAART, IL-2 recipients had a higher proportion of activated $CD4^+$ lymphocytes (17% vs. 10%; $P < .03$) and higher plasma virus loads (4.6 vs. 3.7 $\log_{10}$ copies/mL; $P = .057$) than did subjects who received only HAART, but these differences were not present at immunization. Seventy-one percent of patients had plasma virus loads <50 copies/mL at immunization, and the proportions of subjects with plasma virus loads below this level in each group were comparable at each evaluation.

None of the patients in the HAART-only arm and 1 patient in the HAART/IL-2 arm developed an LPA response to native HIV p24. When we analyzed native p24 responses using less stringent criteria (SI $\geq$3 and $\geq$3-fold increase from baseline), 31% of HAART-only recipients responded, compared with 12% of HAART/IL-2 recipients ($P = .378$). The magnitude of the responses tended to be greater in HAART-only recipients (figure 1*A* and 1*B*). When the less strict definition of response was used, 70% of HAART-only recipients had a response to whole inactivated virus (HZ321) at any time after immunization, compared with 24% of HAART/IL-2 recipients ($P = .049$). The magnitude of proliferative responses to HZ321 tended to be larger in HAART-only recipients than in HAART/IL-2 recipients (figure 1*C* and *D*).

Eleven of 13 HAART-only recipients and 21 of 25 HAART/IL-2 recipients received 2 tetanus immunizations. Although the percentages of HAART-only recipients (46%) and HAART/IL-2 recipients (20%) who developed LPA responses to TT were not significantly different, patients in the HAART-only arm tended to have larger LPA responses after immunization (figure 1*E* and 1*F*).

Sixty-seven percent of HAART-only recipients and 21% of HAART/IL-2 recipients developed a DTH response to inactivated HIV at some time point after immunization ($P < .05$). The results of all other analyses of responses were comparable between groups.

There were no differences between groups in the percentage

Table 1. Baseline immunologic and virologic characteristics of human immunodeficiency virus (HIV)–infected patients enrolled in a study of the effect of interleukin (IL)–2 on responses to immunization.

Study point, variable	Patients receiving HAART		P[a]
	Alone (n = 13)	With IL-2 (n = 25)	
Before initiation of HAART (at enrollment into parent study)			
$CD4^+$ lymphocyte count, median cells/μL (IQR)	242 (229–265)	222 (80–275)	.31
$CD4^+$ lymphocytes expressing CD28, %[b]	96 (87–97)	88 (74–95)	.15
Activated $CD4^+$ lymphocytes, %[b]	10 (9–15)	17 (13–34)	.03
$CD8^+$ lymphocyte count, median cells/μL (IQR)	446 (367–630)	458 (410–557)	.72
Activated $CD8^+$ lymphocytes, %[b]	26 (22–45)	42 (35–52)	.09
HIV-1 RNA load,[c] median $\log_{10}$ copies/mL (IQR)	3.7 (3.2–4.3)	4.6 (4–5.2)	.06
At baseline (enrollment in immunization study; after initiation of HAART)			
$CD4^+$ lymphocyte count, median cells/μL (IQR)	445 (322–536)	865 (434–1466)	.02
Naive ($CD45RA^+CD62L^+$) $CD4^+$ lymphocyte count, median cells/μL (IQR)	215 (105–237)	265 (102–684)	.22
Memory ($CD45RO^+CD45RA^-$) $CD4^+$ lymphocyte count, median cells/μL (IQR)	198 (165–248)	392 (308–669)	.001
$CD4^+$ lymphocytes expressing CD28, %	94 (89–97)	96 (87–99)	.71
Activated $CD4^+$ lymphocytes, %	5 (4–7)	5 (3–7)	.88
$CD8^+$ lymphocyte count, median cells/μL (IQR)	719 (525–887)	969 (754–1422)	.03
Activated $CD8^+$ lymphocytes, %	19 (15–22)	22 (12–31)	.59
HIV-1 RNA load,[c] median $\log_{10}$ copies/mL (IQR)	1.7 (1.7–1.7)	1.7 (1.7–2.6)	
HIV-1 RNA load <50 copies/mL, % of patients	85	64	.27

NOTE. HAART, highly active antiretroviral therapy; IQR, interquartile range.

[a] Wilcoxon rank sum test.

[b] These assays were performed for only 25 patients.

[c] For this calculation, all plasma HIV RNA loads <50 copies/mL were assigned a value of 50.

of subjects who had antibody responses to inactivated gp120-depleted HIV-1 (increase in p24-binding antibodies) or to hepatitis B virus or tetanus immunizations at any time. There was no significant difference between groups when we compared the percentages of patients who developed detectable antibody levels after hepatitis A virus immunization at any single time point. However, when we compared the proportions of patients who developed antibody responses to hepatitis A virus immunization any time point after immunization, 88% of HAART-only recipients and 36% of HAART/IL-2 recipients had responses ($P < .04$).

Because T helper dysfunction may contribute to $CD8^+$ T cell dysfunction in HIV disease [9], we investigated whether induction of HIV-specific proliferative responses (largely a $CD4^+$ T cell response) would enhance HIV-specific $CD8^+$ lymphocyte responses. We identified 3 patients who had consistent responses to HIV-1 antigen and 4 patients who did not respond who had viably stored cells. We tested a median of 26 peptides in patients who had responses and a median of 22 peptides in patients who did not have responses at each time point after immunization when viable cells were available. $CD8^+$ lymphocytes of patients who had responses to HIV-1 antigen recognized a median of 1.3 peptides, whereas $CD8^+$ lymphocytes of patients who did not have responses recognized 0.5 peptides ($P > .1$). In contrast, lymphocytes of chronically HIV-1–infected subjects who start HAART with early-stage HIV disease typically recognize a median of 6.5 of 21 cytotoxic T lymphocyte epitopes (S.A.K., unpublished data).

Discussion. Immunization responses are an in vivo reflection of the ability of the host's immune system to mount defensive responses to a microbial challenge; this is supported by the observation that HIV-1–infected subjects who respond to immunization have a more favorable disease course [10, 11]. We tested immunization responses to investigate whether IL-2–induced increases in $CD4^+$ lymphocytes enhanced immune function in vivo. Although patients who were receiving HAART and IL-2 had nearly twice as many $CD4^+$ lymphocytes at the time of immunization as did patients who received only HAART, the response to immunization among HAART/IL-2 recipients was no better than that among HAART-only recipients. The results for other predictors of responses to immunization in patients with HIV-1 infection either were comparable between groups or favored IL-2 recipients [4]. Several explanations can be postulated to account for these unexpected findings.

Patients who received HAART and IL-2 tended to have higher plasma virus loads and a larger proportion of activated $CD4^+$ lymphocytes before initiation of HAART. Higher plasma virus loads and heightened $CD4^+$ lymphocyte activation at the

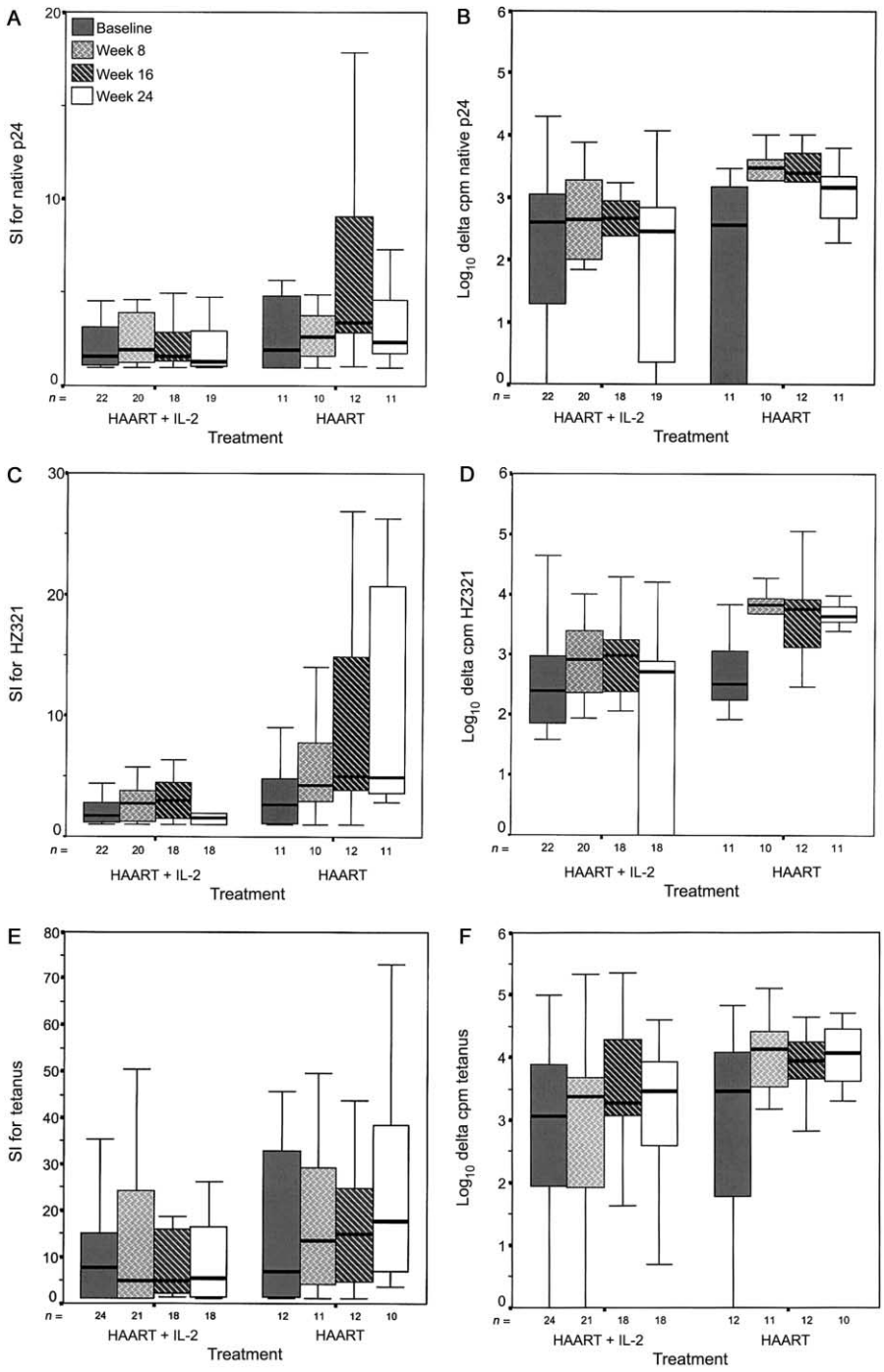

Figure 1. Stimulation indices (SIs) and change in counts per minute in response to native p24, HZ321, and tetanus toxoid (TT) stimulation after immunization among patients who received highly active antiretroviral therapy (HAART) only or HAART and interleukin (IL)–2. *A,* The median SI for native p24 after immunization with inactivated gp120-depleted HIV-1 was significantly higher among HAART-only recipients than among HAART/IL-2 recipients at week 16 (3.4 vs. 1.6; $P = .008$). *B,* The median change in cpm after native p24 stimulation was higher among HAART-only recipients at weeks 16 (2476 vs. 478 cpm; $P = .02$) and 24 (1470 vs. 284 cpm; $P = .06$). *C,* The median SI for HZ321 after immunization with inactivated gp120-depleted HIV-1 was significantly larger among HAART-only recipients at weeks 16 (5 vs. 3; $P = .03$) and 24 (5 vs. 1.6; $P < .001$). *D,* The median change in cpm after HZ321 stimulation was significantly higher among HAART-only recipients at weeks 8 (6696 vs. 887 cpm; $P = .02$), 16 (5799 vs. 949 cpm; $P = .04$), and 24 (4364 vs. 508 cpm; $P < .001$). *E,* The median SI for TT after tetanus immunization was higher among HAART-only recipients at week 24 (18 vs. 5; $P = .06$). *F,* The median change in cpm after TT stimulation was significantly higher among HAART-only recipients at weeks 8 (13,817 vs. 2392 cpm; $P = .03$) and 24 (11,901 vs. 2870 cpm; $P = .03$). Thick horizontal bars represent medians; upper and lower extremes of the boxes represent the 75th and 25th percentiles, respectively.

time of immunization (but not before initiation of HAART) have predicted poorer responses to immunization [4]. We do not think that minor disparities in either of these indices before initiation of HAART accounted for the failure to find an effect of IL-2 on immunization responses, because neither predicted responses in a multivariate model [4]. Earlier studies that compared the immune responses of patients receiving combination NRTI regimens with or without IL-2 showed that IL-2 increases the magnitude of responses to mitogens and antigens but not the proportion of patients who have responses [7, 8]. Thus, cyclic IL-2 treatment may expand responses already present but may not facilitate generation of new responses.

A population of regulatory memory $CD4^+CD25^+$ lymphocytes that suppresses T cell responses has been described elsewhere [12]. Although intermittent administration of IL-2 to HIV-1–infected subjects results in an increased number and proportion of $CD4^+CD25^+$ lymphocytes [13], it is not clear whether these cells have a suppressor phenotype. Unfortunately, neither CD25 expression nor suppressor activity was measured in the present study.

Lower doses of IL-2 close to the time of immunization have been used successfully as a vaccine adjuvant [14]. Our data suggest that high doses of IL-2 used to expand circulating $CD4^+$ lymphocyte populations do not enhance and may actually diminish immunization responses [15]. High doses of IL-2 result in mitogen-like activation and expansion of $CD4^+$ lymphocytes [13]. It is conceivable that these activated and proliferating cells are not capable of maintaining the rounds of high-level antigen-driven proliferation that are necessary to mount a response to immunization.

To explore whether insufficient $CD4^+$ lymphocyte help underlies impaired HIV-specific cytotoxic T lymphocyte activity in patients with HIV-1 infection [9], we compared HIV-specific $CD8^+$ lymphocyte responses in subjects who did and subjects who did not develop lymphoproliferative responses to HIV after immunization with inactivated gp120-depleted HIV-1. We failed to find a difference in the breadth of $CD8^+$ lymphocyte responses to HIV peptides (as assessed by ELISPOT). This suggests that induction of $CD4^+$ lymphocyte help by immunization of HIV-infected persons may not be sufficient to enhance HIV-specific $CD8^+$ lymphocyte responses in persons with advanced HIV infection.

In conclusion, we found that, in subjects who start HAART during moderately advanced HIV-1 infection, coadministration of cyclic high-dose IL-2 did not enhance responses to immunization and may have actually attenuated these responses. Thus, the dramatic $CD4^+$ lymphocyte increases that were seen in patients who received IL-2 did not result in better in vivo immune responses. Whether cyclic high-dose IL-2 administration to HIV-1–infected subjects will confer clinical benefit is currently under study in 2 phase 3 clinical trials.

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