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Differential Effects of Interleukin-12, Interleukin-15, and Interleukin-2 on Human Immunodeficiency Virus Type 1 Replication In Vitro

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Cytokines may have clinical utility as therapeutic agents for human immunodeficiency virus type 1 (HIV-1) infection and as an adjuvant for vaccines. The effect of interleukin-12 (IL-12) and IL-15 on in vitro HIV-1 replication was investigated. IL-12 and IL-15 at doses up to 10 ng/ml had little effect on basal HIV-1 p24 antigen production by chronically HIV-infected T (ACH-2) and monocytic (U1) cell lines. For ACH-2 cells stimulated with phorbol 12-myristate 13-acetate (PMA; 50 ng/ml), IL-12 and IL-15 significantly increased p24 antigen production by 20 and 30%, respectively ($n = 6$). In contrast, IL-12 and IL-15 (10 ng/ml) treatment of PMA-stimulated U1 cells decreased p24 antigen production by 16 and 15%, respectively ($n = 6$). We next studied the effect of IL-12 and IL-15 on HIV-infected peripheral blood mononuclear cells (PBMCs). In 10 HIV-seropositive patients' PBMCs cocultured with mitogen-activated HIV-seronegative donor cells, two patterns of p24 antigen production were observed in response to IL-2: low (p24 antigen production $<10^3$ pg/ml; $n = 8$) and high (p24 antigen production $>10^3$ pg/ml; $n = 2$) response. For the low-response pattern, IL-12 and IL-15 increased viral replication by 97-fold and 100-fold, respectively ($P = 0.05$ and 0.004, respectively). For the high-response pattern, both IL-12 and IL-15 suppressed HIV replication. The effect of IL-2, IL-12, and IL-15 on acute in vitro infection by HIV-1_{JRCSF} was also examined. IL-12 did not increase p24 antigen production above basal levels while IL-2 and IL-15 significantly enhanced p24 antigen production (by ~2-fold). In conclusion, IL-12 and IL-15 may have differential effects on latent and acute HIV infection, and their ability to enhance HIV production may depend on cell activation. Thus, the use of these cytokines may be dictated by the clinical state of the patient.

Depletion of $CD4^+$ T cells and immunodeficiency are the hallmarks of human immunodeficiency virus type 1 (HIV-1) infection and AIDS (7, 39, 41, 50). However, exactly how HIV induces AIDS remains controversial (14, 39, 41). Recently, the onslaught of HIV replication has been shown to be linked to the depletion of $CD4^+$ T cells (28, 51). In addition, control of HIV replication appears to involve cytotoxic T lymphocytes (CTL) in that a strong HIV-specific CTL response is associated with low viral load and symptom-free infection (31). Diminished in vitro production of interleukin-2 (IL-2), IL-12, and gamma interferon (IFN-γ) has been observed in symptom-free HIV-1-infected individuals with relatively normal absolute $CD4^+$-T-cell counts as well as in AIDS patients (11, 13, 15, 37). Moreover, compared with healthy volunteers, HIV-1-infected persons have a significantly higher frequency of $CD4^+$-T-cell clones producing IL-4, IL-5, and IL-10 but a similar frequency of $CD4^+$-T-cell clones producing IFN-γ and IL-2 (15, 21, 37). Studies of murine and human T helper (Th) cells have identified Th phenotypes with distinct cytokine profiles. Th1 cells produce IL-2 and IFN-γ, while Th2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13 (14, 15, 21, 37, 45, 48). In light of the cytokine profiles of cells obtained from HIV and AIDS patients, investigators have hypothesized that a Th shift contributes to the immune dysfunction of HIV (14).

The ability of some cytokines to augment CTL activity and the finding that some cytokines can restore in vitro cytokine production by cells obtained from HIV-1-infected individuals have led to a resurgent interest in cytokines for treatment of HIV disease (11, 32, 33, 36, 38, 39, 44, 50). Two recently cloned cytokines, namely, IL-12 and IL-15, are of particular interest. IL-12 (natural killer [NK] cell stimulatory factor) is a 70-kDa disulfide-linked heterodimer composed of a 35-kDa unit and a 40-kDa unit (6, 10, 18). IL-12 has been shown in vitro to restore the production of IL-2 and IFN-γ by cells obtained from HIV-infected persons and to increase CTL activity (12, 13, 47, 50). IL-12 has also been reported to augment the production of several cytokines, including IFN-γ, IL-10, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor alpha, and IL-2, to enhance the cytolytic activity of NK cells and lymphokine-activated killer cells, and to increase T-cell proliferation (3, 6, 18, 19).

IL-15, a T-cell growth factor, binds to a trimeric receptor consisting of α, β, and γ chains (1, 4, 26). The α chain specifically binds IL-15, while the β and γ chains are shared by both IL-15 and IL-2 (25–29). IL-15 and IL-2 both activate NK cells and stimulate B-cell proliferation and differentiation (2, 8, 9). IL-15 has been reported to enhance in vitro proliferation and production of IFN-γ in response to tetanus toxoid and HIV-1-specific antigen by peripheral blood mononuclear cells (PBMCs) obtained from HIV-1-seropositive patients (46). IL-15 (similarly to IL-2) has also been shown to enhance tumor-specific CTLs and lymphokine-activated killer cell activity, to be an anabolic cytokine for skeletal muscle, and to be a chemotactic factor for polymorphonuclear leukocytes (36, 40, 43, 53).

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These attributes of IL-12 and IL-15 suggest that they may have a role in treatment of HIV disease. The recent finding that HIV-1 replication is the central driving force for loss of $CD4^+$ T cells argues that cytokines with therapeutic potential should be evaluated to delineate their effect on HIV replication (7, 17, 32, 35, 42, 51). Moreover, IL-2 treatment of HIV-infected individuals has been reported to have either beneficial or adverse effects (e.g., increasing in vivo HIV-1 replication during high-dose IL-2 administration (5, 33). The present study was initiated to evaluate the in vitro effect of IL-12 and IL-15 on HIV replication in latent and acute infections. We report the differential effects of IL-12 and IL-15 on HIV production in chronically HIV-1-infected cell lines, PBMCs from HIV-infected patients, and mitogen-activated naive donor PBMCs infected in vitro with HIV-1_{JRCSF}.

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MATERIALS AND METHODS

Materials, reagents, and cell lines. The following materials and reagents were used: RPMI 1640, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer solution (Whittaker Bioproducts, Inc., Walkersville, Md.); penicillin, streptomycin, L-glutamine, and heat-inactivated fetal bovine serum (GIBCO Laboratories, Grand Island, N.Y.); IL-2 (Boehringer Mannheim, Indianapolis, Ind.); Ficoll, phytohemagglutinin (PHA), and phorbol 12-myristate 13-acetate (PMA) (Sigma Chemical Co., St. Louis, Mo.); 76% Hypaque (Winthrop Pharmaceutical, New York, N.Y.); 25-cm^2 tissue culture flasks and 24-well tissue culture plates (Corning Glass Works, Corning, N.Y.); 96-well plates (Falcon Becton Dickinson, Lincoln Park, N.J.); 0.9% NaCl solution, sterile water, and an HIV-1 p24 antigen detection kit (Abbott Laboratories, North Chicago, Ill.); and recombinant human IL-12 from Joseph Sypek (Genetic Institute, Hanover, Mass.). Purified recombinant IL-15 derived from a simian kidney epithelial cell line was a gift from Immunex Corporation, Seattle, Wash. The HIV-1_{JRCSF} isolate stock (10^5 50% tissue culture infective doses per ml) was obtained from David D. Ho (Aaron Diamond AIDS Research Center, New York, N.Y.) (7).

Chronically HIV-infected cell lines, T cells (ACH-2) and monocytes (U1), were obtained from Thomas Folks (Centers for Disease Control and Prevention, Atlanta, Ga.) (23, 24, 30). Cells were cultured in RPMI 1640 medium containing 100 U of penicillin per ml, 100 μg of streptomycin per ml, 25 mM HEPES, 2 mM L-glutamine, and 10% heat-inactivated fetal bovine serum (complete medium) at 37°C under 5% CO_2, and cultures were divided every 2 to 3 days. Cell viability was tested by trypan blue exclusion.

Effect of cytokines on HIV production by cell lines. Chronically HIV-infected cells (2×10^5 in 1.5 ml of complete medium) in 24-well plates were cultured with or without IL-12 or IL-15. In some experiments, chronically HIV-infected cells were activated with PMA (50 ng/ml) for 5 min and further cultured with IL-12 or IL-15. Supernatants were harvested at 24, 48, and 72 h and stored at −70°C until assayed for HIV p24 antigen.

HIV-seropositive patients and isolation and culture of PBMCs. Patients coming for routine follow-up at the Center for Special Studies, The New York Hospital-Cornell Medical Center, New York, or the Cornell-GHESKIO, Port-au-Prince, Haiti, were asked to donate 15 to 20 ml of blood. This study was approved by the Human Rights Committee of Cornell University Medical College. Verbal informed consent was obtained from each patient prior to the donation of blood. Data on clinical status, CD4 T-cell subset, and medications were abstracted, coded, and entered into an EPI-INFO questionnaire. PBMCs were prepared from HIV-seronegative leukocyte-enriched buffy coats (New York Blood Center) and from heparinized peripheral blood of 14 known HIV-1-seropositive patients. HIV-1-seropositive patients' cells were separated typically less than 24 h after phlebotomy (30). To obtain mitogen-activated mononuclear cells, HIV-seronegative PBMCs were stimulated with PHA (5 μg/ml) for 24 to 48 h and washed twice with normal saline (30). Mitogen-activated HIV-seronegative PBMCs (10^5) were cocultured with PBMCs (10^5) from HIV-seropositive patients in complete medium (0.25 ml) in 96-well round-bottom plates. Triplicate cocultures were treated with IL-2, IL-12, or IL-15 and maintained for 14 days at 37°C in 5% CO_2. After centrifugation of the plate ($150 \times g$, 5 min, room temperature), 0.5 volume of medium containing cytokines was exchanged every 2 to 3 days. The supernatants were stored at −70°C until assayed for HIV p24 antigen.

Acute infection of mitogen-activated PBMCs with the HIV-1_{JRCSF} isolate. Mitogen-activated PBMCs (2×10^6) (as described above) in 1.5 ml of complete medium in 24-well plates were treated with cytokines or not treated and infected with HIV-1_{JRCSF} isolate (3,000 50% tissue culture infective doses) (7, 34). Cytokines were added 1 day before, simultaneously with, or 1 day after HIV-1_{JRCSF} infection. At 48 h after infection, cells washed twice to remove free virus were cultured for 14 days at 37°C in 5% CO_2. On days 4, 7, 10, and 14, 0.5 volume of medium was removed and replaced with complete medium containing cytokines. Supernatants were stored at −70°C until assayed for HIV p24 antigen.

HIV p24 antigen. An enzyme immunoassay was performed in accordance with the manufacturer's directions for the quantitation of HIV-1 p24 antigen.

Statistical analysis. Data were analyzed by using the Student *t* test, the Wilcoxon rank sum test, the Kruskal-Wallis test, or analysis of variance.

RESULTS

Effect of IL-12 and IL-15 on chronically HIV-1-infected T cells. We examined the effect of IL-12 and IL-15 on basal and PMA-activated chronically HIV-1-infected T cells (ACH-2 cells). Neither IL-12 nor IL-15 had a significant effect on the kinetics of basal HIV-1 production by ACH-2 cells (2×10^5) at the highest tested concentration (Fig. 1A). In other experiments (data not shown), a similar lack of effect by these cytokines on lower numbers of ACH-2 cells was also observed. We next evaluated the effect of these cytokines on PMA-activated ACH-2 cells. As illustrated in Fig. 1B and C, IL-12 and IL-15 significantly enhanced HIV p24 antigen production by PMA-stimulated ACH-2 cells ($P < 0.03$) in a dose-dependent manner.

Effect of IL-12 and IL-15 on chronically HIV-1-infected monocytic cells. In addition to T cells, monocytes are another major reservoir for HIV-1. Using chronically HIV-1-infected monocytes (U1 cells), we examined the effect of IL-12 and IL-15 on basal and PMA-activated HIV-1 p24 antigen production. As illustrated in Fig. 2, neither IL-12 nor IL-15 had a significant effect on basal HIV-1 p24 antigen production. PMA increased p24 antigen production by sixfold. In contrast, IL-12 (10 ng/ml) and IL-15 (10 ng/ml) significantly diminished p24 antigen release by PMA-activated U1 cells ($P < 0.004$). HIV-1 p24 antigen production at 24 and 48 h was similarly reduced by treatment with either cytokine (data not shown).

Effect of IL-12, IL-15, and IL-2 on HIV replication in patients' PBMCs cocultured with mitogen-activated donor cells. IL-2 is utilized in the standard HIV coculture assay to optimize the induction of virus production (30). We compared the effects of IL-12 and IL-15 with that of IL-2 on HIV p24 antigen production by patients' PBMCs. Illustrated in Fig. 3A are the effects of cytokines on the kinetics of HIV-1 p24 antigen production by PBMCs (10^5 cells per well) from one of four HIV-infected patients. Maximal HIV-1 p24 production in response to cytokines was observed by day 10 of culture in PBMCs from all four patients. We next examined the effect of cytokines on in vitro HIV-1 p24 antigen production by 14 HIV-infected patients. As shown in Fig. 3B, viral replication in response to IL-2 was used to classify patients as low- or high-IL-2 responders according to whether HIV-1 p24 antigen production by their PBMCs in response to IL-2 was $<10^3$ or $>10^3$ pg/ml, respectively. A differential effect of IL-12 and IL-15 was observed depending on the responses to IL-2. In eight patients who were low-IL-2 responders, both IL-12 and IL-15 increased HIV-1 p24 antigen production by 2 log units ($P = 0.05$). For these patients, the mean absolute $CD4^+$-T-cell count was 303 ± 57 μl^{-1}. In contrast, for two patients who were high-IL-2 responders, IL-12 and IL-15 reduced HIV-1 p24 antigen production by 0.5 to 1 log unit. The absolute $CD4^+$-T-cell counts for the two high-IL-2 responders were 200 and 32 μl^{-1} (mean, 116 ± 84 μl^{-1}). For the four remaining patients, HIV p24 antigen was not detected when PBMCs were cultured with either IL-2, IL-12, or IL-15. For these patients, the mean absolute $CD4^+$-T-cell count was 705 ± 398 μl^{-1} (range, 444 to 1,293 μl^{-1}).

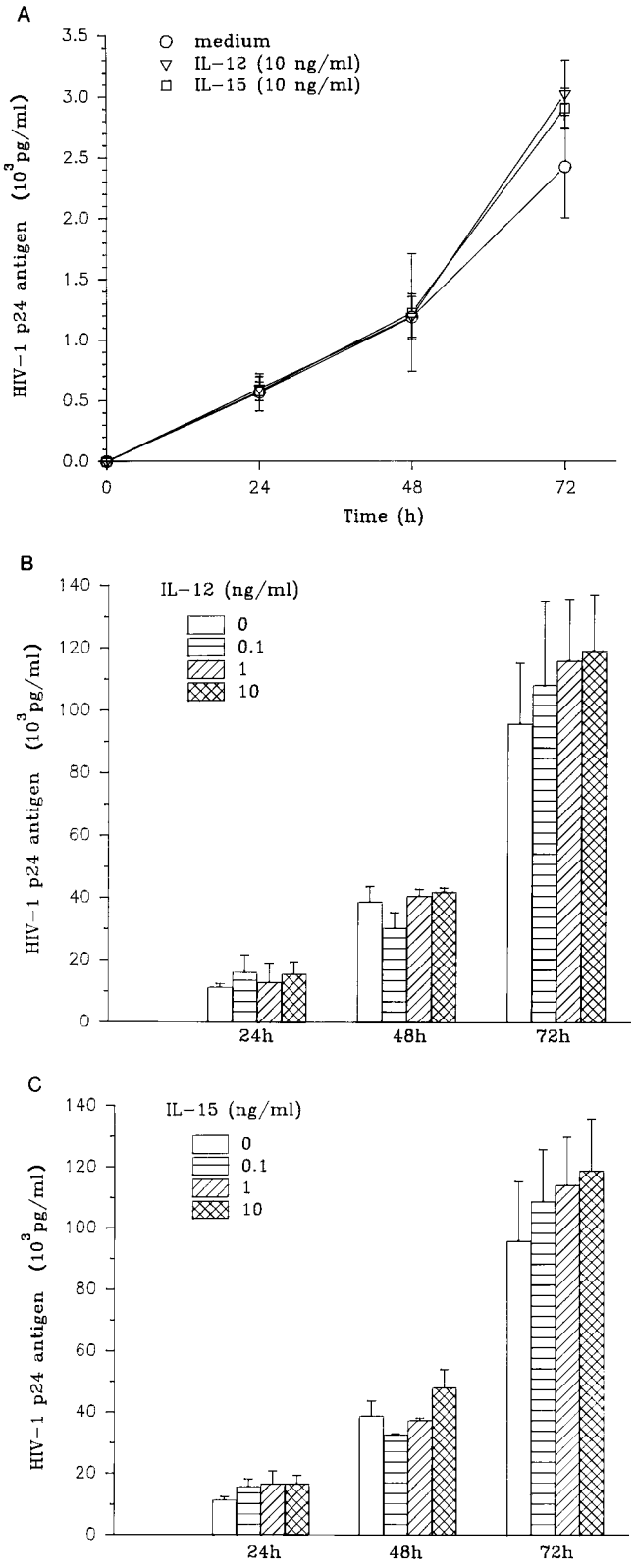

FIG. 1. Effect of IL-12 and IL-15 on basal and phorbol ester-activated HIV-1 replication by chronically HIV-infected T cells. (A) ACH-2 cells (2×10^5) were cultured in medium only or in medium containing IL-12 (10 ng/ml) or IL-15 (10 ng/ml). Results are the means ± standard errors of six experiments. (B) ACH-2 cells (2×10^5) stimulated with PMA (50 ng/ml) were cultured with IL-12. HIV p24 antigen production at 72 h was significantly increased by IL-12 at doses of 1.0 and 10 ng/ml compared with medium only ($P < 0.05$; $n = 6$). Results are means plus 1 standard error. (C) ACH-2 cells (2×10^5) stimulated with PMA (50 ng/ml) were cultured with IL-15. HIV p24 antigen production at 72 h was significantly increased compared with production in medium only ($P < 0.03$; $n = 6$). Results are means plus 1 standard error.

Effect of IL-2, IL-12, and IL-15 on viral replication in mitogen-activated PBMCs acutely infected with HIV-1_{JRCSF}. We additionally examined whether IL-2, IL-12, or IL-15 can affect acute HIV infection. The monocyte-tropic HIV-1_{JRCSF} strain was used to infect mitogen-activated PBMCs from HIV-seronegative donors. Illustrated in Fig. 4 is the HIV p24 antigen production by HIV-seronegative-donor PBMCs cultured in medium only or medium containing IL-12 (10 ng/ml), IL-2 (10 U/ml), or IL-15 (10 ng/ml). A time-dependent increase in HIV p24 production was shown for all culture conditions (analysis of variance, $P < 0.05$, $n = 3$ donors). The time of addition of each of the three cytokines in relation to infection with HIV made little difference in viral production (Kruskal-Wallis test). In contrast, compared with basal p24 antigen production, IL-2, IL-12, and IL-15 had differential effects on viral production in cells acutely infected with HIV-1_{JRCSF}. The production of p24 antigen by cells infected with HIV-1_{JRCSF} in medium only and in medium with IL-12 was similar. In contrast, both IL-2 and IL-15 induced significantly higher viral production (~2-fold) than medium only or IL-12 ($P < 0.01$, Student paired t test, $n = 3$ donors). We have studied three additional donors and similar findings were observed (data not shown).

DISCUSSION

Cytokines may have clinical applications for treatment of HIV infection. Studies of the effect of IL-12 and IL-15 on HIV replication were initiated because these cytokines have features suggesting that they may enhance cellular immunity. In this study, IL-12 and IL-15 had little effect on basal HIV-1 p24 antigen production in chronically HIV-infected T cell (ACH-2) and monocyte (U1) lines. Similarly, IL-2, IL-12, and IL-15 had little effect on basal HIV p24 antigen production by PBMCs from HIV-infected patients (not cocultured with mitogen-activated donors cells) (data not shown). The finding that IL-12 and IL-15 had little effect on HIV production by unstimulated chronically HIV-infected T cells and monocytes or by PBMCs from HIV-infected patients can be interpreted as follows. First, IL-12 and IL-15 receptors are not expressed at basal state but are expressed upon cell activation, thereby leading to a functional response. Second, cell activation may be required because a mechanism(s) not related to receptor expression, such as costimulatory signaling by PMA or PHA and the tested cytokines, is required for up-regulation of HIV replication (52). Consistent with the first interpretation is the finding of Desai et al. (19). Using radiolabeled IL-12, Desai et al. found that the expression of high-affinity IL-12 receptors by PBMCs, $CD4^+$ T cells, $CD8^+$ T cells, and $CD56^+$ NK cells required stimulation with either mitogen (PHA) or IL-2 and that binding of IL-12 to nonactivated cells was undetectable. Although IL-15α receptor mRNA expression is detected in T cells, monocytes, B cells, mast cells, and several other types of cells, receptor number and affinity for IL-15 in basal and activated cells have not been reported (1).

IL-12 and IL-15 significantly enhanced HIV-1 p24 antigen production in PMA-induced ACH-2 cells. In contrast, IL-12 and IL-15 treatment of PMA-stimulated U1 cells decreased p24 antigen production. As noted in Fig. 1B and C, IL-12 and IL-15 increased PMA-stimulated T-cell production of HIV p24 antigen by ~25%. In contrast, the same cytokines reduced

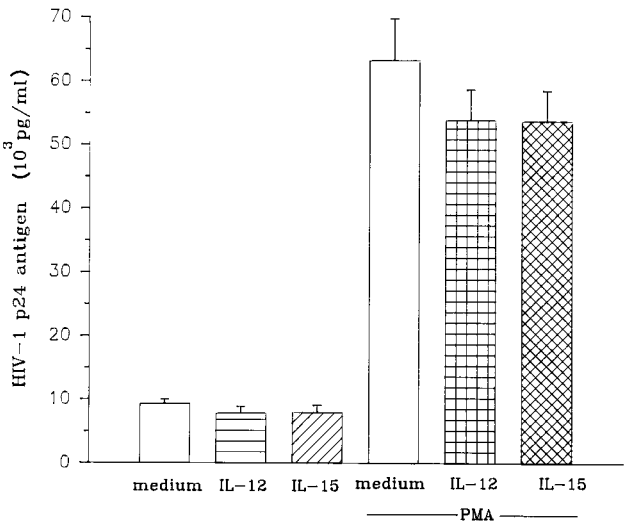

FIG. 2. Effect of IL-12 and IL-15 on basal and phorbol ester-activated HIV-1 replication by chronically HIV-infected monocytes. U1 cells (2×10^5) were cultured in medium only or in medium containing PMA (50 ng/ml) and treated with IL-12 (10 ng/ml) or IL-15 (10 ng/ml) or left untreated. IL-12 and IL-15 decreased HIV p24 antigen production by phorbol ester-activated U1 cells after 72 h of culture (PMA versus PMA plus IL-12, $P = 0.004$; PMA versus PMA plus IL = 15, $P = 0.003$; $n = 6$). Results are the means plus standard error of six experiments.

PMA-stimulated monocyte production of p24 antigen by ~15% (Fig. 2). These changes in HIV p24 antigen production represent changes on the order of $\sim 25 \times 10^3$ and 10×10^3 pg, respectively, of HIV p24 antigen per ml produced by 10^5 patient PBMCs. These changes are significant in light of the finding that the same p24 antigen assay coupled with acid dissociation to increase sensitivity has detected p24 antigen levels that are less than 100 pg/ml of plasma (20). Moreover, antiretroviral therapies reduced viral load in most patients by at most ~20%, as measured by RNA or by branched-chain DNA techniques (16, 20).

The differential effect of IL-12 and IL-15 on viral replication according to the type of HIV-infected cells suggests that a costimulatory signal provided by PMA or PHA and cytokines may be involved. A differential effect of IL-12 and IL-15 was also observed in HIV-infected patient cells cocultured with mitogen-activated HIV-seronegative donor cells. PBMCs from HIV-1-seropositive patients in this assay showed two patterns of p24 antigen production in response to IL-2: low- and high-IL-2 response. For the low-IL-2-response pattern, IL-12 and IL-15 increased HIV p24 antigen production by 100-fold and 97-fold, respectively. For the high-IL-2-response pattern, both cytokines suppressed HIV replication. The differential effects of cytokines on chronically infected cell lines and patient cells are not due to their differential effects on cell viability because the fractions of cells excluding trypan blue in medium only and in medium following cytokine (IL-12 or IL-15) treatment were similar.

The differential effects of IL-12 and IL-15 on HIV production in T cells versus monocytes may be due to differences in receptor signaling between cells. For example, in T cells IL-15 has been reported to signal through JAK-1 and JAK-3 of the Janus kinases with recruitment of STAT-3 and STAT-5 proteins, and the cytoplasmic domain of the IL-15α receptor is not required for function (29, 36). In contrast, IL-15 in mast cells signals through JAK-2 and recruits STAT-5. Moreover, Tagaya and colleagues have observed that mast cells express only the IL-15α chain, require the IL-15α cytoplasmic domain for function, and express no IL-2β and IL-2γ chains (49).

The finding that in HIV-infected patients' cells, IL-12 and IL-15 enhanced HIV production in the low responders to IL-2 but suppressed HIV replication in high responders may be explained by the findings concerning the effects of the two

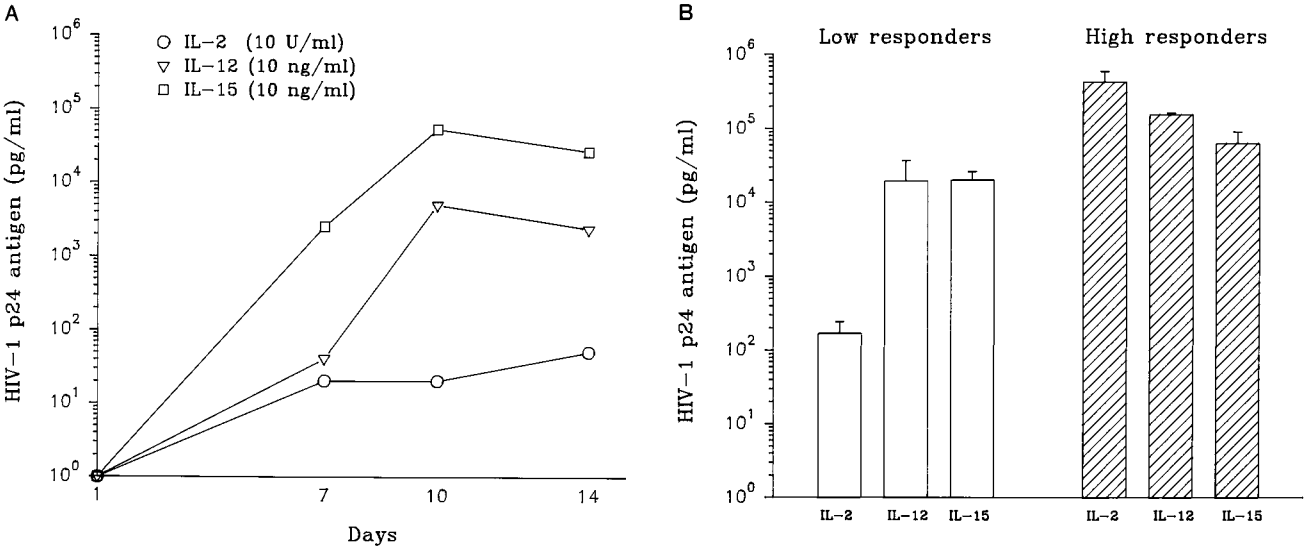

FIG. 3. Effect of IL-12 and IL-15 on HIV p24 antigen production by PBMCs obtained from HIV-seropositive patients. Cocultures of patient PBMCs (10^5/ml) and PHA-stimulated donor PBMCs in the absence of IL-2 produced levels of p24 antigen that were either not detectable or <10 pg/ml. (A) Representative kinetics of HIV p24 antigen production by PBMCs (10^5 cells per well) from one HIV-seropositive patient cocultured with mitogen-activated HIV-seronegative donor PBMCs in the presence of IL-2 (10 U/ml), IL-2 (10 ng/ml), or IL-15 (10 ng/ml) ($n = 4$). On the basis of the level of HIV-1 p24 antigen produced in response to IL-2 (<10^3 pg/ml), the patient is a low responder. (B) Differential production of HIV p24 antigen by HIV-seropositive patients' PBMCs (10^5 cells per well) allowed patients to be classified by their response to IL-2 as low ($n = 8$) and high ($n = 2$) responders. The amount of HIV p24 antigen detected at day 10 of culture is graphically illustrated (and was not significantly different from the amount of p24 antigen produced at day 14 of culture). Results are means plus 1 standard error.

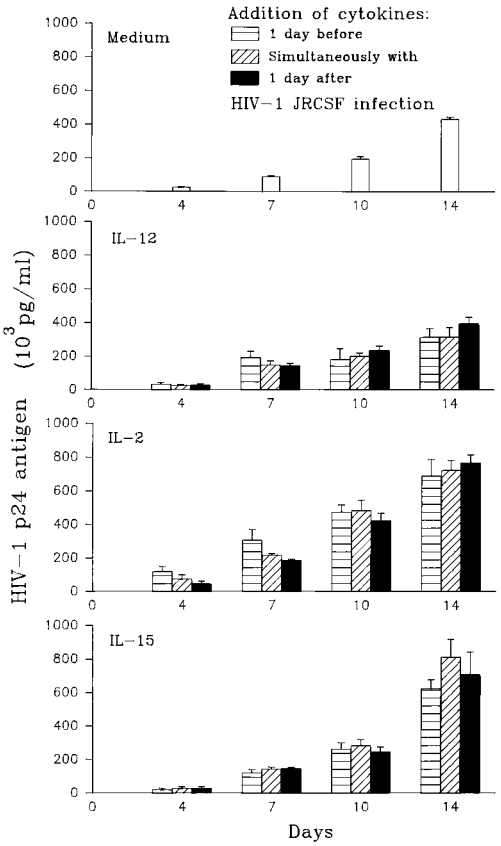

FIG. 4. Effect of IL-12 and IL-15 on acute HIV infection of mitogen-activated PBMCs from HIV-seronegative-donor cells. Mitogen-activated PBMCs (2×10^6) were cultured with IL-2 (10 U/ml), IL-12 (10 ng/ml), or IL-15 (10 ng/ml) either 1 day before, simultaneously with, or 1 day after infection with HIV-1_{JRCSF} (3,000 50% tissue culture infective doses). The results are the means plus standard error of three experiments using cells from different donors. Trypan blue exclusion performed on cells at day 14 did not differ for cells cultured with IL-2, IL-12, and IL-15.

cytokines on chronically HIV-infected T cells and monocytes (compare Fig. 3 with Fig. 1 and 2). Depletion of $CD4^+$ T cells is observed during the course of HIV disease progression, while major fluctuations of monocytes are not observed. High responders have a lower $CD4^+$-T-cell count (mean, 116 μl^{-1}), while low responders have a higher $CD4^+$-T-cell count (mean, 303 μl^{-1}). The finding that monocyte counts for the two groups were similar (276 versus 385 μl^{-1}) implies a higher number of $CD4^+$ T cells in relation to the number of monocytes in low responders than in high responders. This is supported by the finding that the ratios of the absolute number of monocytes to $CD4^+$ T cells in low responders and high responders were 1.3 and 2.3, respectively. Thus, the differential effect of IL-12 and IL-15 on patients' cells may therefore mirror the effect of these cytokines on chronically infected T cells and monocytes. The differential effects of IL-12 and IL-15 on chronically HIV-infected T cells, monocytes, and PBMCs from HIV-infected patients have not previously been reported and provide a potential clue to the contribution by different reservoirs of HIV in HIV pathogenesis.

The effects of cytokines on naive PBMCs acutely infected with HIV-1_{JRCSF} in vitro was also evaluated because in vivo, the major pool of new virus originates from newly infected cells (28, 51). IL-2, IL-12, and IL-15 showed different patterns of response. IL-2 and IL-15 increased HIV p24 antigen production, while basal p24 antigen production was not increased by treatment with IL-12. Our findings, compared with those of Foli et al. on the effect of IL-12 on HIV infection of naive cells, are confirmed in some respects but are in distinct contrast in others (22). Foli et al. studied five subjects and observed that IL-12 enhanced HIV-1 production by naive cells infected in vitro with HIV-1_{LAV}. However, Foli et al. did not examine the effect of IL-12 on HIV-infected patients' cells or cell lines (22). Foli et al. showed that IL-12 (4.8 ng/ml) increased HIV p24 antigen production by ~19-fold. In contrast, we noted no difference or a mild suppressive effect by IL-12 (10 ng/ml) compared with medium only in PHA-stimulated PBMCs infected with HIV-1_{JRCSF}. The major differences between the study of Foli et al. and our study may be due to the viral strain used (BAL and JRCSF, respectively), the viral infective dose (40,000 and 3,000 50% tissue culture infective doses per 2×10^6 PBMCs, respectively), or the cell density of the culture (5×10^4 PBMCs per 200 μl and 2×10^6 PBMCs per 1.5 ml, respectively) (20). Although we cannot exclude the possibility that the differential effects of IL-2, IL-12, and IL-15 on viral replication may be due to their effect on cell proliferation, the findings of Seder et al. (46) argue against this possibility. Although Seder et al. found that all three cytokines increased the proliferative response to tetanus compared with medium only, significant differences between the effects of IL-2, IL-12, and IL-15 on proliferative response to tetanus and HIV peptides were not observed (46).

IL-2 is a potent trigger for viral replication. This is observed in vitro and is suggested by studies of HIV-infected patients given IL-2 (30, 33). The finding that in four persons with the highest $CD4^+$-T-cell counts IL-2 did not induce HIV p24 antigen production may be an indicator of HIV-specific suppressor cell activity or lower viral load (7, 41). In addition, for these individuals IL-12 and IL-15 similarly had no effect. On the basis of our assay, we are not able to distinguish between HIV-specific suppressor cell activity and lower viral load as the cause of no detectable HIV p24 antigen. However, our assay's culture size (100,000 cells) suggests that suppressor cells rather than low viral load played a more prominent role because otherwise we would have to assume that the concentration of virus-infected cells was less than 1 per 100,000 cells (a less probable scenario).

We have observed that IL-12 and IL-15 have differential effects on different types of HIV-infected cells and on chronically infected cells as opposed to acutely newly HIV-1_{JRCSF}-infected cells. Furthermore, the cell activation state may be a very important factor in the ability of these cytokines to effect HIV replication. The recent finding that viral replication may be the major contributor to $CD4^+$-T-cell depletion argues for judicious use of therapies that may have an enhancer impact on viral replication. Our data suggest that during active coinfection, a condition analogous to in vitro activation by mitogen or PMA, these cytokines may enhance viral replication and so they should be used with caution. Thus, the clinical use of IL-12 or IL-15 may be dictated by the clinical state of the patients. The effects of these cytokines on viral load should be investigated during clinical trials.

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