

In Vitro Immunologic and Virologic Effects of Interleukin 15 on Peripheral Blood Mononuclear Cells from Normal Donors and Human Immunodeficiency Virus Type 1-Infected Patients

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Interleukin 15 (IL-15) is a cytokine that shares receptor subunits and functional activity, such as T-cell and B-cell stimulation, with IL-2. The effect of IL-2 on immune function and human immunodeficiency virus (HIV) viral load in HIV-infected patients is being actively studied. Thus, we examined how IL-15 compares with IL-2 in several in vitro immunologic and virologic assays in order to explore whether a rationale exists for pursuing initial clinical therapeutic trials with IL-15. The effects of IL-15 on induction of lymphokine-activated killer (LAK) cells, gamma interferon (IFN- γ) production from HIV-positive peripheral blood mononuclear cells (PBMCs), and HIV production from PBMCs were studied. Induction of LAK cells by IL-15 was found in eight of eight HIV-positive donors. Incubation of PBMCs from some donors with IL-15 (1, 10, 50, and 100 ng/ml) induced production of IFN- γ . The effect of IL-15 was compared with that of IL-2 on HIV replication in PBMCs from five HIV-positive patients and four HIV-negative donors whose PBMCs were infected in vitro with HIV. Levels of HIV p24 antigen were moderately lower in the presence of 10 ng of IL-15 per ml than with 10 ng of IL-2 per ml, but they were similar for 100 and 500 ng of each cytokine per ml. In summary, IL-15 can induce LAK cell activity in HIV-seropositive patients and can stimulate IFN- γ production from PBMCs of some donors. IL-15 stimulates levels of HIV production from PBMCs which are similar to or moderately lower than those obtained with IL-2, depending on cytokine concentration.

Interleukin 15 (IL-15) is a 14- to 15-kDa cytokine initially reported in 1994 by Grabstein and colleagues (19) that stimulates T-cell proliferation, lymphokine-activated killer (LAK) cells, alloantigen-specific cytotoxic lymphocytes, and IL-5 production from allergen-specific human CD4⁺ T-cell clones (26). IL-15 has also been reported to induce B-cell differentiation and proliferation in a manner similar to that of IL-2 (1). IL-15 mRNA is found in many tissues and in lipopolysaccharide-stimulated monocytes but not in T cells (19). Activated human monocytes have also been shown to produce IL-15 protein, which in turn optimizes gamma interferon (IFN- γ) production from human NK cells (7).

IL-15 can bind to NK cells, T cells, B cells, and activated monocytes. IL-15 binds the IL-2 receptor beta and gamma chains but not the alpha chain (17, 19). The complete IL-15 receptor (IL-15R) also includes a third subunit, IL-15R alpha chain (13, 17-19). Taken together, these initial studies demonstrate that IL-15 and IL-2 share two receptor subunits and have overlapping functional activities with respect to human T cells, LAK cells, and B cells.

A cytokine with several properties identical to those of IL-15 was described at the same time as IL-15 (2, 6). Provisionally named interleukin T (IL-T), like IL-15 it stimulates proliferation of human T cells and the murine CTLL cell line, induces LAK cells, is 14 kDa in size, and binds to the IL-2R beta chain.

This cytokine is produced by the adult T-cell leukemia HuT-102-B2 cell line and has been postulated to contribute to the IL-2-independent proliferation of some human T-cell leukemia virus type 1-associated adult T-cell leukemia cells (6).

During the course of human immunodeficiency virus (HIV) disease, IL-2 production and proliferation of peripheral blood mononuclear cells (PBMCs) in response to recall antigens, alloantigens, and mitogens can be lost (11, 12, 15, 23-25), possibly as an effect of the HIV envelope protein gp160 (21). Such impairment of IL-2 production is thought by some investigators to play a critical role in the loss of cell-mediated immunity characteristic of progressive HIV infection and AIDS (15, 23-25). Accordingly, IL-2 has been studied and is currently under study as a therapy for HIV-infected patients (10, 22, 28) with the objective of enhancing immune function and antiretroviral activity of the immune system. On the other hand, IL-2 also serves to enhance isolation of HIV in vitro from mitogen-activated PBMCs (16), and IL-2 therapy increases plasma HIV RNA in some patients (14, 22). Thus, the risk/benefit ratio for IL-2, and cytokines in general, requires consideration of both immunologic and virologic consequences.

Sera from HIV-infected patients, as well as synthetic peptides from the gp41 transmembrane protein, impair LAK cell activity (8). LAK cell responses have been shown to provide a functional correlate of HIV type 1 (HIV-1) disease progression, where LAK cells were defined as IL-2-inducible counterparts of NK cells (5). NK cell function has also been reported to decrease with advancing HIV disease (4, 9). Impairment of IL-2 production and responsiveness has been implicated in loss

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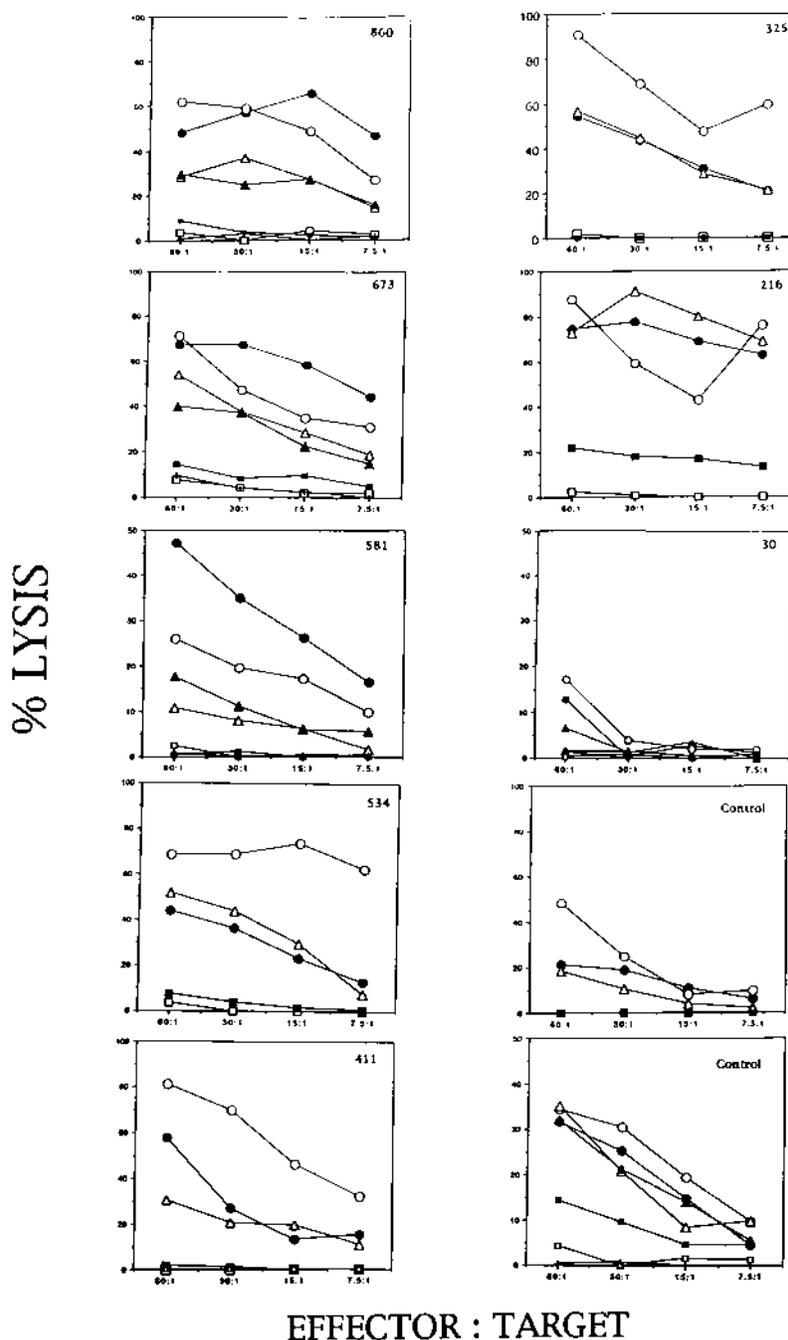


FIG. 1. IL-15 induction of LAK cell activity in response to IL-15 in 10 donors (8 HIV-seropositive donors with peripheral blood CD4⁺ T-cell counts ranging from 860 to 30/ μ l as noted in the upper right corner of each panel and 2 healthy, HIV-seronegative controls). NK-resistant Daudi targets were used at E:T ratios of 60:1, 30:1, 15:1, and 7.5:1. Open circles, 100 ng of IL-15 per ml; closed circles, 10 ng of IL-2 per ml; open triangles, 30 ng of IL-15 per ml; closed triangles, anti-Tac and 30 ng of IL-15 per ml; plus sign, Mik β 1 and 30 ng of IL-15 per ml; open square, medium only; closed square, 3 ng of IL-15 per ml. Donors with CD4⁺ T-cell counts of 860, 673, 581, 411, and 30/ μ l and both controls were tested by using Mik β 1 antibody. The ordinate on three graphs has a maximum percent lysis of 50, rather than 100, in order to allow clearer comparison of the percent lysis under the different culture conditions.

of both LAK and NK cell activity with HIV disease progression (4, 5, 20).

Initial in vitro studies of IL-15 and HIV have shown that in HIV-positive persons, IL-15 increases the proliferative response of PBMCs stimulated by HIV envelope-specific peptides, tetanus toxoid, and mitogen (27). The virologic effects of IL-15 on HIV production from mononuclear cells have not

been reported, however, nor have the in vitro effects of IL-15 on LAK cell production from PBMCs of HIV-positive patients. We studied the effects of IL-15 on LAK cell induction and cytokine production from HIV-positive PBMCs and assessed the virologic effect of IL-15 on HIV production in order to explore whether a rationale exists for pursuing initial clinical therapeutic trials with IL-15.

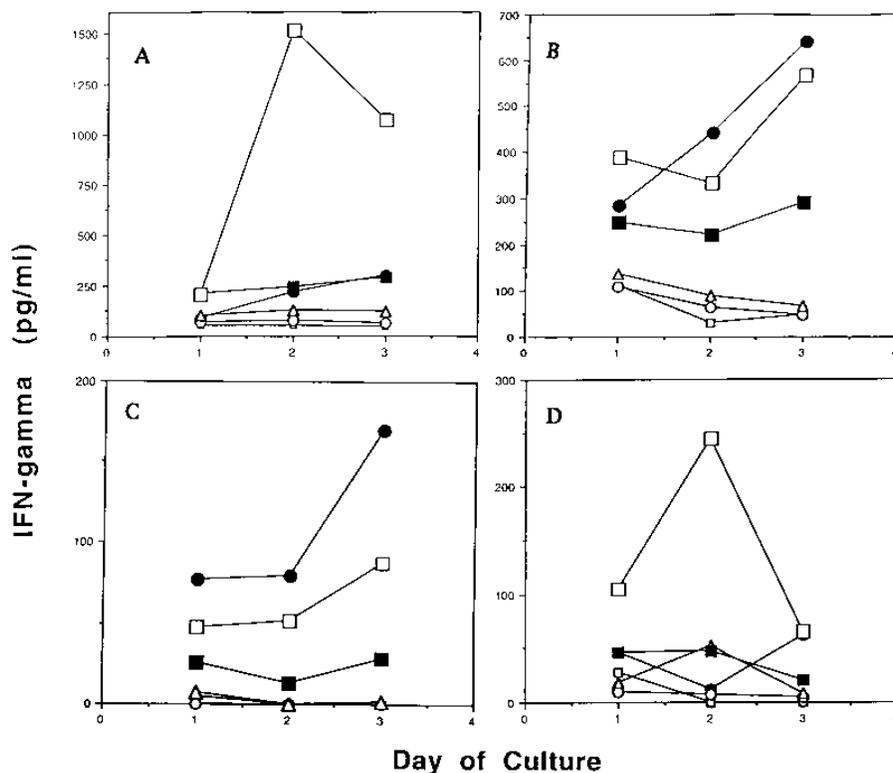


FIG. 2. IFN- γ production by PBMCs of four donors (two HIV-positive donors [A and B] and two HIV-negative donors [C and D]) stimulated with IL-15 or IL-2. Culture supernatants were assayed at 24, 48, and 72 h. Large open squares, 100 ng of IL-15 per ml; closed squares, 50 ng of IL-15 per ml; open triangles, 10 ng of IL-15 per ml; open circles, 1 ng of IL-15 per ml; closed circles, 5 ng of IL-2 per ml; small open squares, medium alone.

MATERIALS AND METHODS

Study subjects. PBMCs were obtained from HIV-infected patients at Wilford Hall USAF Medical Center, San Antonio, Tex., and from Rush-Presbyterian-St. Luke's Medical Center, Chicago, Ill. Informed consent and Institutional Review Board (IRB) approval were obtained. HIV-negative blood donations were obtained from the Transfusion Medicine Department at the National Institutes of Health.

Cytokines and antibodies. Recombinant human IL-2 was purchased from Boehringer Mannheim (Indianapolis, Ind.). Recombinant simian IL-15, which is 97% homologous to human IL-15, was obtained from Immunex, Seattle, Wash., as previously described (18). Monoclonal antibody to the IL-2R beta chain (Mik β 1) was a gift of T. A. Waldmann (2), and antibody to the IL-2R alpha chain (anti-Tac) was a gift of David Winkler, National Cancer Institute.

HIV-1 p24 Ag EIA. Antigen (Ag) capture enzyme immunoassays (EIAs) were purchased from Coulter Corporation (Hialeah, Fla.) and from Program Resources Inc./DynCorp., National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Md.

LAK assay. Effector cells were generated from PBMCs (3×10^6) incubated in 2 ml of RPMI 1640 with 5% human AB serum (Sigma, St. Louis, Mo.), 2 mM glutamine, 100 U of penicillin per ml, and 100 μ g of streptomycin per ml in 24-well plates (Linbro; Flow Laboratories, McLean, Va.). IL-15 (3, 30, and 100 ng/ml), IL-2 (20 U/ml = 10 ng/ml), or medium alone was added to each well, and the cells were incubated at 37°C in 7% CO₂ for 6 days. The cells were then harvested, washed once, counted, and resuspended in RPMI 1640 with 10% fetal calf serum (FCS). The cells were then added to 96-well plates with targets at effector-to-target cell (E:T) ratios of 60:1, 30:1, 15:1, and 7.5:1.

Both Daudi lymphoblastoid cells and K-562 cells were used as targets in a standard ⁵¹Cr release assay for LAK cell activity. The cells were labeled with ⁵¹Cr (200 mCi/10⁶ cells) (ICN, Irvine, Calif.) at 37°C in 7% CO₂ in RPMI 1640 with 10% FCS. After three washes in RPMI 1640, the target cells (5×10^3) were added to effector cells in 96-well, round-bottom tissue culture plates (Costar, Cambridge, Mass.). Following incubation at 37°C in 7% CO₂ for 4 h, the plates were centrifuged, then the supernatants were harvested, and radioactivity was measured with a gamma spectrometer. Results were expressed as the percent target lysis above background by using the formula % specific lysis = 100 \times (experimental release in cpm - spontaneous release in cpm)/(maximal release in cpm - spontaneous release in cpm). Spontaneous release was defined as counts per minute from wells containing 100 μ l of ⁵¹Cr-labeled target cells and 100 μ l

of RPMI 1640 with 10% FCS. In all experiments, spontaneous release was <10%. Maximal release was from 100 μ l of ⁵¹Cr-labeled target cells to which 100 μ l of 5% Triton X-100 detergent in water had been added. We defined a positive LAK cell response as >9.5% lysis at an E:T ratio of 60:1 because this value was 3 standard deviations above the mean background lysis of cultures without any added cytokine.

Cytokine EIAs. PBMCs (3×10^5 per well) were cultured in 96-well tissue culture plates (Costar) in 200 μ l of RPMI 1640 (Gibco, Gaithersburg, Md.) with 5% human AB serum (Sigma) and 100 U of penicillin per ml, 100 μ g of streptomycin per ml, and 2 mM glutamine in the presence of IL-15 (1, 10, 50, or 100 ng/ml) or IL-2 (10 ng/ml). Culture supernatants were harvested after 24, 48, and 72 h, frozen at -30°C, and analyzed by cytokine EIAs (Endogen, Cambridge, Mass.) for IL-2 and IFN- γ .

HIV isolation from patient PBMCs. HIV was isolated from PBMCs of HIV-seropositive donors as previously described (16). In brief, PBMCs (10^6 /ml) from five HIV-positive patients (blood CD4⁺ T-cell counts per μ l, 824, 600, 484, 326, and 145) were stimulated with phytohemagglutinin (PHA) (5 μ g/ml) for 3 days. A total of 3×10^6 cells/2 ml of RPMI 1640 with 10% FCS were then cultured in 24-well plates (Linbro; Flow Laboratories) with either IL-2 or IL-15 at 10, 100, or 500 ng/ml (a total of six cultures per donor). At 4-day intervals over the course of 3 weeks, the cultures were harvested and supernatants were frozen for p24 Ag assay.

In vitro HIV infection protocol. In vitro infection of HIV-negative PBMCs was performed as previously described (16). In brief, PBMCs from four HIV-seronegative blood bank donors were stimulated for 3 days at 10^6 cells/ml with PHA-P (5 μ g/ml) (Difco Laboratories, Detroit, Mich.) infected with HIV-1 (10 50% tissue culture infective doses of the Ba-L strain; gift of Suzanne Gartner) in vitro and cultured in either recombinant human IL-2 or recombinant simian IL-15 at 10, 100, or 500 ng/ml (total of six cultures per donor). The HIV Ba-L strain was used for this study because it is a primary patient isolate that has not been passaged in neoplastic cell lines and that infects both T cells and monocyte-macrophages. At 3- to 4-day intervals over the course of 3 weeks, the cultures were harvested and supernatants were frozen for HIV-1 p24 Ag assay. The cells were counted and resuspended at 10^6 /ml in T-25 flasks (Costar) with the same three concentrations of IL-2 or IL-15 in order to control for potentially confounding effects of differences in cell numbers resulting in differences in p24 Ag concentrations.

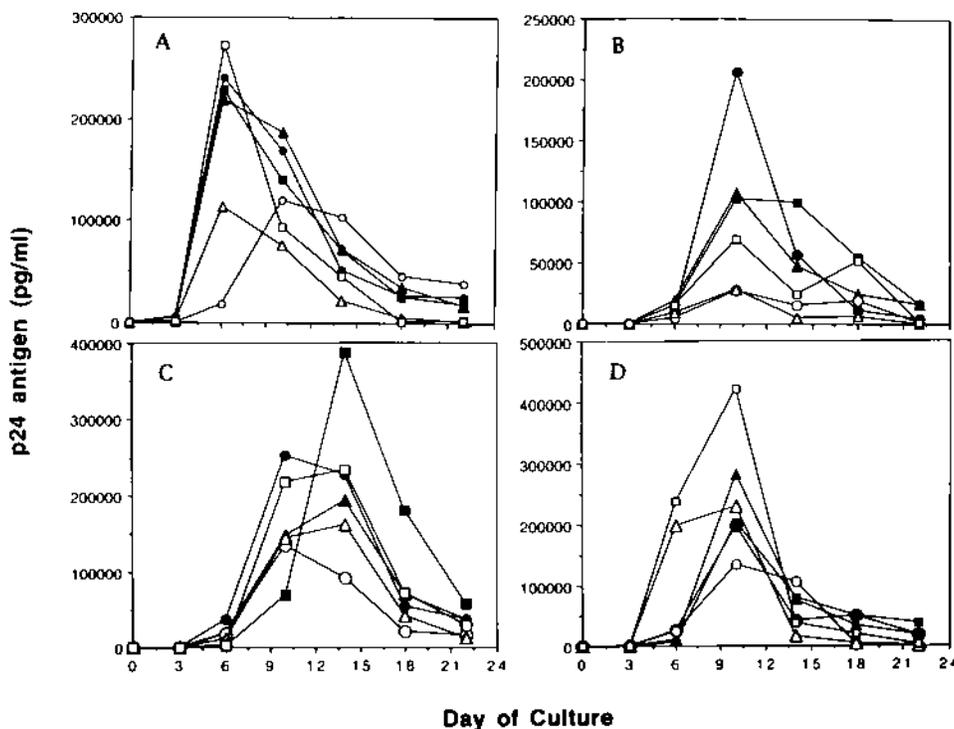


FIG. 3. Quantitative comparison of HIV-1 p24 Ag production after PHA stimulation of PBMCs and in vitro infection with HIV strain Ba-L followed by 3 weeks in culture with IL-15 or IL-2. Closed circles, 10 ng of IL-2 per ml; open circles, 10 ng of IL-15 per ml; closed triangles, 100 ng of IL-2 per ml; open triangles, 100 ng of IL-15 per ml; closed squares, 500 ng of IL-2 per ml; open squares, 500 ng of IL-15 per ml.

RESULTS

LAK cell induction. PBMCs from eight of eight HIV-seropositive donors ($CD4^+$ T-cell counts, 30 to $860/\mu\text{l}$) and two of two HIV-negative donors demonstrated induction of cytolytic activity against the Daudi lymphoblastoid LAK targets in an IL-15 concentration-dependent manner (Fig. 1). Induction of this cytolytic activity could be inhibited by an antibody against the IL-2 beta chain (Mik β 1) but not by antibody against the alpha chain (anti-Tac) (Fig. 1). The patient with the lowest $CD4^+$ T-cell count (30 cells/ μl) had only a minimal LAK cell response at an E:T ratio of 60:1 for 100 ng of IL-15 per ml and a negative response at 30 ng of IL-15 per ml (Fig. 1). Of the other seven patients ($CD4^+$ T-cell counts, 216 to $860/\mu\text{l}$), six had maximal IL-15-induced LAK cell killing of >60%, whereas the two controls had maximal killing of 35 to 50%. Cytolytic responses were very similar for each of the patients and controls when K-562 targets instead of Daudi targets were used (data not shown).

Measurement of IFN- γ and IL-2. PBMC supernatants from an additional eight donors (four HIV seropositive; $CD4^+$ T-cell counts of 54, 300, 416, and $450/\mu\text{l}$) were assayed by EIA for IL-2 and IFN- γ after 1, 2, and 3 days of culture. IL-2 production was not detected in response to IL-15 in any of the eight donors. IFN- γ was found in two of four HIV-positive donors ($CD4^+$ T-cell counts, 300 and $450/\mu\text{l}$) and two of four HIV-negative donors, being maximal at 100 ng of IL-15 per ml and minimal or absent at 1 and 10 ng/ml (Fig. 2). Levels of IFN- γ were higher at 48 or 72 h than at 24 h for all four donors.

In vitro HIV infection of PBMCs. HIV replicated in vitro in the PBMCs from all four HIV-negative donors at the three concentrations of IL-15 and IL-2 studied. Virus production peaked between days 6 and 13 and declined during the third week of culture (Fig. 3). Virus was produced throughout the 3

weeks of culture for all three concentrations of both IL-2 and IL-15. Comparison of p24 Ag levels in cultures using 100 ng of IL-15 versus IL-2 per ml or 500 ng of IL-15 or IL-2 per ml showed significant overlap and donor variability in the 3-week cultures. At 10 ng/ml, however, the IL-15 cultures yielded lower peak p24 Ag levels for all four donors than did IL-2 at the same concentration.

Isolation of HIV-1 from HIV-infected individuals. The HIV-1 p24 Ag results of the PBMC cultures from five consecutive HIV-positive patients demonstrated virus production in three patients (blood $CD4^+$ T-cell counts, 600, 326, and $145/\mu\text{l}$) from cultures to which IL-15 or IL-2 had been added (Fig. 4). HIV-1 could be detected from PBMCs of these donors at all three concentrations of IL-15 and IL-2 studied, except for one patient (patient 293) who showed no detectable p24 Ag production in the culture with 10 ng of IL-15 per ml. Moreover, as with the in vitro HIV-1 infections, the peak p24 Ag levels were lower in cultures for all three patients with 10 ng of IL-15 per ml than with the same concentration of IL-2. HIV p24 Ag levels with 100 and 500 ng of IL-15 versus IL-2 per ml did not differ. No virus was detected in any of the IL-15 or IL-2 cultures from the other two patients ($CD4^+$ T-cell counts of 824 and $484/\mu\text{l}$), both of whom were asymptomatic.

DISCUSSION

Given the functional similarities between IL-15 and IL-2 and the ongoing therapeutic trials of IL-2 for HIV disease, the in vitro study of HIV interactions with IL-15 may provide a rationale for initial clinical trials with IL-15. In this regard, several observations on the immunologic and virologic effects of IL-15 are provided in this study. First, IL-15 can induce LAK cells from PBMCs of HIV-seropositive patients in a dose-

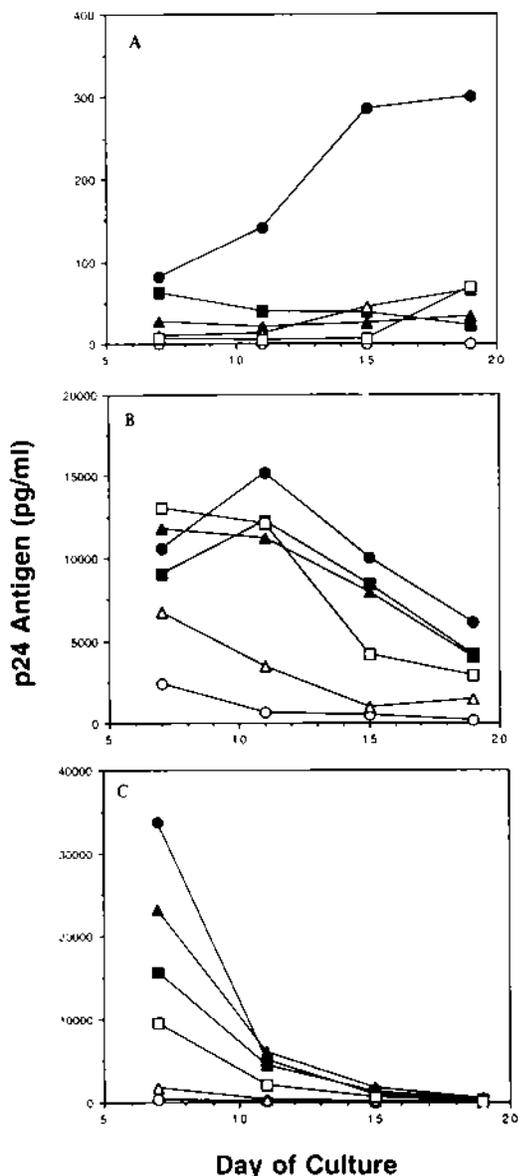


FIG. 4. Quantitative comparison of HIV-1 p24 Ag from HIV patients' PBMCs stimulated with PHA for 3 days and then cultured with either IL-15 or IL-2. Five patients were studied in separate experiments. In two patients, no virus was detected in any of the IL-15 or IL-2 cultures, whereas p24 Ag was detected for the other three patients (A, patient 293; B, patient 738; and C, patient 1413). Closed circles, 10 ng of IL-2 per ml; open circles, 10 ng of IL-15 per ml; closed triangles, 100 ng of IL-2 per ml; open triangles, 100 ng of IL-15 per ml; closed squares, 500 ng of IL-2 per ml; open squares, 500 ng of IL-15 per ml.

dependent manner. IL-2 was used as a positive control at a concentration known to induce LAK cell activity, and the method of Grabstein et al. was used to parallel their study of IL-15-mediated LAK cell activity in normal donors (19). Thus, donor PBMCs were incubated for 6 days with IL-15, implying that the concordant results for Daudi (NK cell-resistant, LAK cell-sensitive) and K-562 (NK and LAK cell-sensitive) targets are most consistent with induction of LAK cell activity. IL-15 stimulated LAK cell activity to similar degrees in patients with $CD4^+$ T-cell counts of 200 to 500/ μ l and $>500/\mu$ l. The single patient with a $CD4^+$ T-cell count of 30/ μ l had the lowest level of LAK cell activity.

LAK cell responses have been shown to provide a functional correlate of HIV-1 disease progression, where LAK cells were defined as IL-2-inducible counterparts of NK cells (5). Impairment of IL-2 production and responsiveness has been implicated in loss of both LAK and NK cell activity with HIV disease progression (4, 5). Whether IL-15 levels or IL-15 bioactivity also decreases during the course of HIV infection is still unknown. The present study demonstrates that IL-15 induces LAK cell activity in HIV-positive persons. This LAK cell activity may derive from NK cell precursors. Because NK cells express the IL-2R beta and gamma chains and can bind IL-15 (17), this cytokine may be able to stimulate NK cells directly as well as induce them to become LAK cells.

IL-15 was also found to induce modest amounts of IFN- γ in PBMCs from some HIV-seropositive and HIV-negative donors in a dose-dependent manner. The highest concentration of IL-15 (100 ng/ml) stimulated the highest level of production of IFN- γ in the four of eight donors who produced IFN- γ . No IL-2 production from these PBMCs was detected. Thus, IL-15 is unlikely to function solely via induction of IL-2. Our findings are consistent with those of Seder and colleagues (27), who also found that at high concentrations of IL-15 (e.g., 100 ng/ml) IFN- γ could be produced from human PBMCs. Moreover, Carson and colleagues have recently made the important finding that human monocytes can produce IL-15 protein and that this endogenous IL-15 contributes to optimal IFN- γ production by NK cells (7). Further study is required to determine whether IL-15 stimulates T cells, as well as NK cells, to produce IFN- γ .

With respect to virologic effects, at the three concentrations studied, IL-15 did not stimulate higher levels of p24 Ag production than IL-2 from either HIV-seronegative PBMCs infected in vitro or PBMCs of HIV-1-infected patients. In fact, at a standard concentration of IL-2 (10 ng/ml) used to culture HIV (16), a higher peak level of p24 Ag production was found with IL-2 than with IL-15 (10 ng/ml) in both the in vitro HIV infections and PBMCs from HIV-infected donors. This difference in HIV p24 Ag production could have one or more possible explanations. For example, lower levels of p24 Ag production could reflect less T-cell proliferation with IL-15 than with IL-2 at 10 ng/ml. In an effort to control for this effect in the four in vitro HIV infection experiments, cells were counted and resuspended at identical concentrations under each of the six culture conditions when the supernatants were harvested at 3- to 4-day intervals. Differences in expression of the complete receptors for IL-15 and IL-2 on T cells and monocytes, as well as differences in cytokine-receptor affinity or cytokine-receptor signal transduction, could also account for variations in virus replication at some concentrations of IL-15 and IL-2 (e.g., 10 ng/ml). Alternatively, IL-15 and IL-2 may vary in their effects on HIV replication by differential induction of antiviral cytotoxic T cells, NK cells, or LAK cell activity.

In conclusion, IL-15 shares some of the immunologic and virologic effects of IL-2 in the setting of HIV-1 infection, such as LAK cell induction, IFN- γ production, and T-cell proliferation with associated HIV replication. At low concentrations, IL-15 may stimulate moderately less HIV replication than IL-2. Notably, IL-15 did not increase viral replication more than IL-2 at any of the three concentrations tested. If IL-15 enters clinical trials as an immune therapy for HIV disease, however, it should be studied in conjunction with antiretroviral chemotherapy, as has recently been done in IL-2 clinical trials (22).

ADDENDUM

After submission of the manuscript, Bayard-McNeeley and colleagues published their findings (3) on the in vitro effects of IL-2, IL-12, and IL-15 on HIV replication. They reported that the effect of IL-15 on HIV replication may be different in acute versus latent infection, possibly due to the degree of cell activation. With 10 ng of IL-15 per ml and 10 U of IL-2 per ml, they found similar levels of HIV production after acute infection of PBMCs. Methodologically, their work differs from ours in terms of the use of T-cell cocultures, different infecting strains of HIV-1 and 50% tissue culture infective doses, the number of PBMCs per well, the duration of the cultures, and the number of concentrations of IL-15 studied.

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