Interleukin-18 Inhibits Human Immunodeficiency Virus Type 1 Production in Peripheral Blood Mononuclear Cells

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Interleukin (IL)–18 is an interferon (IFN)–γ-inducing factor and contributes to the Th1 immune response. IL-18 added after infection of peripheral blood mononuclear cells (PBMC) with monocyte-tropic human immunodeficiency virus type 1 (HIV-1) inhibited p24 antigen production by a maximum of 72%. IFN-γ levels in these cultures were increased, and a significant inverse relationship between HIV-1 production and IFN-γ levels was observed. A neutralizing anti–IFN-γ antibody reversed the IL-18 inhibitory effect. Preincubation of PBMC with IL-18 before infection inhibited p24 without additional IL-18 (64%). However, compared with the degree of IL-18 inhibition observed after a 4-day culture, no additional IL-18 inhibitory effect was observed during days 5–13. IL-18 also reduced cell surface expression of the HIV-1 receptor CD4. These results demonstrate that IL-18 inhibited HIV-1 production in PBMC through intermediate IFN-γ. Furthermore, inhibition was present during the early stages of viral infection and was associated with reduced HIV-1 receptor expression.
ferred into a 50-mL polypropylene tube. After centrifugation at 400 g, the medium from the tube was decanted, and the PBMC pellet was infected with virus.

Three hundred TCID₉₀ of M-tropic virus per 10⁶ PBMC was added to the cell pellet, followed by resuspension and incubation for 3 h at 37°C in 5% CO₂, as described elsewhere [26]. RPMI (20 mL) was added to the 50-mL tube, and the cells were resuspended and pelleted by centrifugation at 400 g. This washing step removed free virus from the infected PBMC. Cells (2 × 10⁶/mL) were suspended in fresh R3 medium. A 250-μL aliquot of infected PBMC suspension was transferred to a 1.5-mL polypropylene tube (BD Falcon) and was lysed with an equal volume of R3 medium containing Triton X-100 (1% final concentration). This aliquot was frozen at −70°C and was designated as the time 0 aliquot. The remaining infected PBMC suspension was aliquoted (250 μL) into separate wells of a 24-well polystyrene tissue culture plate (BD Falcon), and 250 μL of R3 medium alone or 250 μL of medium containing IL-18 was added to each well. Cultures were incubated (37°C, 5% CO₂) for 4 days; Triton X-100 (final concentration, 1% vol/vol) then was added, and the cultures were frozen (−70°C) until assayed. PBMC obtained from separate donors were infected with T-tropic A018A HIV-1 strain (300 TCID₉₀/10⁶ cells). These PBMC assayed. PBMC obtained from separate donors were infected with M-tropic virus and were added to polystyrene wells, as described in the text.

For IFN-γ neutralization studies, PBMC were infected with M-tropic virus and were added to polystyrene wells, as described above. The infected cells were incubated for 1 h in medium alone (spontaneous), in the presence of neutralizing anti–human IFN-γ antibody, or with isotype control antibody. After the 1-h incubation, IL-18 was added (final concentration, 5 nM) to the cultures that included antibody but not to the spontaneous one, and then cells were cultured for 4 days.

For time-course investigations of HIV-1 production, PBMC were infected with M-tropic virus and then were aliquoted into 4 separate 5-mL capped polypropylene tubes at 10⁶ cells/mL in a final volume of 2.0 mL. Each tube received R3 medium alone or medium containing sufficient IL-18 to produce final concentrations of 0.002, 1.0, or 5.0 nM. After a 4-day culture, the cells were pelleted, and the half the culture supernatant volume (1.0 mL) was aspirated into a separate polypropylene tube. Triton X-100 (final concentration, 1% vol/vol) was added to the supernatants, and the samples were frozen at −70°C. The remaining cells then received 1.0 mL of fresh medium alone or fresh medium containing IL-18 sufficient to restore the initial IL-18 concentration, and then they were returned to the incubator. These steps were repeated on days 7, 10, and 13 of culture.

**Measurement of p24 antigen and IFN-γ.** HIV-1 p24 antigen was quantified by use of an ELISA kit with a limit of detection of 37 pg/mL (National Cancer Institute–Frederick Cancer Research and Development Center). IFN-γ was quantified using electrochemiluminescence (ECL) [27, 28]. The amount of ECL was determined by use of an Origen Analyzer (Igen), with a lower limit of quantification of 12 pg/mL IFN-γ.

**Cell viability.** A toxic or cell-proliferative effect of IL-18 was assessed in 4-day cultures of HIV-1–infected PBMC. PBMC infected with M-tropic HIV-1 were aliquoted into wells of a 96-well polystyrene tissue culture plate at 10⁶ cells/mL in a final volume of 200 μL. Infected PBMC were cultured in the absence or presence of 5.0 nM IL-18, and the cultures were incubated for 4 days, as described above. After incubation, 20 μL of reagent (CellTiter 96 AQueous One Solution Reagent; Promega) was added to each well containing PBMC. Using this reagent, we quantified the capacity of mitochondrial dehydrogenase of viable cells to reduce tetrazolium compound to formazan [29]. After a 2-h incubation, the color change in the culture medium was measured by use of an ELISA reader (490 nm).

**Flow cytometry for CD4 and CCR5.** Uninfected PBMC at 2 × 10⁶ cells/mL were incubated in 1.0 mL of R3 medium (control) or in medium containing 5 nM IL-18 for 4 days in polypropylene tubes. The cell-containing tubes were centrifuged, decanted, and washed. The pellet in each of the 2 tubes was resuspended in PBS, and 100 μL was aliquoted into each of 2 separate polypropylene tubes. One of the control aliquots and one of the IL-18–exposed aliquots received fluorescein isothiocyanate (FITC)–conjugated anti–human CD4 MAb (BD PharMingen) and phycoerythrin (PE)–conjugated anti–human CCR5 MAb (BD PharMingen). The second control aliquot received isotype-matched control antibody to FITC-CD4 and PE-CCR5. The 3 tubes were incubated in the dark for 30 min at room temperature. After the cells were washed, each of the 3 PBMC-containing aliquots received 7-aminoactinomycin D (7-AAD; final concentration, 2 μg/mL; Molecular Probes) in a final volume of 500 μL of PBS. 7-AAD is excluded from the interior of viable cells, but nonviable cells admit 7-AAD to the cell interior. PBMC then were analyzed by use of an EPICS XL flow cytometer (Beckman Coulter).

**Statistical analysis.** All data are presented as the mean ± SEM. Differences were considered significant for P < 0.05. Selected p24 results were expressed as percentage of inhibition. For experiments conducted in each donor's PBMC, p24 measured in the time 0 (T = 0) sample was subtracted from the p24 values measured in each additional sample. The percentage of p24 production in each sample was calculated using the following formula: [(sample p24 concentration – (p24 concentration at T = 0))/((spontaneous p24 concentration) – (p24 concentration at T = 0))] × 100%.

Using this calculation, we set the p24 measured in each spontaneous culture at 100%. To calculate the percentage of p24 inhibition, the percentage of production for each sample was subtracted from 100%. Group means were compared by use of analysis of variance with Fisher's least significant difference, except where indicated in the text.

**Results**

**Exogenous IL-18 inhibits p24 antigen levels and induces IFN-γ production in PBMC infected with M-tropic HIV-1.** To examine the effect of IL-18 on HIV-1 production in freshly infected primary cells, we isolated PBMC from healthy donors and infected the cells with HIV-1. IL-18 was added to PBMC immediately after the infection procedure, and the infected cells were cultured in the absence or presence of IL-18. As shown in figure 1A, PBMC infected with M-tropic HIV-1 contained 116 ± 28 pg/mL p24 antigen at time 0, representing the cell-associated HIV-1 before culture. After the PBMC were incubated for 4 days with medium alone, the level of spontaneous p24 antigen in the cultures was 1309 ± 291 pg/mL, an 11-fold
increase, compared with time 0. Exogenous IL-18 added at 0.2, 1, and 5 nM dose dependently reduced 4-day p24 levels by 42%, 59%, and 72% (final concentrations, 803 ± 178, 610 ± 130, and 443 ± 97 pg/mL, respectively), compared with p24 antigen produced in spontaneous cultures (medium alone).

IFN-γ can suppress HIV-1 production in monocyte-derived macrophages infected with M-tropic virus [30–32]. Since IL-18 is an IFN-γ–inducing factor, we considered the possibility that IL-18–induced HIV-1 inhibition was associated with an increase in IFN-γ. IFN-γ production was assessed in the PBMC infected with M-tropic HIV-1 described for figure 1A. IFN-γ was below the limit of detection in the PBMC cultures from 3 of the 6 donors. In figure 1B, the results are presented for the 3 donors whose PBMC produced detectable levels of IFN-γ. The HIV-1–infected PBMC from these donors demonstrated a dose-dependent increase in IFN-γ production in cultures treated with IL-18, compared with spontaneous cultures. IFN-γ measured at time 0 was 229 ± 162 pg/mL, which increased over 4 days of incubation by 10-fold (2317 ± 626 pg/mL) in spontaneous cultures. Compared with spontaneous cultures, IL-18 increased IFN-γ production in the infected PBMC at each concentration tested. A 2.8-fold increase in IFN-γ was observed at 1 nM IL-18 (6503 ± 1132 pg/mL), and a maximum 3.4-fold increase was obtained at 5 nM IL-18 (7788 ± 1578 pg/mL).

We assessed the HIV-1–infected PBMC cultures for a possible toxic effect of IL-18. The presence of 5 nM IL-18 in 4-day cultures did not affect the proliferation of cells, compared with that in 4-day spontaneous cultures, as assessed using metabolically induced formazan production (see Materials and Methods) in 3 separate experiments.

**Exogenous IL-18 inhibits p24 antigen levels and induces IFN-γ production in PBMC infected with T-tropic HIV-1.** The effect of IFN-γ on HIV-1 production may depend on viral tropism. Several reports have demonstrated an HIV-1–inhibitory effect of IFN-γ in infected monocyte-derived macrophages, but an inhibitory effect was not observed in infected lymphocytes [32, 33]. Therefore, we examined the effect of IL-18 on HIV-1 production in PBMC infected with T-tropic HIV-1 (figure 2A). After PBMC were infected with T-tropic HIV-1 for 4 days, p24 antigen increased from 58 ± 13 pg/mL at time 0 to 58 ± 13 pg/mL at time 0.

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**Figure 1.** Effect of interleukin (IL)–18 added to peripheral blood mononuclear cells (PBMC) after infection with monocyte (M)–tropic human immunodeficiency virus type 1 (HIV-1). After infection with M-tropic HIV-1, time 0 (T = 0) samples were obtained, and PBMC were cultured without (spontaneous [spont]) or with IL-18. Concentrations of IL-18 are shown under the horizontal axes. After 4 days, concentrations of p24 (A) and interferon (IFN)–γ (B) were determined in the same cultures. Data (mean ± SEM) were derived using PBMC from 6 donors (A) and from 3 of these 6 donors (B). *P < .05; **P < .01; ***P < .001, compared with spontaneous cultures.

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**Figure 2.** Effect of interleukin (IL)–18 added to peripheral blood mononuclear cells (PBMC) after infection with T lymphocyte (T)–tropic human immunodeficiency virus type 1. PBMC were infected with T-tropic virus, time 0 (T = 0) samples were obtained, and PBMC were cultured without (spontaneous [spont]) or with IL-18. Concentrations of IL-18 are shown under the horizontal axes. After 4 days, concentrations of p24 (A) and interferon (IFN)–γ (B) were determined in the same cultures. Data (mean ± SEM) were derived using PBMC from 7 donors. *P < .05; **P < .01; ***P < .001, compared with spontaneous cultures.
PBMC infected with M- or T-tropic HIV-1. An inverse relationship between the degree of HIV-1 inhibition and IFN-γ production suggested a suppressive effect of IL-18 through intermediate production of IFN-γ. In figure 3A, we compared mean p24 antigen levels with the mean IFN-γ concentrations obtained in PBMC infected with M-tropic virus and cultured in medium alone (spontaneous) or exposed to different concentrations of IL-18 (data obtained from the 3 donors with detectable IFN-γ shown in figure 1). The correlation coefficient for this relationship was \(-.91\) \((P = .01)\), which indicates a significant but inverse relationship between HIV-1 production and IFN-γ levels in infected PBMC.

Figure 3B depicts the correlation of mean p24 antigen and mean IFN-γ concentrations obtained in IL-18–treated or –untreated PBMC infected with T-tropic HIV-1 (data obtained from figure 2). Although this relationship did not achieve statistical significance \((r = -.43; P = .19; n = 7)\), an inverse relationship between p24 levels and IFN-γ production was again observed.

Neutralization of endogenous IFN-γ reverses the IL-18 inhibitory effect. Since we observed an inverse correlation between IL-18–mediated HIV-1 inhibition and IFN-γ synthesis, we examined the possibility that IL-18–induced IFN-γ accounted, in part, for the HIV-1 inhibitory effect. As shown in figure 4, spontaneous p24 production in 4-day PBMC cultures infected with M-tropic virus was 2913 ± 963 pg/mL. The addition of 5.0 nM IL-18 alone reduced p24 production to 625 ± 360 pg/mL, a mean 82% inhibition compared with that in spontaneous cultures.

As shown in figure 2B, IFN-γ production was assessed in PBMC cultures shown in figure 2A. Cultures exposed to IL-18 had a dose-dependent increase in IFN-γ production, compared with that in untreated cultures. At time 0, cultures contained 55 ± 26 pg/mL IFN-γ, which increased 20-fold \((1088 ± 379\) pg/mL in the PBMC incubated in medium alone (spontaneous cultures). The addition of various amounts of IL-18 increased IFN-γ production at each concentration tested, with a 2.1-fold increase at 1 nM IL-18 \((2280 ± 626\) pg/mL) and a maximum 3.8-fold increase at 5 nM IL-18 \((4140 ± 482\) pg/mL), compared with increases in spontaneous cultures.

Comparison of HIV-1 production and IFN-γ synthesis in PBMC infected with M- or T-tropic HIV-1. An inverse relationship between the degree of HIV-1 inhibition and IFN-γ production suggested a suppressive effect of IL-18 through intermediate production of IFN-γ. In figure 3A, we compared mean p24 antigen levels with the mean IFN-γ concentrations obtained in PBMC infected with M-tropic virus and cultured in medium alone (spontaneous) or exposed to different concentrations of IL-18 (data obtained from the 3 donors with detectable IFN-γ shown in figure 1). The correlation coefficient for this relationship was \(-.91\) \((P = .01)\), which indicates a significant but inverse relationship between HIV-1 production and IFN-γ levels in infected PBMC.

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cultures. The presence of anti–IFN-γ antibody in addition to IL-18 in the cultures resulted in dose-dependent reversal of the IL-18 inhibitory effect. Combined IL-18 and anti–IFN-γ antibody at 0.5 or 2.0 μg/mL resulted in 4-day p24 antigen concentrations of 1092 ± 848 and 2626 ± 767 pg/mL, respectively. The 82% maximum IL-18 inhibitory effect observed in the absence of anti–IFN-γ antibody was reduced to 65% and 10% (P < .05) at 0.5 and 2.0 μg/mL of anti–IFN-γ antibody, respectively. A nonimmune isotype control antibody was also added to IL-18–containing cultures but did not affect IL-18–induced p24 inhibition.

**Pretreatment with IL-18 2 days before HIV-1 infection inhibits p24 production in PBMC.** We performed experiments to determine whether IL-18 inhibited early events associated with viral infection of PBMC. PBMC isolated from separate donors were incubated in medium alone or in the presence of 1.0 or 5.0 nM IL-18 for 48 h before infection. After PBMC were infected and washed, cells from each of the 3 conditions were resuspended in fresh medium. Time 0 aliquots were obtained and frozen. Infected PBMC from each condition were cultured without IL-18 for 4 days. As shown in figure 5A, after 4 days of incubation, p24 antigen increased 11.6-fold (934 ± 280 pg/mL) in cultures conducted in medium alone (spontaneous), compared with p24 amounts (81 ± 12 pg/mL) in time 0 cultures. On day 4, compared with spontaneous cultures, exposure to 1.0 and 5.0 nM IL-18 resulted in 43% and 64% HIV-1 inhibition, respectively (final concentrations, 568 ± 21 and 389 ± 12 pg/mL, respectively). In figure 5B, IFN-γ concentrations were measured in the same cultures shown in figure 5A. IFN-γ production at time 0 was 32 ± 11 pg/mL, which increased 16.3-fold to 527 ± 76 pg/mL in the spontaneous cultures. Preincubation with 1.0 and 5.0 nM IL-18 during the 2 days before infection increased 4-day IFN-γ production by 3.6-fold (1918 ± 28 pg/mL) and 5.1-fold (2678 ± 306 pg/mL), respectively, compared with spontaneous cultures.

**Effect of prolonged exposure to IL-18 on p24 antigen and IFN-γ production in infected PBMC cultures.** The results shown in figure 5 demonstrated an early inhibitory effect of IL-18 exposure on HIV-1 production in PBMC. Therefore, these studies were extended to investigate the effect of prolonged IL-18 exposure in HIV-1–infected PBMC. As shown in figure 6A, after 4 days of incubation, supernatants obtained from the PBMC cultures exposed to medium alone (spontaneous) contained 2034 ± 1091 pg/mL p24 antigen, which was a 16-fold increase over levels in time 0 cultures (125 ± 25 pg/mL). One or 5 nM IL-18 reduced p24 production by 37% (1333 ± 826 pg/mL) or 58% (933 ± 557 pg/mL), respectively, compared with spontaneous cultures.

On day 7 of incubation, the p24 concentration in the spontaneous cultures further increased by 19-fold (37,632 ± 10,990 pg/mL), compared with levels in the 4-day spontaneous cultures. IL-18 dose dependently reduced p24 production by 32% (25,583 ± 9744 pg/mL) at 1.0 nM, and a maximum 46% reduction (20,219 ± 8803 pg/mL) was obtained using 5.0 nM IL-18. After 10 days, the p24 concentration in the spontaneous cultures had increased only 1.7-fold, compared with the level in 7-day spontaneous culture (65,022 ± 15,353 pg/mL). In these cultures, IL-18 dose dependently reduced p24 production by 40% (39,285 ± 8800 pg/mL) at 1.0 nM and by a maximum of 52% (31,268 ± 8708 pg/mL) at 5.0 nM.

After 13 days of incubation, spontaneous p24 antigen was essentially unchanged from the day-10 level (1.1-fold increase). IL-18–induced p24 reduction was also similar to that at day 10 (40% at 1 nM and 52% at 5 nM, respectively). The presence of 0.002 nM IL-18 did not significantly affect spontaneous HIV-1 production at each time point examined.

Figure 6B shows IFN-γ concentrations measured in the same culture supernatants as in figure 6A. On day 4, PBMC contained 548 ± 77 pg/mL IFN-γ in cultures conducted in medium alone, a 2.4-fold increase, compared with time 0 cultures (229 ± 162 pg/mL). IL-18 increased IFN-γ production by 51-
Figure 6. Effect of prolonged presence of interleukin (IL–18) in peripheral blood mononuclear cells (PBMC) infected with human immunodeficiency virus type 1 (HIV-1). After infection with monocyte-tropic HIV-1, PBMC were washed and cultured without (spontaneous) or with IL–18. The concentrations of added IL–18 are indicated by symbols: \( \bullet \), 0.002 nM; \( \square \), 1 nM; \( \bigcirc \), 5 nM. IL–18 was added on days 0, 4, 7, and 10. p24 Antigen (A) and interferon (IFN)–\( \gamma \) (B) were determined on the days indicated under the horizontal axes. The inset figure in panel A shows p24 antigen obtained 4 days after infection. Data (mean ± SEM) were derived using PBMC from 6 donors.

IL–18 decreases cell surface expression of CD4. Since pretreatment with IL–18 for 2 days before infection with virus inhibited 4-day spontaneous HIV-1 production (figure 5), early infection–associated events were likely affected by IL–18. Productive infection of cells with HIV-1 involves the initial co-binding of the virus to the cell surface CD4 molecule and to one of the \( \alpha - \) or \( \beta - \) chemokine coreceptors [34, 35]. The chemokine coreceptor used by M-tropic virus is CCR-5 [36]. Since the results shown in figure 5 were obtained using an M-tropic HIV-1, we tested the hypothesis that IL–18–induced inhibition was associated with reduced cell surface expression of CD4 or CCR-5. We analyzed cell surface expression of CD4 and CCR5 in PBMC by flow cytometry. Uninfected PBMC were incubated for 4 days in the absence or presence of 5 nM IL–18. As shown in figure 7, the percentage of cells positive for CD4 (FITC) in PBMC cultured in the absence of IL–18 (control) was 17%. PBMC incubated in the presence of IL–18 reduced the percentage of CD4-positive cells to 8%, a 53% reduction. However, the percentage of CCR5-positive cells was not significantly affected by IL–18 (data not shown). The PBMC aliquot incubated with isotype-matched nonimmune antibody showed no significant antibody-associated FITC or PE binding (data not shown).

Discussion

We demonstrated that exogenous IL–18 inhibited HIV-1 production in acutely infected PBMC 4-day cultures. The inhibitory effect of IL–18 was observed in PBMC infected with either M- or T-tropic HIV-1. Since IL–18 generates a Th1 type response [13] and induces IFN–\( \gamma \) production in vitro and in vivo [37, 38], we investigated the IL–18–exposed infected PBMC for IFN–\( \gamma \) production.

In PBMC infected with M- or T-tropic virus, there was an inverse correlation between HIV-1 production and IFN–\( \gamma \) synthesis. However, this inverse correlation was statistically significant only for PBMC infected with the M-tropic strain. Several reports have documented an HIV-1–suppressive effect of exogenous IFN–\( \gamma \) in monocyte-derived macrophages and in PBMC infected with M-tropic virus [30, 32, 39–42]. The inverse association between p24 levels and IFN–\( \gamma \) production that we observed in PBMC infected with M-tropic HIV-1 is consistent with these reports.

IFN–\( \gamma \) was not detectable in infected PBMC from 3 of the 6 donors whose cells were infected with M-tropic HIV-1. We have previously observed variability in IFN–\( \gamma \) production in PBMC from certain individuals whose cells produced an undetectable level of IFN–\( \gamma \). The inability to detect IFN–\( \gamma \) by ECL in 3 of these 6 PBMC donors does not exclude the biological
The effect of IFN-γ on HIV-1 production may depend on viral tropism. Previous studies showed an HIV-1-inhibitory effect of exogenous IFN-γ in monocyte-derived macrophages infected with M-tropic HIV-1 but no inhibitory effect of IFN-γ in lymphocytes infected with T-tropic HIV-1 [32, 33]. We detected a difference in the maximum IL-18 inhibitory effect in PBMC infected with M- or T-tropic HIV-1 (72% vs. 57%, respectively). Since IFN-γ may mediate IL-18–induced inhibition of HIV-1, these results suggest an increased component of antiretroviral activity due to IFN-γ in cells infected with M-tropic HIV-1. However, in our studies of M- and T-tropic HIV-1 production in PBMC, we did not infection PBMC from the same donors with both types of viruses. Therefore, the differential inhibitory effect of IL-18 in PBMC infected with M- or T-tropic virus may have resulted from donor variability.

In addition to IFN-γ–dependent IL-18 inhibition of HIV-1 replication in PBMC, IL-18 effects independent of IFN-γ production are possible. For example, IL-18 has been shown to protect mice against acute herpes simplex virus type 1 infection via both IFN-γ–dependent and –independent pathways [45]. IL-18-deficient mice showed a profound attenuation in the Th1 type response, but this defect was not observed in mice deficient in the IFN-γ receptor [46], which indicates an IFN-γ–independent IL-18 effect. Anti-IL-18 antibody protected IFN-γ–deficient mice against lethal endotoxemia [47], which further indicates IL-18 effects that are both IFN-γ–dependent and –independent.

What explains the discrepancy between IL-18 stimulation of HIV-1 production in chronically infected U1 monocytic cells [43] and in Hut 78 lymphocytic cells [48] but the reduced HIV-1 production in acutely infected PBMC observed in our studies? It is possible that IL-18 stimulates virus production in chronically infected cells that do not produce IFN-γ or β-chemokines, whereas these natural HIV-1 antagonists are produced in PBMC. In fact, IFN-γ was undetectable in the chronically HIV-1–infected monocytic cell line and in the infected Hut 78 T cell line [43, 48].

A similar dichotomy (opposite effects in acute vs. chronic HIV-1 infection) has been observed using LPS or TNF-α [16, 18–20, 30]. HIV-1 production was increased in chronically inf-
fected U1 cells stimulated with TNF-α or LPS [16–18] but was reduced in acutely infected primary cells exposed to TNF-α or LPS [19–21, 30, 49]. The TNF-α– or LPS-induced inhibition of HIV-1 production in acutely infected primary cells occurred through blockade of early infection-associated events in these reports. Exposure to IL-18 after HIV-1 infection of Hut 78 T cells increased virus production, but incubation of the cells with IL-18 before infection resulted in reduced HIV-1 production [48]. This report extends the concept of IL-18–inhibitory effects during early infection to an infectible cell line. For this reason, we examined whether the time of exposure to exogenous IL-18 relative to infection with HIV-1 affected virus production.

Two-day exposure of PBMC to IL-18 before infection inhibited HIV-1 production in the 4-day PBMC cultures (figure 5A). This inhibitory IL-18 effect was observed despite the absence of IL-18 during the 4 days of culture after infection with virus. Furthermore, compared with inhibition in spontaneous cultures, the maximum inhibition in the PBMC exposed to IL-18 before infection (64%) was comparable to that observed in PBMC infected with the same M-tropic virus and cultured in the presence of IL-18 throughout the 4-day incubation (72%). In contrast, the maximum inhibitory effect of IL-18 was not enhanced in prolonged 13-day PBMC cultures infected with this virus. In these studies, IL-18 was present immediately after infection and throughout the 13-day culture. The inhibition of p24 antigen production in the presence of 5 nM IL-18 was 58%, 46%, 52%, and 52% on days 4, 7, 10, and 13, respectively, compared with the p24 in medium alone (spontaneous) cultures at each time point (figure 6).

Considered together, these results imply a preferential suppressive effect of IL-18 during the early stages of infection. Several mechanisms of HIV-1 inhibition induced by TNF-α and LPS have been described that involve inhibition of early infection-associated events. These include blockade of viral genome nuclear integration, augmented β-chemokine secretion, and reduced CCR-5 coreceptor expression [20, 21, 49, 50]. Another possible early event affected by IL-18 is modulation of the HIV-1 receptor CD4 expression, since previous reports showed that HIV-1 inhibition by exogenous IFN-γ involved down-regulation of CD4 expression in PBMC [39, 51, 52]. Using flow cytometry analysis, we showed that IL-18 reduced cell surface expression of CD4 in the PBMC. However, IL-18 did not affect cell surface CCR5 expression. Therefore, we speculate that IL-18, like TNF-α and LPS, inhibits the early stages of HIV-1 infection through reduced CD4 expression.

In conclusion, these studies demonstrate that IL-18 inhibited HIV-1 production in PBMC and that this inhibitory effect was associated with increased IFN-γ production. For PBMC infected with M-tropic virus, experiments incorporating a neutralizing anti–IFN-γ antibody indicated a functional inhibitory role for IL-18–induced IFN-γ. A preferential inhibitory effect of IL-18 during the early stages of PBMC infection was suggested, since exposure to IL-18 only before infection resulted in viral inhibition. Flow cytometry studies revealed a likely mechanism for IL-18–induced HIV-1 inhibition during early infection. Since IL-18–exposed PBMC demonstrated reduced cell surface CD4 expression, virus entry into the cells may have been blocked by this mechanism.

All currently approved antiretroviral drugs target the virus-specific aspartyl protease or the viral reverse transcriptase [53], and viral mutations can result in resistance to these drugs. On the other hand, targeted modulation of host factors presents the possibility of therapy unaffected by viral mutation. IL-18, an inducer of the Th1 type response, may be a therapeutic candidate to inhibit HIV-1 in infected patients.

Acknowledgments

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References


