Interleukin-12 Enhancement of Antigen-Specific Lymphocyte Proliferation Correlates with Stage of Human Immunodeficiency Virus Infection

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The effect of interleukin (IL)-12 on T lymphocyte function was assessed in 47 human immunodeficiency virus (HIV)-infected persons of different disease stages and 16 seronegative controls. Lymphoproliferative responses (LPR) were measured to various HIV and non-HIV antigens and mitogens using peripheral blood mononuclear cells cultured with or without IL-12. Without exogenous IL-12, 96% of HIV-seropositive persons responded to mitogens, 77% to ≥1 non-HIV antigen, and 11% to ≥1 HIV antigen. Supplementation with IL-12 augmented LPR of HIV-seropositive persons to non-HIV antigens; however, the effect was greatest for those with higher CD4 cells (40% vs. 9% for those with >200 vs. ≤200 CD4 cells/mm³). Addition of IL-12 also enhanced LPR to HIV antigens in 30% of subjects. This effect was most pronounced for those with >500 CD4 cells/mm³ (56% [P < .05]). These findings suggest that impaired T lymphocyte recognition of foreign antigen, including HIV, can be reconstituted in part for selected HIV-seropositive persons.

The course of human immunodeficiency virus (HIV) disease is characterized by a progressive decline in host cellular immunity, most notably a loss of T4 lymphocyte responsiveness to HIV and non-HIV recall antigens and, ultimately, alloantigen and mitogens [1–5]. This progressive anergy possibly relates to a Th1 to Th2 imbalance, whereby the latter host response prevails [6]. It is plausible that a deficiency in one or more cytokines may contribute to this anergy. The cytokine interleukin (IL)-12 has multiple biologic effects [7, 8], including differentiation of naive T cells toward the Th1 subset [9]. Persons infected with HIV show decreased IL-12 production by peripheral blood mononuclear cells (PBMC) [10]. Supplementation with exogenous IL-12 could prove therapeutically beneficial through its ability to induce or augment a Th1 response to foreign antigen, in particular HIV. A limited number of studies have shown that supplementation in vitro with IL-12 may augment HIV-seropositive patients’ peripheral blood lymphocyte recognition of foreign antigen [11–15]. Most of these studies have used lymphocyte recognition (LPR) as their measure of T4 lymphocyte responsiveness to antigen. Although the studies have addressed a similar question, the results have differed, primarily relating to frequency of enhancement in LPR. The discrepancies reflect a variety of factors, including differences in study population and spectrum of antigens selected for study. Response to HIV antigen was assessed in only three of these studies, and in each case was limited to recognition of the same pool of envelope peptides [11–13]. We have further investigated the effect of IL-12 on LPR of HIV-seropositive subjects to foreign antigen. We hypothesized that this effect might depend on degree of immunocompetence. Thus, we recruited subjects from a broader range of HIV disease than has previously been studied. Our primary aim was to examine the effect of IL-12 on LPR to HIV itself, employing a variety of subcomponent antigens. We also sought to further characterize the effect of IL-12 on non-HIV recall antigens and mitogens, using a more diverse group of in vitro stimulants than previously used in any of the aforementioned studies.

Materials and Methods

Study population. Forty-seven HIV-infected and 16 HIV-seronegative persons were enrolled. The seropositive subjects were outpatients without non-HIV related medical problems and were stratified as follows by numbers of CD4 cells/mm³: stratum A, asymptomatic, >500; stratum B, asymptomatic, 200–500; stratum C, symptomatic, 200–500; stratum D, ≤200; and stratum E (control group), healthy uninfected persons.

Reagents. Human recombinant IL-12 (Genetics Institute, Cambridge, MA) was utilized at a concentration of 10 U/mL (2.2 ng/mL) at or above which maximal proliferation was observed by dose-response determination assay. Mitogens included phytohemagglutinin (VWR Scientific, Boston), 5 μg/mL; concanavalin A (Boehringer Mannheim, Indianapolis), 10 μg/mL; and pokeweed mitogen (Gibco, Grand Island, NY), 1:100 dilution. Recall antigens have been used.
included purified protein derivative (PPD) (Connaught Laboratories, Swiftwater, PA), 1 μg/mL; candida (Gree Labs, Lenoir, NC), 2 μg/mL; tetanus (Wyeth-Ayerst Laboratories, Marietta, PA), 1:50 dilution; and heat-inactivated herpes simplex virus (HSV), 1: 20 dilution. Recombinant HIV antigens included yeast-derived proteins HIV1gp25 (1 μg/mL), HIV1gp31 (1 μg/mL), and HIV1gp24; env2-3 (0.5 μg/mL) (gifts of Chiron, Emeryville, CA); CHO cell-derived HIV1gp120 (1 μg/mL; AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases, NIH); and baculovirus-derived proteins HIV1gp120, HIV1gp160, and HIV1gp160 (1 μg/mL; MicroGeneSys, Meriden, CT). All assays included relevant control proteins.

Lymphocyte proliferation (LP) assays. PBMC were isolated from whole blood by ficoll-hypaque methodology, resuspended in complete medium, RPMI 1640 supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin (Gibco), and 10% human AB serum (Gemini Bioproducts, Calabasas, CA), plated at 2 × 10^5 cells/well in a total volume of 200 μL using 96-well, round-bottom microtiter plates, and cultured for 6 and 3 days, respectively, with soluble antigens and mitogens in the presence and absence of IL-12. Each well was labeled with 1 μCi of [3H]thymidine (DuPont New England Nuclear, Boston), cells were collected by cell harvester, and [3H]thymidine incorporation, expressed as change in counts per minute (Δcpm), was calculated as the difference between mean cpm of 4 replicate-stimulated wells and the mean of 4 unstimulated control wells. The stimulation index (SI) was calculated by dividing the mean cpm of the 4 replicate-stimulated wells by the mean of the unstimulated control wells.

Data analysis. Statistical analysis was performed using analysis of variance and unpaired t test corrected for multiple groups by Bonferroni method.

Results

Study population. Studies were carried out on blood specimens from 47 HIV-seropositive persons. Numbers of subjects by stratum and mean CD4 cells/mm^3 and ranges were as follows: stratum A, 18 (787, 520–1550); stratum B, 10 (366, 210–500); stratum C, 8 (355, 260–430); and stratum D, 11 (91, 10–200). One patient was a long-term nonprogressor, and 1 was a recent seroconverter. Nine patients were anergic by skin testing, and 2 had a history of tuberculosis. Seven patients were receiving combination therapy with two nucleoside analogue reverse transcriptase inhibitors.

Responses to mitogens. In the absence of IL-12, all controls responded to all 3 mitogens. In the absence of IL-12, all HIV-infected subjects from strata A–C responded to ≥2 of the 3 mitogens. Two of 11 persons from stratum D, who had the fewest CD4 cells compared with all other participants (<10/ mm^3), failed to respond to any mitogens. Absence of mitogen responsiveness correlated with decreasing CD4 cells for phytohemagglutinin, concanavalin A, and pokeweed mitogen, respectively: A—0%, 0%, 11%; B—0%, 0%, 30%; C—0%, 13%, 25%; and D—18%, 27%, 45%. IL-12 did not significantly effect mitogen responses of HIV-seronegative or -seropositive donors.

Responses to recall antigens. Among controls, in the absence of IL-12, all responded to ≥1 and 88% to ≥3 recall antigens. In the absence of IL-12, most (94%) HIV-infected subjects from strata A–C responded to ≥1 recall antigen. In contrast, of 11 donors from stratum D, only 2 (18%) responded. The frequency of LP to ≥3 antigens correlated with CD4 cell status (stratum A, 72%; B, 60%; C, 37.5%; and D, 0%). The antigen recognized most frequently was HSV (62%). The magnitude of mean LP was highest to HSV and diminished with advancing disease stage. This was true for the other antigens as well (figure 1). In the presence of IL-12, 5 (31%) of 16 controls evidenced an enhanced LPR to ≥1 recall antigen. Three of these subjects had an enhanced response to PPD.

For the seropositive group, addition of IL-12 to cultures augmented LPR to ≥1 recall antigen for 7 (39%) of 18 subjects in stratum A, 4 (40%) of 10 in stratum B, and 3 (38%) of 8 in stratum C. The frequency with which an enhanced response was seen was similar for the 4 recall antigens. In contrast, only 1 (9%) of 11 donors from stratum D demonstrated an enhanced response. Neither donor with a history of tuberculosis responded to PPD or any other recall antigen in the presence of IL-12. For each stratum, a trend toward enhancement in level of mean responses to recall antigens was observed in the presence of IL-12 (figure 1).

Responses to HIV antigens. None of the control donors responded to HIV antigens in the absence or presence of IL-12. Of all study participants, only 5 (4 [22%] from stratum A and 1 [12.5%] from stratum C) responded to ≥1 HIV antigen in the absence of IL-12. No subject responded to gp120. To investigate the possibility that CD4 cell binding by gp120 was responsible for the lack of gp120 response observed, responses were assessed to the envelope protein env2-3SF2, which does not bind CD4 cells. None of the 26 subjects assessed responded to env2-3SF2.

For the seropositive group, supplementation with exogenous IL-12 had a notable effect on LPR to HIV antigens. Responsiveness to HIV antigens in the presence of IL-12 correlated with stage of HIV infection as indicated by ≥500 CD4 cells/ mm^3 (P < .05). For stratum A, in the presence of IL-12, 10 donors (56%) had an enhanced response to ≥1 HIV antigen; of note, the majority lacked a response in the absence of IL-12. Individual donors developed responses to up to 4 antigens (donors A2, A5, and A6) (figure 2). For donors from stratum A, the frequency of enhanced response to each HIV antigen was as follows: gp120, 11%; gp160, 22%; p24, 28%; p25, 22%;
Figure 1. Mean lymphoproliferative response (LPR) to various recall antigens or medium alone in presence (+) or absence (−) of IL-12 for each study stratum. All data are mean response for peripheral blood mononuclear cells from all donors within each stratum. Frequency of donors developing or augmenting LPR in presence of IL-12 was 0±3 (0±19%) for all strata. Wide variation in level of response among donors accounts for large SDs (e.g., mean level of response in Dcpm to herpes simplex virus [HSV] in absence of IL-12 for each stratum) was as follows: A, 35,631 (range, 685–118,028); B, 31,673 (range, 1250–127,910); C, 13,447 (range, 227–51,729); D, 204 (range, 7–699); and E, 14,206 (range, 1154–34,910). PPD, purified protein derivative.

Figure 2. Mean lymphoproliferative response (LPR) of stratum A HIV-positive donors who developed response to HIV subcomponent antigens in presence of IL-12. LPR to HIV antigens representative of envelope (gp120, gp160), core (p24, p25), and pol (p31, p66) in absence (−) or presence (+) of IL-12 for 1 representative donor, patient A3, were as follows: gp120, 181/2057; gp160, 72/10,985; p24, 791/13,333; p25, 913/5659; p31, 327/12,091; p66, 1304/4751. Responsiveness to HIV antigens in presence of IL-12 correlated with >500 absolute CD4 cells/mm$^3$ ($P < .05$).

Discussion

Our results corroborate and expand the findings of others that HIV-specific LPR are absent in most HIV-seropositive persons, including those with relatively preserved CD4 cell status [11, 13]. In the absence of IL-12, only 11% of the subjects responded to HIV antigens, and the response was weak (mean Dcpm, 5638). As noted by others [4], response to gag protein was most common.

Addition of IL-12 disclosed additional subjects with preserved ability to respond to HIV antigen at the bulk culture level. Ten (56%), 3 (30%), and 1 (12.5%) subjects from strata A, B, and C, respectively, responded to >1 HIV antigens in the presence of IL-12. However, the responses were relatively weak (mean Dcpm, 7204). No subject with <200 CD4 cells/mm$^3$ was responsive to HIV, even after supplementation with IL-12. These results corroborate the findings of two other groups,
where augmentation of LPR to HIV by use of a pool of HIV envelope peptides was limited to persons with >200 CD4 cells/mm³ [11, 13]. In our study, this finding was statistically significant only for persons with >500 CD4 cells/mm³. The finding of a correlation between restoration of responsiveness to HIV antigen and disease stage may indicate that patients with higher CD4 lymphocyte counts are preferable candidates for receipt of IL-12 in vivo, at least when IL-12 is used to enhance HIV-specific, rather than nonspecific, recall responses.

This study extends the results of other groups in that we examined LPR to a more comprehensive array of HIV and non-HIV recall antigens and mitogens than previously used in any other single study. We evaluated 8 separate HIV antigens representative of envelope, core, and polymerase viral proteins. We failed to find a statistically significant difference in responsiveness to these viral proteins in the absence versus presence of IL-12. Nonetheless, it is viewed as important to have looked for such a difference, as were a defect seen, this might further our understanding of the HIV-specific T4 lymphocyte defect that occurs in HIV disease.

The mechanism for this HIV anergy is incompletely understood. It has been hypothesized that this anergy relates to early clonal deletion of HIV-specific T4 effectors, which occurs during the initial and appropriate encounter of these cells with circulating virus following acute infection [16]. It remains to be demonstrated whether complete clonal deletion of HIV-specific effectors actually occurs or if the resultant precursor frequency falls below the sensitivity of bulk culture assessments, such as LP assays. Alternatively, these cells may be qualitatively impaired in a way that precludes detection of their presence by routine in vitro measures. If the latter is the more common sequela, then therapeutic interventions aimed at increasing the precursor frequency or the function of these host effectors may prove useful. With that goal in mind, results of this investigation with the soluble mediator IL-12 should be viewed as both encouraging and potentially therapeutically important.

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References