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Inhibition of CCR5 Expression by IL-12 Through Induction of β-Chemokines in Human T Lymphocytes

Jinhai Wang,1 Ennan Guan, Gregory Roderiquez, and Michael A. Norcross1

IL-12 induces initiation of the differentiation of naive CD4+ T lymphocytes into Th1 cells and is important for the control of cell-mediated immunity. β-Chemokines serve to attract various types of blood leukocytes to sites of infection and inflammation. The specific receptor for the β-chemokines (macrophage-inflammatory protein (MIP)-1α, MIP-1β, and RANTES), CCR5, also functions as the primary coreceptor for macrophage-tropic isolates of HIV-1. IL-12, but not IL-4, IL-10, or IL-13, now has been shown to down-modulate the surface expression of CCR5 induced by IL-2 on both CD4+ and CD8+ T lymphocytes. Decreased CCR5 surface expression was not secondary to transcriptional inhibition, given that CCR5 mRNA was enhanced in cells cultured in IL-12/IL-2 compared with those cultured in IL-2 only. The effect of IL-12 in down-modulation of CCR5 surface expression was shown to be mediated by soluble factors secreted from the T cells. Rapid and transient intracellular Ca2+ mobilization was induced in monocytes by IL-12-induced supernatants, which desensitized the response of monocytes to MIP-1α, but not their response to stromal cell-derived factor-1α. Neutralization with specific Abs identified these factors as MIP-1α and MIP-1β from most donors. IL-4, IL-10, IFN-γ, and IL-18 primarily inhibited MIP-1β secretion and also weakly suppressed MIP-1α secretion. HIV-1 replication was inhibited in IL-2/IL-12-containing cultures that correlated with chemokine and chemokine-receptor levels. These data suggest that the effects of IL-12 on β-chemokine production and chemokine-receptor expression may contribute to the immunomodulatory activities of IL-12 and may have potential therapeutic relevance in controlling HIV-1 replication. The Journal of Immunology, 1999, 163: 5763–5769.

Interleukin-12 is a heterodimeric cytokine that consists of disulfide-linked p35 and p40 subunits. Bioactive IL-12 (p70) is produced predominantly by neutrophils and activated APC such as dendritic cells and macrophages. IL-12 induces the secretion of IFN-γ by T lymphocytes and NK cells and promotes the development of the Th1 phenotype of CD4+ cells (2–4). Th1 lymphocytes secrete IFN-γ, but not IL-4, and mediate cellular immunity, whereas Th2 lymphocytes secrete IL-4 and IL-5, but not IFN-γ, and promote humoral immunity. Studies of Th1 and Th2 clones reveal that expression of the β-chemokine receptor CCR5 is characteristic of Th1 cells (5), whereas the CCR3 receptor is preferentially expressed by Th2 clones (6). However, another study showed that the expression of CCR5 did not differ between Th1 and Th2 subsets (7).

Various cytokines have been shown to modulate the expression of chemokine receptors on both monocytes and lymphocytes (8–11). IL-2 induces expression of CCR5 in cultured lymphocytes, whereas IL-4 increases predominantly the expression of the chemokine receptor CXCR4 in these cells. In monocyte and macrophage cultures, M-CSF and GM-CSF up-regulate CCR5 expression, whereas IL-4 and IL-13 inhibit expression of this receptor (11). At high concentrations, GM-CSF also inhibits CCR5 expression (12). IFN-γ increases the expression of CCR1, CCR3, and CCR5 in U937 monocytic cells (8).

Cytokines also play a central role in modulating immune function and in the pathogenesis of HIV infection. Because of defects in the production of Th1 cytokines during the course of HIV infection, several cytokines, including IL-2 and IL-12, are being considered for use as immunotherapeutic agents in individuals with AIDS, both to increase the number of CD4+ cells and to enhance immune function. In vitro, IL-2 stimulates virus replication, whereas IL-12 can either enhance virus production when added with mitogens (13) or suppress replication in the presence of IL-2 (14). In clinical trials, IL-2 has been shown to increase CD4+ cell number in HIV-1-infected patients, but it also stimulates virus replication in the absence of antiviral drugs (15, 16). This latter effect is consistent with the activation of T cells by IL-2 and with the stimulatory effect of this cytokine on CCR5 expression in cultured lymphocytes, given that CCR5 functions as a coreceptor with CD4 for macrophage-tropic (M-tropic)2 isolates of HIV-1. Deficiencies in the production of IL-12 have also been demonstrated in individuals infected with HIV-1. Because of the potential of IL-12 to reconstitute immune function in HIV-1-infected individuals, we have now investigated the effect of IL-12, as well as those of other cytokines, on CCR5 expression in human lymphocytes. Unlike the other cytokines examined (IL-4, IL-10, and IL-13), IL-12 down-regulated the expression of CCR5 on T cells through the induction of β-chemokines.

Materials and Methods

Cells and reagents

Human PBLs and monocytes were isolated from individual donors by countercurrent centrifugal elutriation and PBLs were cultured in RPMI

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2 Abbreviations used in this paper: M-tropic, macrophage-tropic; rhIL-2, recombinant human IL-2; MIP, macrophage-inflammatory protein; SDP, stromal cell-derived factor; FCA, flow cytometric analysis; MFI, mean fluorescence intensity.
CCR5 (18), PE-conjugated F(ab')2 fragments of goat polyclonal Abs to mouse IgG (Caltag, South San Francisco, CA), and then either with FITC-conjugated OKT4 mAb to CD4 (Ortho Diagnostics, Raritan, NJ) or with mouse IgG (Caltag, South San Francisco, CA). We next investigated the effect of IL-12 on cells stimulated to express CCR5, two-color staining was performed with the 2D7 mAb to CCR5 and subjected to FCA as described previously (11). For analysis of CD4+ and CD8+ subsets expressing CCR5, two-color staining was performed with the 2D7 mAb to CCR5 (18), PE-conjugated F(ab')2 fragments of goat polyclonal Abs to mouse IgG (Caltag, South San Francisco, CA), and then either with FITC-conjugated OKT4 mAb to CD4 (Ortho Diagnostics, Raritan, NJ) or with FITC-conjugated mAb to CD8 (Caltag).

Chemokine secretion

The amounts of RANTES (BioSource International, Camarillo, CA), MIP-1α, and MIP-1β (R&D Systems) secreted by PBLs into culture supernatants were determined with the use of ELISA kits.

Cell surface immunofluorescence staining

Cells were stained with the 5C7 (17) mAb to CCR5 and subjected to FCA as described previously (11). For analysis of CD4+ and CD8+ subsets expressing CCR5, two-color staining was performed with the 2D7 mAb to CCR5 (18), PE-conjugated F(ab')2 fragments of goat polyclonal Abs to mouse IgG (Caltag, South San Francisco, CA), and then either with FITC-conjugated OKT4 mAb to CD4 (Ortho Diagnostics, Raritan, NJ) or with FITC-conjugated mAb to CD8 (Caltag).

RT-PCR

Total cellular RNA of human PBL was isolated by using Trizol reagent, treated with DNase I, and equal amounts of total RNA were subjected to first-strand DNA synthesis using RNase minus Superscript II reverse transcriptase (Life Technologies, Rockville, MD). Amplification of cDNA was performed for 30 cycles of PCR with human CCR5-specific sense primer 5'-CCT CCG TCT TAC TCA CTG GTG TTC (nt 100–123) and antisense primer 5'-GCA GGT GTA AAG ACC TTC TT (nt 534–514), and human GAPDH sense and antisense-specific primers 5'-CAC CAC CAT GGA GAA GGC TGG (nt 306–326) and 5'-GCC ATG CCA GTG AGC (nt 695–675). PCR products were separated and stained with SYBR Green 1 (Molecular Probes, Eugene, OR).

Measurement of cytosolic-free Ca2+

Cells were labeled with the fluorochrome fura-2 acetoxymethyl ester (Molecular Probes, Eugene, OR). In brief, fura-2 acetoxymethyl ester (5 mM) and 10% Phuronic F-127 were premixed, added to cell suspension (1 × 10^6/ml) in Ca2+ buffer (136 mM NaCl, 4.8 mM KCl, 1 mM CaCl2, 5 mM glucose, and 20 mM HEPES, pH 7.4), and incubated at 37°C in the dark for 45 min. Cells were washed and resuspended at 2 × 10^6/ml in Ca2+ buffer with 0.1% BSA. Cytosolic-free Ca2+ was measured using excitation at 340 and 380 nm with emission measured at 510 nm with a FS scan (Photon Technology International, Princeton, NJ), and data were recorded as the relative ratio of 340 and 380 nm measurements. The supernatants were concentrated (30%) and dialyzed against PBS buffer before testing.

Virus infection

After culture in medium containing IL-2, or IL-12 and IL-2 for 7 days, PBLs were infected with M-tropic HIV-1 strain Ba-L (equivalent to 50 ng/ml) in Ca2+ buffer (136 mM NaCl, 4.8 mM KCl, 1 mM CaCl2, 5 mM glucose, and 20 mM HEPES, pH 7.4) and incubated at 37°C for 90 min. Cells were stained with the 5C7 mAb to CCR5 (filled trace) or with an isotype control (dotted line), and then were subjected to FCA (A). Similar staining patterns of IL-12-treated cells were obtained with six different PBL donors. CCR5 expression (MFI) for these donors is shown in B.

Results

Effect of IL-12 on CCR5 expression by PBLs

To investigate the effects of IL-12 and other cytokines on CCR5 expression by T lymphocytes, we used a simple culture system in which PBLs were incubated in the absence of polyclonal mitogens. Initial experiments revealed that incubation of the cells with IL-12 markedly inhibited the expression of CCR5 induced by IL-2 (Fig. 1A). In contrast, the Th2 cytokines IL-4, IL-10, and IL-13 each slightly increased the CCR5 fluorescence intensity of IL-2-treated cells. The inhibitory effect of IL-12 on CCR5 expression was dose-dependent, being less apparent at a concentration of 1 ng/ml. CCR5 expression (mean fluorescence intensity (MFI)) after IL-2 or IL-2 plus IL-12 treatment from six different PBL donors. CCR5 expression (MFI) for these donors is shown in B.

Virus infection

After culture in medium containing IL-2, or IL-12 and IL-2 for 7 days, PBLs were infected with M-tropic HIV-1 strain Ba-L (equivalent to 50 ng/ml of p24) overnight. After extensive washing with PBS, PBLs were refed with medium containing IL-2 (150 U/ml) and were cultured for 7 days. Supernatant was collected and assayed for p24 by ELISA (Coulter, Hialeah, FL).

Effects of IL-12 on CCR5 expression by CD4+ and CD8+ T cells

Both CD4+ and CD8+ T cells previously have been shown to express CCR5 after sequential stimulation with Abs to CD3 and
and upper right
the isotype control mouse IgG2a (FCA. Quadrants were set according to the staining pattern obtained with left panels CD4 (ng/ml). The cells were stained with mAb 2D7 to CCR5 and mAbs either to CCR5 surface expression by T cells is mediated by IL-12-induced
We next investigated whether the inhibitory effect of IL-12 on released soluble factor Mediation of the effect of IL-12 on CCR5 expression by a
CCR5 surface expression is not a consequence of CCR5 transcrip-
mRNA in cultured T cells, and demonstrates that inhibition of that IL-12 does not directly inhibit, but rather enhances, CCR5
from CCR5 genome DNA (data not shown). These data indicate the CCR5 band detected is specific for CCR5 mRNA and is not was detected without adding reverse transcriptase, indicating that band in Fig. 3. With the addition of IL-12, increased levels of CCR5 mRNA, as shown as an expected CCR5-specific 435-bp
expression of CCR5 on activated T cells (9), and T cells po-
CR5 expression in response to IL-12 in our system was second-
lar to effects on mRNA synthesis, cytokine-treated cells were an-
alyzed for CCR5 mRNA by RT-PCR. Consistent with previous
reports, PBLs cultured in medium containing IL-2 expressed CCR5 mRNA, as shown as an expected CCR5-specific 435-bp band in Fig. 3. With the addition of IL-12, increased levels of CCR5 mRNA were detected in the treated cells. No CCR5 band was detected without adding reverse transcriptase, indicating that the CCR5 band detected is specific for CCR5 mRNA and is not from CCR5 genome DNA (data not shown). These data indicate that IL-12 does not directly inhibit, but rather enhances, CCR5 mRNA in cultured T cells, and demonstrates that inhibition of CCR5 surface expression is not a consequence of CCR5 transcrip-
tional regulation.

Mediation of the effect of IL-12 on CCR5 expression by a released soluble factor
We next investigated whether the inhibitory effect of IL-12 on CCR5 surface expression by T cells is mediated by IL-12-induced secretion of a soluble factor (or factors) that induces down-regu-
lation of CCR5. PBLs were cultured in IL-2-containing medium for 8 days to induce CCR5 expression; then they were washed and cultured overnight in medium conditioned by incubation of PBLs either in the absence or presence of IL-12. PBLs incubated overnight with the culture supernatant of IL-12-treated cells from the same donor (Fig. 4A) or from an unrelated donor (Fig. 4B) showed a marked decrease in the extent of CCR5 expression. In contrast, the extent of CCR5 expression by PBLs similarly incubated with medium conditioned by culture of cells in the absence of IL-12 did not differ substantially from that CCR5 expression of cells incubated overnight with control medium. These results indicate that IL-12 induced the secretion of a soluble factor that is capable of directly down-modulating the surface expression of CCR5 in homologous or heterologous PBLs.

Effect of IL-12 on the secretion of β-chemokines by PBLs
Given that chemokines have been shown to induce down-regu-
ation of their receptors through endocytosis (20), we next examined whether IL-12 stimulates the secretion of chemokines that interact specifically with CCR5 (21, 22). Therefore, we measured the amounts of the β-chemokines MIP-1α, MIP-1β, and RANTES in the culture supernatants of PBLs incubated for 8 days in medium containing IL-2, either in the absence or presence of IL-12. Only

FIGURE 2. Effects of IL-12 on CCR5 expression by CD4+ and CD8+ T lymphocytes. PBLs were cultured for 8 days in medium containing IL-2 in the absence (center panels) or presence (lower panels) of IL-12 (10 ng/ml). The cells were stained with mAb 2D7 to CCR5 and mAbs either to CD4 (left panels) or to CD8 (right panels), and then were analyzed by FCA. Quadrants were set according to the staining pattern obtained with the isotype control mouse IgG2a (top panels). The pairs of numbers in the upper right and upper left quadrants represent the percentage of CD4+ or CD8+ T cells positive for CCR5 (left) and the corresponding MFI (right). Similar staining patterns were obtained with three different PBL donors.

FIGURE 3. Transcriptional regulation of CCR5 gene by IL-12. PBLs were cultured in medium containing IL-2 (Med), or IL-12 and IL-2 (IL-12) for 8 days; total RNA was isolated and subjected to RT-PCR (described in Materials and Methods). Representative results from two donors are shown.

FIGURE 4. Effects of IL-12-treated PBLs medium on CCR5 expression of autologous and heterologous cells. PBLs from each of two donors (A and B) were cultured for 8 days in medium containing IL-2; they were then washed and incubated overnight with medium containing IL-2 (Med) or with the culture supernatant of autologous (A, from donor 3) or heterolo-
gous (B, from donor 5) PBLs that had been cultured for 8 days in the absence or presence of IL-12. Cells were stained with the 5C7 mAb to CCR5 or with an isotype control (IgG), as indicated, and then subjected to FCA.
small amounts of these three chemokines were produced by IL-2-treated PBLs from eight unrelated donors (Fig. 5). However, culture of the PBLs in the presence of IL-12 induced a marked increase in the amounts of MIP-1α, MIP-1β, and RANTES in the culture supernatants; the maximal concentrations detected were ∼40, 90, and 10 ng/ml, respectively, and there was some individual variability in the total amount of chemokines produced. The concentrations of MIP-1α and MIP-1β in the culture supernatants were higher than that of RANTES for PBLs from most donors; however, cells from one donor (donor 5) secreted substantial amounts of RANTES but little MIP-1α or MIP-1β. These data suggested that the inhibition of CCR5 expression by IL-12 might be attributable to IL-12-induced secretion of β-chemokines into the medium.

**Down-regulation of CCR5 expression by β-chemokines released by IL-12-stimulated PBLs**

We investigated whether MIP-1α, MIP-1β, or RANTES released from IL-12-treated PBLs were responsible for the inhibitory effect of IL-12 on CCR5 expression. PBLs were incubated with IL-2 for 8 days, washed, and then incubated overnight with conditioned medium from 8-day cultures of IL-12-treated PBLs from donor 3 (Fig. 5) in the absence or presence of neutralizing Abs to each of the three β-chemokines. Abs to either MIP-1α or MIP-1β reversed the inhibition of CCR5 expression induced by the conditioned medium, with the effect of the former Abs being most pronounced, whereas Abs to RANTES had no marked effect (Fig. 6). These results show that MIP-1α and MIP-1β were primarily responsible for the inhibition of CCR5 expression induced by this culture supernatant. When the Abs to the three chemokines were combined, CCR5 expression recovered to a level higher than that apparent for PBLs incubated with IL-2-containing medium or with conditioned medium from cells not exposed to IL-12. This observation suggests that low concentrations of endogenous β-chemokines limit CCR5 expression even in the absence of exogenous IL-12. RANTES also appears to contribute to inhibition of CCR5 expression when present at high concentrations, as indicated by the observation that the inhibitory effect of medium conditioned by IL-12-treated PBLs from donor 5 (Fig. 5) was reversed by Abs to RANTES (data not shown).

**Inhibition of β-chemokine secretion by Th2-type cytokines, IL-4 and IL-10, and by IFN-γ and IL-18**

Some effects of IL-12, such as its anti-tumor activity (23), involve the induction of IFN-γ. IL-12 has been reported to stimulate the production of other cytokines from PBLs (24), including IL-4 and IL-10. To examine the contribution of cytokines previously associated with IL-12 effects, IFN-γ, IL-4, and IL-10 were tested on β-chemokine production in the presence of IL-2. Because IL-18 is a potent IFN-γ inducer (25) and because TNF-α has been reported to induce β-chemokines from fibroblasts, these two cytokines were also tested.

As shown in Fig. 7, IL-12 is the only potent inducer of MIP-1α and MIP-1β secretion in this set of cytokines, although TNF-α did have a small stimulatory effect on MIP-1α and MIP-1β. In contrast, IL-4, IL-10, IFN-γ, and IL-18 strongly inhibited MIP-1β production and had less of an inhibitory effect on MIP-1α production. These data suggest that the Th2 cytokines, IL-4 and IL-10, and Th1 cytokine, IFN-γ, do not induce MIP-1α and MIP-1β secretion from PBLs and are therefore unlikely to be indirectly mediating IL-12 effects on chemokine production.

**Calcium mobilization and desensitization**

We were next interested in determining whether the chemokines secreted in response to IL-12 were biologically active in triggering cells. As shown in Fig. 8A, control IL-2-cultured supernatant (S)
did not stimulate cytosolic-free Ca\(^{2+}\) in monocytes or desensitize responses to MIP-1\(\alpha\) or SDF-1\(\alpha\) (30 nM each). A rapid and transient rise in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) was seen with IL-12 supernatant (Fig. 8B), which desensitized the response of monocytes to MIP-1\(\alpha\), but not to SDF-1\(\alpha\). These data indicate that IL-12 supernatant contains ligands for CCR1 and/or CCR5, but not ligands capable of stimulating through CXCR4. IL-12 supernatant also desensitized the response of monocytes to RANTES, but not to MCP-1 (data not shown). Complete desensitization of monocytes to IL-12 supernatant by MIP-1\(\alpha\) was observed (Fig. 8C). Because MIP-1\(\alpha\) desensitizes monocytes to MIP-1\(\alpha\), MIP-1\(\beta\), and RANTES, but not to MCP-1, MCP-2, and MCP-3 (26), the above data suggest that IL-12 supernatant does not contain MCP-1, MCP-2, or MCP-3. A rapid and transient rise in [Ca\(^{2+}\)]\(_i\) was also seen with IL-12 supernatant in macrophages, which desensitized the response of macrophages to MIP-1\(\alpha\), but not to SDF-1\(\beta\) (data not shown).

Inhibition of M-tropic HIV-1 infection of T cells by IL-12

Because IL-12 down-modulated surface CCR5 expression through induction of MIP-1\(\alpha\) and MIP-1\(\beta\), we next examined its impact on M-tropic HIV-1 infection of T cells. PBLs cultured in medium containing IL-2 produced 1697 pg/ml of p24, whereas PBLs cultured in medium containing both IL-12 and IL-2 before infection only produced 200 pg/ml of p24, an 88% reduction (Fig. 9).

Discussion

We have shown that IL-12 inhibits CCR5 expression on the surface of IL-2-activated CD4\(^+\) and CD8\(^+\) T lymphocytes through induction of \(\beta\)-chemokine secretion. The Th2 cytokines IL-4, IL-10, and IL-13 slightly increased CCR5 expression by PBLs. Given that IL-2 and IL-12 are considered classical Th1-polarizing cytokines, our data showing that IL-12 induces the secretion of substantial amounts of MIP-1\(\alpha\) and MIP-1\(\beta\) support an association between the type 1 immune response and \(\beta\)-chemokine production that was suggested in a previous study (27). Th2 cytokines IL-4 and IL-10 may function to oppose this effect by suppressing chemokine production from activated T cells.

CCR5 and CCR3 were identified as markers for Th1 and Th2 cells on the basis of their expression by PHA- and IL-2-stimulated clones polarized by treatment with IL-12 or IL-4, respectively (5, 6). In accordance with the reported data, our RT-PCR results showed increased CCR5 mRNA in PBLs cultured with IL-12- plus IL-2-containing medium, a condition standard for Th1 differentiation. However, because of the dramatically elevated production of \(\beta\)-chemokines in our culture conditions, ligand-induced endocytosis of CCR5 results in down-modulation of the receptor from the cell surface. Conditions where Th1 clones continue to express CCR5 (5) suggest to us that cells in long-term culture may not continuously secrete CCR5-specific chemokines or that prior exposure to mitogenic (PHA) stimulation alters receptor modulation.
Overall, our data indicate that surface CCR5 expression is not a stable marker for Th1 differentiation in primary cell culture. Our results are consistent with another study on murine Th1 and Th2 cells (28) where only Th1 cells, and not Th2 cells, expressed high levels of MIP-1α, MIP-1β and MCP-1.

We have previously shown that cytokines affect HIV-1 entry and replication in monocytes/macrophages through modulation of CCR5 expression. M-CSF and GM-CSF increased HIV-1 entry and production through up-regulation of CCR5, whereas IL-4 and IL-13 reduced HIV-1 production through down-modulation of CCR5 (11). Our present data indicate that CCR5 expression is regulated differently in macrophages and T lymphocytes. Thus, whereas IL-4 and IL-13 directly reduce CCR5 expression on macrophages, these cytokines slightly increased CCR5 expression by T cells. In addition, unlike their effects on macrophages (11), M-CSF and GM-CSF did not induce CCR5 expression by T cells (data not shown).

The effect of a specific cytokine on chemokine receptor expression therefore depends both on the type of cytokine receptor present on the target cell and on the array of chemokines induced by the cytokine. On the basis of our results, we propose that IL-12 selectively enhances Th1 cell responses through the following two mechanisms: 1) a direct effect on cell differentiation associated with Th1 cytokine expression, and 2) stimulation of T cells to produce chemokines that attract Th1 cells through CCR5 engagement.

The ability of IL-12 to stimulate chemokine production may have relevance to other effects of IL-12 such as in stimulating anti-tumor immune responses and as a Th1 adjuvant for prophylactic and therapeutic vaccines.

Cytokine induction of chemokines has been the focus of several recent reports. Perera et al. (29) reported that IL-12 induces low levels of chemokines in T lymphocytes. Fehniger et al. (30) reported that IL-12 in combination with IL-12 induces chemokines and β-chemokines in human NK cells. Our results showing the secretion of chemokines from PBLs in response to IL-12 plus IL-2 in the absence of prior Ag sensitization suggest a possible role for T cell-derived β-chemokines in early responses to infection.

IL-12 promotes lymphocyte replication (31–33), induces the production of β-chemokines, and down-regulates the expression of CCR5 on CD4+ T cells. Given that β-chemokines play a pivotal role in protection against HIV-1 infection (34, 35) and that CCR5 is the primary coreceptor for M-tropic HIV-1 (36–40), IL-12 may contribute to control of HIV infection through its effects on chemokines and their receptors. Our results showing that M-tropic HIV-1 replication is inhibited in T cells cultured in medium containing IL-12 plus IL-2 (compared with that containing IL-2 alone), in conjunction with results showing that IL-12 also inhibited SI virus infection of T cells (41), suggest that a combination of IL-2 and IL-12 may be useful as an adjunct to current antiviral therapies for HIV infection. Whether combination of IL-2, IL-12, and IL-13 can simultaneously control HIV-1 infection of both T cells and macrophages warrants further investigation.

The role of IL-12 to stimulate chemokine production may have relevance to other effects of IL-12 such as in stimulating antitumor immune responses and as an adjuvant for prophylactic and therapeutic vaccines. The observation that combinations of IL-12 and IL-2 are more effective in controlling tumor growth than either cytokine alone (42, 43) raises the possibility that β-chemokine induction may be involved in the antitumor activity of these cytokines.

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