Elevated Levels of Circulating Interleukin-18 in Human Immunodeficiency Virus-Infected Individuals: Role of Peripheral Blood Mononuclear Cells and Implications for AIDS Pathogenesis

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Originally identified as the gamma interferon-inducing factor, interleukin-18 (IL-18) was rediscovered as a proinflammatory cytokine related to the IL-1 family of cytokines that plays an important role in both innate and adaptive immune responses against viruses and intracellular pathogens. Despite its importance in inducing and regulating immune responses, relatively little is known about its production in HIV infection. We report here significantly (P < 0.05) elevated levels of this cytokine in the sera of human immunodeficiency virus (HIV)-infected/AIDS patients compared to those of HIV-seronegative healthy persons. Surprisingly, the peripheral blood mononuclear cells (PBMC) from HIV-infected/AIDS patients were compromised in the ability to upregulate IL-18 gene expression and produce this cytokine with and without lipopolysaccharide (LPS) stimulation. A significant positive correlation (P < 0.05) existed between the concentration of IL-18 in serum and its production from PBMC of HIV-seronegative healthy individuals but not those of HIV-infected/AIDS patients. Furthermore, the patients’ PBMC expressed relatively reduced levels of activated caspase-1 constitutively as well as in response to LPS stimulation. Our data suggest the involvement of transforming growth factor beta (TGF-β) in suppressing the production of IL-18 from PBMC. (i) In vitro studies it suppressed the production of IL-18 from PBMC. (ii) Its levels were significantly higher in the plasma of patients compared to that of control subjects. (iii) A significant negative correlation existed between the concentrations of TGF-β in plasma and of IL-18 in serum of the patients. The elevated levels of IL-18 in the serum of HIV-infected individuals may contribute to AIDS pathogenesis, whereas its compromised production from their PBMC in response to stimuli may reduce their innate defense to opportunistic intra-cellular pathogens.

AIDS in humans is the ultimate outcome of an infection with human immunodeficiency virus type 1 (HIV-1). This viral infection causes several immune abnormalities in the infected host, rendering him or her unable to control the infection. It also makes the host unusually susceptible to a variety of opportunistic infections. These immune abnormalities occur, at least in part, as a result of the deregulated expression of several immunologically important cytokines (6, 9, 36, 37). For example, aberrant production of tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), interleukin-12 (IL-12), and IL-15, etc., has been well documented in HIV-infected/AIDS patients (1, 7, 8, 21). Optimal production of these cytokines is essential not only for innate host resistance to pathogens but also for the induction, amplification, and maintenance of pathogen-specific immunity. The qualitative nature of the immune response is also determined by the type of cytokines induced in the host, i.e., whether they are of T-helper 1 (TH1) or of TH2 type. A predominance of TH2-type cytokines has been reported in HIV-infected/AIDS patients and is believed to contribute to the pathogenesis of AIDS (36). Finally, cytokines induce and/or activate transcription factors directly or indirectly, which may regulate HIV-1 replication in human cells. Despite the ability of IL-18 (see below) to enhance innate immunity, regulate TH1- and TH2-type immune responses, and enhance HIV-1 replication, little is known about its expression in HIV-infected/AIDS patients.

Previously known as the gamma interferon (IFN-γ)-inducing factor, IL-18 was rediscovered as a novel cytokine that plays an important role in promoting TH1 responses by its ability to induce IFN-γ from T and natural killer (NK) cells (24, 25, 28; reviewed in references 11 and 26). This pleiotropic cytokine is produced by activated macrophages, dendritic cells, Kupffer cells, keratinocytes, and enterocytes as well as by the adrenal cortex and neurohypophysis. These cells usually express the IL-18 gene and protein constitutively and increase their expression in response to stress, infection, cold, and lipopolysaccharide (LPS). IL-18 and IL-1β have similar tertiary structures (all β-pleated folded forms). Furthermore, both these cytokines are produced as inactive precursors without a signal...
peptide and require proteolytic cleavage by the IL-1β-converting enzyme (ICE or caspase-1) to become mature biologically active molecules, which are then readily released from the cells (14). ICE cleaves the 193-amino-acid precursor form of IL-18 on the carboxyl side of aspartate 36 (14, 17). IL-18 uses a unique heterodimeric receptor, which comprises the ligand-binding α chain and the signal-transducing β chain, and belongs to the IL-1 receptor superfamily. IL-18 potentiates innate immunity by increasing the cytolytic potential of NK and T cells and regulates adaptive immune responses to pathogens (4, 29). Its protective role for the host against intracellular pathogens, including viruses, has been well documented (11, 31). Being a proinflammatory cytokine, its deregulated production has also been implicated in several chronic autoimmune disorders (23, 44). Induction of IL-18 production seems to be an integral part of the host’s innate response to viral pathogens (26, 32, 41). However, little is known about the regulation of this cytokine in HIV-1 infection. This issue is addressed in this report.

MATERIALS AND METHODS

Patients. Peripheral blood was obtained for serum collection from 70 HIV-infected/AIDS patients of both genders (50 males and 20 females) from local AIDS clinics after their written informed consent. The clinical characteristics of the patients were: age, 28 to 65 years (median = 44); CD4⁺ T cells, 16 to 77 per μl (median = 242); viral load, 10⁻⁷⁰ to 10⁻⁶⁶ (median = 10⁻²⁴); infection duration, 2 to 10 years (median = 6 years).

The viral load in plasma (i.e., number of HIV-1 RNA copies per ml) was determined using a colorimetric reverse transcription-PCR-based commercial kit (HIV-1 monitor test; Roche Diagnostic Systems, Branchburg, N.J.). The patients’ CD4⁺ T-cell counts in the peripheral blood (number of cells per microliter) were determined by flow cytometry using the whole blood lysis method (BD Bioscience, Mississauga, Ontario, Canada). All the patients except six were receiving highly active antiretroviral therapy (HAART) that comprised at least one protease inhibitor (saquinavir, ritonavir, or indinavir) and one or two reverse transcriptase inhibitors ( stavudine [D4T], lamivudine [3TC], zidovudine [AZT], or didanosine). Blood samples from 28 age-matched HIV-seronegative healthy persons were used as controls.

Separation of serum and PBMC. For the collection of serum samples, peripheral blood was obtained in vacuum tubes without any additive. The blood was allowed to clot at room temperature for 4 to 6 h. Thereafter, clear straw-colored serum was obtained by centrifugation, divided into aliquots, and stored at −80°C until used. Peripheral blood mononuclear cells (PBMC) were obtained from the peripheral blood of some of these patients (detailed in individual experiments). For this purpose, blood was collected in heparinized vacuum tubes by centrifugation over Ficol-Hyphaque (Pharmacia, Montreal, Ontario, Canada) as described earlier (2). The buffy coat at the interface of Ficol-Hyphaque and blood was collected, washed, and resuspended in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum and antibiotics (culture medium) as described previously (2).

PBMC cultures. PBMC (2 × 10⁶) were incubated in triplicate in the wells of a round-bottom 96-well microculture plate in the culture medium with or without the presence of LPS (from Escherichia coli O55:B5; Sigma, St. Louis, Mo.; catalog no. L5629; 10 μg/ml). Supernatants from these microcultures were collected 12, 24, 48, and 72 h later, filtered through low protein binding 0.45-μm-pore-size filters (Millipore; Millipore, Bedford, Mass.), divided into aliquots, and stored at −80°C.

ELISA for IL-18. The concentrations of IL-18 were determined in serum samples and culture supernatants using a commercial enzyme-linked immunosorbent assay (ELISA) kit (MBL, Naka-ku, Nagoya, Japan), which has a detection limit of 12.5 pg/ml. The kit is based upon a sandwich ELISA that utilizes two monoclonal antibodies directed against two different epitopes of human IL-18. One antibody is coated onto the wells of the microculture plate to capture IL-18 from biological fluids, and the second antibody is conjugated with horseradish peroxidase. After being washed, the chromogenic substrate tetramethylbenzidine was added and the optical density was measured in an ELISA reader (Easy Reader EAR 400/AT; STL Lab-instruments, Salzburg, Austria) at 450 nm as directed by the manufacturer.

Biological activity of IL-18. The biological activity of IL-18 in the culture supernatants was tested by its ability to induce IFN-γ in mouse splenocytes after their suboptimal stimulation with concanavalin A (ConA) (0.2 μg/ml; Sigma) as described previously (39). Briefly, the culture supernatants were concentrated (10 ml; Amicon, Beverly, Mass.), added to a 50-μl volume to the ConA-pretreated 5 × 10⁵ mouse splenocytes. After 24 h, the quantity of IFN-γ secreted into the culture supernatants was measured with a commercial ELISA kit (R & D Systems, Minneapolis, Minn.). The specificity of the IFN-γ production to IL-18 in this assay was confirmed by preincubating the supernatants with an IL-18-neutralizing monoclonal antibody (clone 2F7; R&D Systems) and 30 min isotype-matched (IgG1κ) control antibody (1 μg/ml; Serotec, Toronto, Ontario, Canada).

Determination of TGF-β. The contents of transforming growth factor beta (TGF-β) in the sera and plasma samples were determined with a commercial ELISA kit (TGF-β1 Emax immunoassay system; Promega, Madison, Wis.) by following the manufacturer’s recommendations. The kit determines the biologically active form of the cytokine in an antibody sandwich format. The first (capture) antibody, used to coat the ELISA plates, is a mouse monoclonal antibody that is specific for the activated form of TGF-β1. For determining total TGF-β1, the samples were first treated with acid and neutralized as detailed in our previous publication (47).

Western blotting. The expression of different proteins within PBMC was analyzed by Western blotting as described in our earlier publications (3, 46). Briefly, 2 × 10⁶ PBMC were incubated in the culture medium with and without treatment as detailed in individual experiments. Twenty-four hours after incubation, cells were washed with phosphate-buffered saline and lyzed in a lysis buffer containing Tris HCl (pH 6.8; 50 mM), sodium dodecyl sulfate (SDS) (2%), leupeptin (1 mg/ml), phenylmethylsulfonyl fluoride (1 mM), and pepstatin (1 mg/ml). The lysates were clarified by centrifugation at 14,000 × g for 15 min. Protein concentrations were determined in the lysates by using a commercial kit (Bio-Rad, Richmond, Calif.). Forty micrograms of the lysate proteins was mixed with 2× SDS-polyacrylamide gel electrophoresis (PAGE) sample loading buffer containing 1 mM dithiothreitol, boiled, run on SDS–12% PAGE gels, and electroblotted onto polyvinylidene difluoride membranes (Millipore; Millipore). After blocking of the membranes in 1% casein for 1 h at room temperature, they were incubated on a shaker with protein-specific antibodies, i.e., antihuman IL-18 (Clone 52713.11; R & D Systems), anti-caspase-1 (clone B24; 2; Pharmingen BD, Mississauga, Ontario, Canada), or anti-β-actin (Sigma), at room temperature for 1 h. The protein bands were revealed by autoradiography by using biotinylated goat antimouse antibodies and a commercial chemiluminescent kit (Vectastain ABC-Amp; Vector Labs, Burlington, Calif.). Individual bands on the X-ray films were quantified by densitometry.

Fluorescence-activated cell sorter analysis. The percentage of PBMC expressing CD14 on their surface was determined by membrane immunofluorescence. For this purpose, 0.5 × 10⁶ PBMC were incubated with phycoerythrin-conjugated antihuman CD14 (Pharmingen BD) on ice for 45 min. After being washed with phosphate-buffered saline, cells were resuspended in 2% paraformaldehyde and analyzed by flow cytometry.

RPA. IL-18 transcripts were measured by using RNase protection assay (RPA). For this purpose, total cellular RNA was extracted from 10⁶ PBMC, using Trizol reagent (Life Technologies, Burlington, Ontario, Canada), which is essentially a monophasic solution of phenol and guanidine isothiocyanate. RNA was stored as an ethanol precipitate at −80°C until used. High-specific-activity antisense probe for detecting IL-18 mRNA was prepared by in vitro transcription with a commercial kit (Promega) and DNA-dependent RNA polymerase from SP6 bacteriophage. The template vector pGEM-3zf(+) containing IL-18 cDNA has been previously described and was linearized with ScaI (32). One million counts per minute of the 3²P-labeled antisense probe was hybridized overnight at 56°C with the total cellular RNA and treated with RNase A using a kit from Pharmingen BD (RiboQuant). The reaction mixture was run on a 5% denaturing polyacrylamide gel. The protected probe was revealed and quantitated by phosphorimaging. For normalization, the transcripts of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were also determined along with the IL-18 transcripts. The antisense probe for GAPDH was prepared from a commercially available template (Pharmingen BD) as described above for IL-18; however, the DNA-dependent RNA polymerase from the T3 bacteriophage was used. The ratio between IL-18 and GAPDH transcripts was determined and compared between different PBMC samples by the ImageQuant software of Molecular Dynamics (Sunnyvale, Calif.).

Statistical analysis. The viral loads (number of copies of HIV-1 RNA per ml of plasma) were log₁₀ transformed. The differences between means of different parameters were compared using Student’s unpaired t test. The forward correlation between two variables was determined using Pearson’s method. The for-
ward stepwise multiple linear regression between IL-18 (independent variable) and dependent variables (CD4⁺ T-cell count, viral load, CD8⁺ T-cell counts, and CD4⁺/CD8⁺ T-cell ratios) was determined by standard methods. For all analyses, statistical software SPSS (Statistical Package for Social Sciences; Graph Pad Inc., Chicago, Ill.) was used. P values ≤0.05 were considered significant.

RESULTS

IL-18 concentration in the sera of HIV-infected/AIDS patients. We measured IL-18 protein in the sera of 70 HIV-infected/AIDS patients and 28 HIV-seronegative individuals. As shown in Fig. 1, the average IL-18 concentration was significantly higher in these patients’ sera compared to that of the control sera (364.60 ± 35.11 versus 113.50 ± 19.45 pg/ml; P = 0.0002). It was approximately threefold higher in the patients’ sera. When we grouped patients on the basis of duration of their infection, the patients with 3 to 6 years of duration showed the highest levels (Fig. 2), although all three groups of patients had significantly (P < 0.05) higher concentrations of IL-18 in serum compared to those of the control samples. No significant correlation (P > 0.05) was found between levels of IL-18 in serum and CD4⁺ T-cell counts, viral load in plasma, CD8⁺ T-cell counts, or CD4⁺/CD8⁺ T-cell ratios (P > 0.05; data not shown). Furthermore, we tested these parameters for multiple linear regression with levels of IL-18 in serum and found no statistically significant (P > 0.05) results (data not shown). We were also unable to find any significant difference (P > 0.05; data not shown) in mean levels of IL-18 in the sera of patients who were or were not undergoing HAART. We made this comparison because HAART has been reported to inhibit cytokine production in human PBMC (43). Since monocytes and macrophages are the main cell types that produce IL-18, we sought to determine whether levels of IL-18 in serum correlated with peripheral blood CD14⁺ cell numbers. No significant (P > 0.05) correlation existed between these two parameters both in HIV-infected/AIDS patients and HIV-seronegative control individuals. The average concentration of IL-18 was approximately threefold higher in the patients’ sera despite a nonsignificant decrease in the percentage of CD14⁺ cells in their PBMC (Fig. 1B). No correlation was found between these concentrations and absolute numbers of these cells in these subjects as well (data not shown).

Production of IL-18 from PBMC. In order to determine whether increased concentrations of IL-18 in the serum of infected individuals were due to an increased production of this cytokine from their PBMC, we investigated its production from these cells from randomly selected control (seven) and HIV-infected (eight) individuals.

For this purpose, 2 × 10⁵ PBMC from each donor were

FIG. 1. Concentration of IL-18 in the sera of HIV-infected/AIDS patients. IL-18 concentrations were determined in serum samples by using a commercial ELISA kit. (A) IL-18 concentrations in individual sera. A dot indicates an individual serum concentration and the horizontal line in each column indicates group mean. (B) Average ± standard error concentrations of IL-18 in the sera of HIV-infected/AIDS patients and control subjects and the average ± standard error percentages of CD14⁺ monocytes in PBMC. N and P, HIV-seronegative healthy and HIV-infected donors, respectively. The average concentrations of IL-18 differed significantly between the two groups of donors (P = 0.0002).

FIG. 2. Concentrations of IL-18 in serum and duration of HIV infection. Average ± standard error concentrations of IL-18 in serum of the three groups of patients with different durations of infection. Note the highest serum concentration in the group that has been infected for <3 years, 3 to 6 years, and >6 years of HIV infection, respectively.
incubated in 200 μl of the culture medium in triplicate in 96-well microculture plates with and without the addition of LPS. LPS is known to increase the production of IL-18 in human PBMC (14) and, therefore, was used as a stimulus in these experiments. The culture supernatants from these microcultures were assayed for IL-18 concentrations as described in Materials and Methods. Results from these experiments are shown in Fig. 3. At each time point tested, both LPS-stimulated and unstimulated PBMC from HIV-infected/AIDS patients produced less IL-18 than their control counterparts. As expected, LPS enhanced the production of this cytokine in the cells from both control and HIV-infected individuals. This enhancement was more pronounced in the PBMC of HIV-infected subjects compared to the cells from control donors. Despite this relatively greater enhancement of IL-18 production, the LPS-stimulated PBMC from HIV-infected/AIDS patients produced significantly less IL-18 even when compared to unstimulated PBMC from HIV-seronegative controls.

We also tested these culture supernatants for IL-18 bioactivity, i.e., its ability to induce IFN-γ production from ConA-stimulated mouse splenocytes, as described in Materials and Methods. As shown in Table 1, the culture supernatants from the PBMC of HIV-infected patients induced significantly lower (P < 0.05) levels of IFN-γ in mouse splenocytes than the control supernatants. We further determined whether there was any correlation between in vitro production of IL-18 from PBMC and the concentrations of this cytokine in sera of control and HIV-infected individuals. As shown in Fig. 4, these two parameters correlated significantly (P < 0.05) in healthy control subjects but not in HIV-infected/AIDS patients.

Since IL-18 exists as an inactive precursor in the cytoplasm and is converted into a biologically active secreted (mature) form after cleavage by caspase-1 (14), we compared intracellular production of IL-18 in the PBMC from HIV-infected/AIDS patients and control subjects by Western blotting. The IL-18-specific antibody used in these blots can recognize both the precursor and mature forms of this cytokine. However, we could detect the precursor form only in the lysates of human PBMC (Fig. 5), which is consistent with results of a previously published report (33). As shown in Fig. 5, the patients’ PBMC expressed significantly less (P < 0.05) IL-18 in their cytoplasm compared to those of the control cells. These data also reveal less production of the cytoplasmic form of the cytokine in the patients’ PBMC upon stimulation with LPS. These results are in accord with those obtained with secreted IL-18.

Expression of the IL-18 gene. In order to determine whether a decreased production of IL-18 in the patients’ PBMC was due to decreased expression of the IL-18 gene, we measured the number of IL-18 transcripts in the PBMC by RPA with IL-18-specific antisense probes. The production of transcripts for the GAPDH housekeeping gene was also determined by RPA for normalization. As shown in Fig. 6, the PBMC from the patients had significantly fewer (P < 0.05) numbers of IL-18 transcripts than the control subjects’ PBMC both with and without LPS stimulation.

Taken together, these results strongly suggest that PBMC from HIV-infected/AIDS patients are relatively compromised in their ability to produce IL-18 constitutively upon in vitro culture as well as in response to LPS stimulation.

Activation of caspase-1. As stated above, caspase-1 cleaves the IL-18 precursor protein into a mature secreted form. A

The PBMC of seven control subjects were cultured in vitro with and without the presence of LPS, and their culture supernatants were collected at the indicated time points and assayed for IL-18 with a commercial ELISA kit. Average ± standard error induction of IFN-γ in three replicate cultures of mouse splenocytes is shown.
reduced level of expression and/or activation of caspase-1 may also cause decreased processing and secretion of IL-18 from cells. Therefore, we wanted to know whether a decreased level of production of this cytokine from the patients' PBMC was due to decreased expression and/or activation of this protease.

FIG. 5. Expression of IL-18 in PBMC. The expression of IL-18 in PBMC was determined by Western blotting using an IL-18-specific monoclonal antibody in 75 µg of cellular lysate. (A) Western blot showing the precursor form of IL-18 (24 kDa) in PBMC with (+) and without (−) stimulation with LPS in HIV-infected/AIDS patients (P) and HIV-seronegative healthy control (N) subjects. The mature form of IL-18 (18 kDa) could not be detected on the blots. (B) Densitometric analysis of the blot showing expression of IL-18 after normalization with β-actin. The 1, 2, and 3 in panels A and B represent data from different donors. (C) Average ± standard error expression of IL-18 in the PBMC.

FIG. 6. Expression of the IL-18 gene in PBMC. The expression of IL-18 gene in PBMC was determined by RPA with α-32P-labeled cRNA and normalized with respect to GAPDH gene expression (also determined by RPA). (A) Autoradiograph of the polyacrylamide gel showing protected IL-18 and GAPDH-specific probes in the PBMC of HIV-infected/AIDS patients and control subjects with (+) and without (−) stimulation with LPS. Probe and Y tRNA, migration of the labeled cRNA without incubation with mRNA and protection of yeast tRNA (negative control), respectively. (B) Densitometric analysis of the protected probes. Ratio of protected IL-18 and GAPDH probes for each individual. The numbers 1, 2, and 3 in panels A and B represent data for different donors. (C) Average ± standard error expression of the ratio of IL-18 and GAPDH-specific protected probes (IL-18 gene expression). N and P, HIV-seronegative and HIV-infected donors, respectively.
in these cells. For this purpose, we determined the expression of activated caspase-1 in the PBMC by Western blotting. As shown in Fig. 7, the expression of activated caspase-1 was significantly reduced in the PBMC of the patients compared to that of the PBMC of the controls. These data suggest that a reduced expression of activated caspase-1 may, at least in part, be responsible for the reduced production of IL-18 by the patients' PBMC.

**Effect of TGF-β on IL-18 production from PBMC.** Although there may be several reasons for a decreased level of production of IL-18 from human PBMC, we investigated the effect of TGF-β. This pleiotropic cytokine is known to inhibit the production of several proinflammatory cytokines from human cells, and its unusually higher levels have been reported in the circulation of HIV-infected AIDS patients (5). As shown in Fig. 8, neutralization of TGF-β in the PBMC cultures significantly \((P < 0.05)\) enhanced IL-18 production. Addition of recombinant TGF-β did not decrease the rate of IL-18 production in these experiments, which could be due to saturation of the culture system with this cytokine (either from the culture medium and/or from the PBMC).

**Correlation between TGF-β and IL-18.** After determining that TGF-β can suppress the production of IL-18 from human PBMC cultures, we investigated the status of this cytokine in the circulation of HIV-infected/AIDS patients. For this purpose, we measured the levels of TGF-β in the plasma from these patients. No significant \((P > 0.05)\) differences were observed for the activated form of this cytokine in the plasma between HIV-infected and HIV-seronegative individuals (data not shown). However, the plasma from the infected persons contained significantly higher amounts of total TGF-β than that from the HIV-seronegative individuals (Fig. 9). Furthermore, we observed a significant \((r = -0.50; P = 0.006)\) nega-
tive correlation between the level of TGF-β in plasma of the patients and the concentrations of IL-18 in serum (Fig. 10).

Collectively, these data suggest a role for TGF-β in the compromised ability of PBMC from HIV-infected/AIDS patients to produce IL-18.

**DISCUSSION**

We have demonstrated here elevated levels of IL-18 in the sera of HIV-infected/AIDS patients compared to those of control subjects. The patients’ sera contained, on average, three-fold higher concentrations of this cytokine. The serum concentrations, however, did not show significant ($P > 0.05$) correlation with CD4$^+$ T-cell counts, CD8$^+$ T-cell counts, ratios between these two cell counts, or the viral loads in plasma of the patients. These results are in agreement with those of an earlier report of elevated IL-18 levels in the sera of AIDS patients (42). We do not know if HIV-1 or any of its gene products plays a direct role in the enhanced concentration of this cytokine in the sera of HIV-infected individuals. A close cousin of HIV-1, simian immunodeficiency virus has been shown to stimulate IL-18 production in rhesus macaques (15). Several other viruses and intracellular pathogens are also known to induce IL-18 production both in vivo and in vitro (30–32, 40). An early stimulation of IL-18 production by these pathogens represents an important defense mechanism of the host meant to enhance both its innate and pathogen-specific adaptive immunities and has been shown to play an important role in controlling these infections (11, 12, 31, 41). Not surprisingly, several viruses have evolved strategies to evade this host response by encoding IL-18 binding proteins which bind and inactivate IL-18 (27, 38). Despite its role in potentiating immune responses, increased levels of IL-18 in the circulation of HIV-infected/AIDS patients may also contribute to AIDS pathogenesis (see below).

IL-18 is a proinflammatory cytokine that was originally named IFN-γ-inducing factor because of its ability to induce this interferon from T cells and NK cells (11, 25, 26, 28). By virtue of this capacity, IL-18 plays an important role in the development of TH1 CD4$^+$ T cells. These cells are crucial in inducing cellular immunity against viral and intracellular pathogens. IFN-γ, itself, is also known to induce an antiviral state in human cells, activate macrophages, and promote apoptosis of virus-infected cells by modulating the expression of TNF-related apoptosis-inducing ligand (TRAIL) and TRAIL receptors (34). Not surprisingly, IL-18 has been shown to play a role in protective immune responses against these pathogens (12, 40, 41). However, chronic excessive production of this cytokine has also been implicated in autoimmune phenomena (13, 16, 41). IL-18-induced production of IFN-γ and TNF-α from T cells, NK cells, and macrophages may cause destruction of the central nervous system and other tissues, leading to the manifestations of alevolitis and HIV-associated dementia in these patients. In the context of HIV infections, IL-18 may also play a pathogenic role by enhancing viral replication, as it has been shown in vitro studies to enhance HIV-1 replication in both monocytic and T cells by activating p38 MAPK and NF-κB as well as by inducing the production of TNF-α and IL-6 (19, 35). The elevated levels of IL-18 in HIV-infected/AIDS patients may, at least theoretically, cause enhanced HIV replication in both these cell types. Second, recent studies have shown that IL-18 per se does not induce the differentiation of TH1 CD4$^+$ T cells; it causes the proliferation of IL-12-induced TH1 CD4$^+$ T cells. IL-12 induces the expression of IL-18 receptor on these TH1 cells and hence makes them susceptible to the proliferative effects of IL-18. The receptor does not occur on TH2 CD4$^+$ T cells (45). In the absence of IL-12, IL-18 actually promotes the differentiation of TH2 CD4$^+$ T cells and the induction of immunoglobulin E (IgE) (reviewed in reference 26). Furthermore, IL-18 induces the production of IL-4 and the release of histamine from basophils and mast cells, which constitutively express IL-18 receptor (48). A reduced production of IL-12 in HIV infections has been well documented (8, 20). Thus, IL-18 may, in fact, be promoting...
TH2-type responses and the production of IgE in HIV infection especially when IL-12 levels are decreased in the later stages of the infection. This is supported by the facts that TH2-type responses predominate and there is enhanced production of IgE in HIV-infected/AIDS patients (22, 36, 37). The predominance of TH2-like responses in these infections has been implicated in the pathogenesis of AIDS (9, 36). The elevated levels of IL-18 in the sera of HIV-infected/AIDS patients, shown here, may play a role in promoting TH2-like responses in these patients.

IL-18 is produced mainly by monocytes/macrophages in the human body. Apart from these cell types, Kupffer cells, keratinocytes, dendritic cells, and cells of the adrenal cortex have also been reported to produce this cytokine (reviewed in reference 11). Surprisingly, our results show that despite elevated levels of IL-18 in HIV-infected/AIDS patients, their PBMC are compromised in the ability to produce this cytokine. They not only produced less IL-18 spontaneously upon in vitro stimulation, but also did so after stimulation with LPS, which is well known to upregulate IL-18 production in PBMC. The reduced production of IL-18 from these PBMC was not due to reduced numbers of monocytes, because we did not find any significant difference in the percentages and absolute numbers of monocytes in PBMC from infected versus control subjects. These results are in accord with those of a previous report (10) that described a decreased production of IL-18 and IL-18 mRNA in the PBMC of HIV-infected/AIDS patients compared to those of control subjects. However, He et al. (18) reported a decreased production of this cytokine from the PBMC of HIV-seropositive hemophiliacs but not from PBMC of HIV-seronegative individuals in response to stimulation with Staphylococcus aureus Cowan strain 1. These differences may result from the use of a different stimulating agent.

We also found a decreased expression of activated caspase-1 in the PBMC of HIV-infected individuals. Because of the involvement of this protease in the processing of precursor IL-18 into a biologically active secreted form, its decreased expression may result in a decreased secretion of this cytokine from the PBMC of HIV-infected individuals. These findings suggest that PBMC may not be the source of elevated IL-18 in these patients. It is possible, however, that IL-18-producing monocytes/macrophages may have migrated out of the circulation into body tissues. Alternatively, cell types other than PBMC, e.g., Kupffer cells, keratinocytes, dendritic cells, etc., may be producing more IL-18 in these patients. This is supported by a lack of correlation between serum concentration and the amount of production of this cytokine from the PBMC of HIV-infected donors. It is noteworthy that these two parameters correlated significantly (P < 0.05) with each other in HIV-seronegative healthy donors. A likely reason for the reduced production of IL-18 from the patients’ PBMC could be an increased production of immunosuppressive cytokines, e.g., TGF-β and IL-10 in HIV-infected individuals.

We investigated the role of TGF-β in this study. As reported in the literature (5; reviewed in reference 21), we found elevated levels of this cytokine in the circulation of the patients. In our in vitro experiments, TGF-β neutralization increased IL-18 production from cultured human PBMC. Furthermore, the concentrations of IL-18 in serum of the patients correlated negatively with the concentration of TGF-β in plasma. Collectively, these results strongly implicate TGF-β in the compromised production of IL-18 from the patients’ PBMC. In fact, this immunosuppressive cytokine is known to inhibit the production of several other proinflammatory cytokines from PBMC and has been implicated in AIDS pathogenesis (reviewed in reference 21). In addition to TGF-β, IL-10 may also be involved in the reduced production of IL-18 from the patients’ PBMC. Clearly, further studies are required to learn more about the role of these cytokines in the regulation of IL-18 production in HIV-infected individuals.

In summary, we have demonstrated elevated levels of IL-18 in the sera of HIV-infected/AIDS patients, which do not correlate with their viral loads in plasma or with their CD8+ or CD4+ T-cell counts. We further demonstrated that despite elevated levels of this cytokine in their sera, the PBMC from HIV-infected/AIDS patients are compromised in the ability to produce IL-18 in vivo cultures both constitutively and in response to LPS stimulation. Elevated levels of TGF-β in the circulation of these patients may be, at least in part, responsible for this compromise. Our results discount the role of PBMC in these patients as a source of this enhanced IL-18. Further studies are needed to identify the cells and tissues that produced the elevated levels of IL-18 in these patients.

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