BRIEF REPORT

Whole-Blood Interleukin-18 Level during Early HIV-1 Infection Is Associated with Reduced CXCR4 Coreceptor Expression and Interferon-γ Levels

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Interleukin (IL)–18 generates T helper 1–type immunity and inhibits human immunodeficiency virus type 1 (HIV-1) in primary cells in vitro. Because IL-18 may participate in HIV-1 containment, whole-blood IL-18 levels were measured in 20 healthy control subjects and longitudinally in 28 subjects with early HIV-1 infection. Compared with those in control subjects, IL-18 levels were higher during early HIV-1 infection, and IL-18 levels predicted reduced CXCR4 HIV-1 coreceptor expression and diminished interferon (IFN)–γ levels. By contrast, a direct association between IL-18 and IFN-γ levels was observed in blood stimulated with lipopolysaccharide. During early HIV-1 infection, IL-18 may regulate HIV-1 coreceptor expression and have antiretroviral activity.

Interleukin (IL)–18 is an 18.3-kDa cytokine with proinflammatory and immune-enhancing properties that is produced in monocytic cells [1, 2]. Synthesized as an inactive precursor, active IL-18 is generated by IL-1β–converting enzyme (caspase 1) [3]. Unlike other proinflammatory cytokines, substantial circulating IL-18 is present in healthy subjects [4]. IL-18 enhances cell-mediated immunity by inducing NK cell development, biasing T cells toward a Th1 phenotype, and stimulating interferon (IFN)–γ production [2]. In vitro and in vivo investigations have indicated that IL-18 is critical for inducing Th1-type responses. Because Th1-type immunity is associated with reduced HIV-1 production and disease progression [2, 5], IL-18 may play a role in controlling HIV-1 in vivo.

In peripheral blood mononuclear cells (PBMCs), exogenous IL-18 inhibited HIV-1 production and reduced expression of the HIV-1 receptor molecule CD4 [6]. In addition to CD4, 2 primary HIV-1 chemokine coreceptors enable virus entry into cells [7]. The β-chemokine receptor, CC chemokine receptor 5 (CCR5), is the coreceptor for monocyte-tropic (R5) HIV-1 strains, whereas the α-chemokine receptor, CXC chemokine receptor 4 (CXCR4), is the coreceptor for lymphocyte-tropic (X4) strains [7]. Initial infection usually occurs with R5 strains, and persons with a genetic defect that reduces CCR5 expression are resistant to HIV-1 infection [7]. The mechanism of preferential infection with R5 virus is uncertain. Studies in a rhesus macaque model revealed that coexposure to R5 and X4 HIV-1 resulted in initial infection with both strains [8]. However, R5 viruses achieved dominance shortly after infection. $CD8^+$ T cell depletion after infection resulted in the emergence of X4 virus, which suggests that $CD8^+$ T cells preferentially suppressed X4 virus during early infection [8]. Therefore, early dominance of R5 viruses may result from selective anti-X4 resistance.

Because IL-18 induces a Th1-type immune response and may affect HIV-1 coreceptor expression, IL-18 may participate in preferential X4 virus suppression soon after infection. To assess a potential IL-18 role in biasing initial infection with R5 viruses, we examined the relationship between circulating IL-18 and HIV-1 coreceptor expression during early infection.

Subjects, materials, and methods. A prospective multicenter phase 2 clinical trial was conducted through the Acute Infection and Early Disease Research Program (AIEDRP-03-005) in 55 patients with early (acute or recent) HIV-1 infection. Participating institutional review boards approved the study, and informed consent was obtained from participants. Acute infection was defined as a plasma HIV-1 RNA level ⩾2000 copies/mL within 14 days of study entry and (1) negative HIV-1 antibody ELISA of plasma within 14 days of entry, (2) positive ELISA with negative or indeterminate Western blot (WB) re-

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sults within 14 days of entry, or (3) positive ELISA with positive WB results within 14 days of entry and negative ELISA or HIV-1 RNA levels <2000 copies/mL within 30 days of entry. Recent HIV-1 infection was defined as positive HIV-1 ELISA and positive WB results within 14 days of entry and either negative ELISA or HIV-1 RNA levels <2000 copies/mL within 31–90 days of study entry or nonreactive detuned HIV-1 ELISA within 14 days of entry. These definitions were designed to enroll patients infected with HIV-1 within 14 days of study entry (acute) or between 31 and 90 days of entry (recent). Subjects elected to receive or defer antiretroviral therapy (ART) consisting of atazanavir (600 mg once daily), enteric coated didanosine (250–400 mg once daily based on weight), and stavudine (40 mg twice daily, adjusted by weight). Substitution within the nucleoside reverse-transcriptase inhibitor class was permitted. The total study duration was 104 weeks.

Whole blood was collected in sodium heparin Vacutainer tubes (Becton Dickinson). Two milliliters of blood was transferred into 5.0-mL polypropylene tubes (BD Falcon; Becton Dickinson) that contained Triton X-100 (final concentration, 0.5% vol/vol; Fisher Scientific), and the lysed samples were frozen at −70°C until they were assayed. An additional 1.0-mL aliquot of blood was transferred to a tube that contained 1.0 mL of RPMI 1640 (Mediatech) with lipopolysaccharide (LPS; *Escherichia coli* 055:B5; Sigma-Aldrich) at a final concentration of 10 ng/mL. The LPS-stimulated sample was incubated for 24 h at 37°C in 5% CO_2, transferred to a tube that contained 0.5% Triton X-100, and frozen until it was assayed. The samples were coded, and total (intra- and extracellular) IL-18 and IFN-γ levels were measured using ELISA kits (MBL; Naka-ku). The IL-18 ELISA measured the active form of IL-18 and had a detection limit of 12.5 pg/mL. The IFN-γ ELISA had a detection limit of 4 pg/mL. Fresh blood samples from 20 HIV-1–seronegative healthy donors served as controls.

We quantified IL-18 levels in whole blood and examined 5 prospectively defined outcomes, including HIV-1 RNA level, $CD4^+$ T cell count, cell-surface CXCR4 and CCR5, and IFN-γ level. Immunostaining for CD3, CD4, CXCR4, and CCR5 was done as described elsewhere [9]. Briefly, whole blood was incubated at room temperature for 20 min with anti-CD3–fluorescein isothiocyanate, anti-CD4–phycoerythrin, anti-CXCR4–peridinin-chlorophyll-protein, and anti-CCR5–allophycocyanin (all from BD Pharmingen). To compensate for nonspecific fluorescence, staining with IgG_{2a} isotype control antibodies (all from BD Pharmingen) for each fluorochrome was performed in parallel using the same concentrations as the antibodies of interest. Four-color flow-cytometric analysis was performed using a FACSCaliber flow cytometer with CellQuest Pro software (version 3.2.1F1; Becton Dickinson). CXCR4 and CCR5 results were calculated as the average number of coreceptors per $CD4^+$ T cell or $CD14^+$ monocyte, respectively. Quantitative polymerase chain reaction (PCR; Roche Ultrasensitive reverse-transcription PCR kits 1.0 and 1.5) was used to measure HIV-1 RNA levels.

All analyses assumed a 2-sided test of hypothesis with an overall significance level of .05. Analyses were conducted using SAS (version 9.1; SAS Institute) and Splus (version 7.0; Insightful) software. The IL-18 effect on each outcome was analyzed using longitudinal mixed-effects regression models that controlled for ART. The duration of therapy was considered to be a predictor. Five prospectively defined outcomes (HIV-1 RNA, CD4, CXCR4, CCR5, and IFN-γ levels) were analyzed in fresh whole blood and in blood stimulated with LPS. No adjustments were made for multiple comparisons. Before longitudinal analysis, one observation (time point) was removed from data for each of 5 treated subjects because of stopped or interrupted ART; all other time points were retained. No initially untreated subject started treatment.

Results. Twenty-eight of 55 HIV-1–infected enrollees (24 white and 4 Hispanic) participated in the present substudy. The median age of the 28 subjects was 32.5 years (range, 21–59 years); the median follow-up period of the study was 104 weeks (range, 40–104 weeks). Substudy blood samples were obtained at weeks 0, 4, 12, 24, 48, and 96; a median of 4 samples was collected per patient (range, 1–6 samples).

Twenty-one subjects were receiving ART (7 acute and 14 recent), and 7 were not receiving ART (all recent). At study entry, median $CD4^+$ T cell counts in treated and untreated patients were 604.5 cells/μL (range, 320.5–1111 cells/μL) and 875 cells/μL (range, 502–1193.5 cells/μL), respectively. Median baseline HIV-1 RNA levels in patients receiving or not receiving

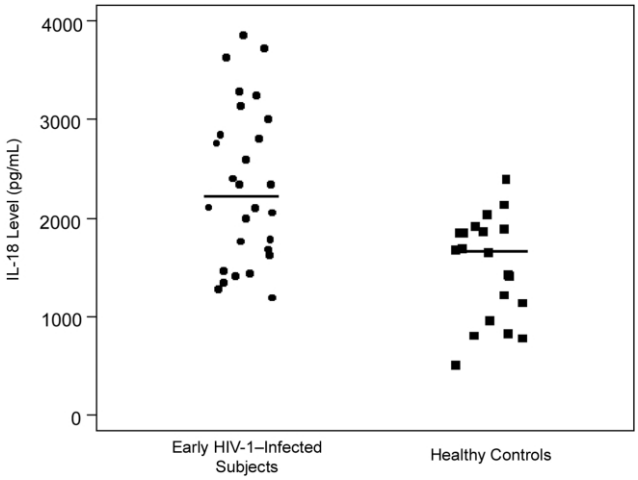

Figure 1. Interleukin (IL)–18 levels in HIV-1–infected and healthy control subjects. Whole-blood IL-18 levels are shown for 28 acute or recent (early) HIV-1–infected subjects *(circles),* compared with levels in 20 healthy control subjects (healthy controls; *squares*). $P = .002$, Wilcoxon rank sum test. Horizontal lines represent group medians.

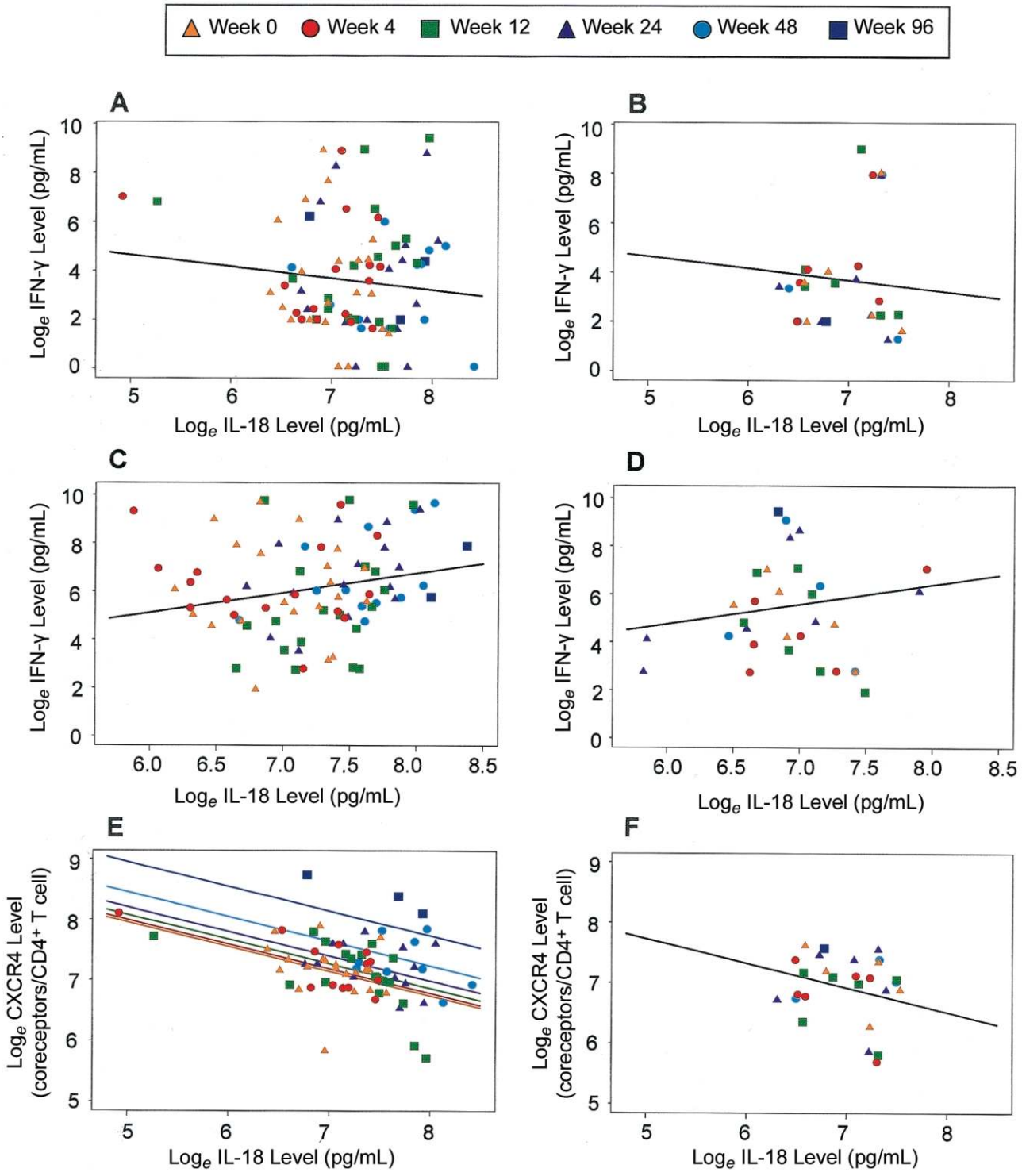

Figure 2. Relationships between circulating whole-blood interleukin (IL)–18 levels and interferon (IFN)–γ production and CXCR4 expression. Population predicted values *(lines)* are shown with raw data points that indicate the study week. Longitudinal mixed-effects regression models that controlled for antiretroviral therapy (ART) were used. An inverse association was observed between circulating IL-18 and IFN-γ concentrations in patients receiving *(A)* and not receiving *(B)* ART ($P = .015$). A direct association was observed between IL-18 and IFN-γ levels in whole blood stimulated for 24 h with lipopolysaccharide in patients receiving *(C)* and not receiving *(D)* ART ($P = .010$). For panels A–D, the $\log_e$ IL-18 level is shown on the horizontal axes and the $\log_e$ IFN-γ level is shown on the vertical axes. For panels E and F, the $\log_e$ IL-18 level is shown on the horizontal axes, and the $\log_e$ average no. of CXCR4 molecules per CD4$^+$ T cell is shown on the vertical axes. An inverse association was observed between IL-18 level and CXCR4 cell-surface expression in patients receiving *(E)* and not receiving *(F)* ART ($P < .0001$). Time receiving the study drug was also a significant predictor in this model, such that CXCR4 expression increased with time receiving ART *(E)*. Each time-specific line is depicted using the corresponding color for each study week.

ART were 67,350 copies/mL (range, 774–636,316 copies/mL) and 3577 copies/mL (range, 226–5138 copies/mL), respectively.

Whole-blood IL-18 levels in the 28 subjects at study entry were compared with those in 20 healthy control subjects (16 male and 4 female white individuals; median age, 35.5 years; range, 21–61 years). HIV-1–infected persons had significantly higher IL-18 levels than healthy control subjects (figure 1) ($P = .002$, Wilcoxon rank sum test). The median IL-18 level in the HIV-1 cohort was 2274 pg/mL (range, 1188–4687 pg/mL), whereas that in healthy control subjects was 1663 pg/mL (range, 498.8–2395 pg/mL).

Whole-blood IFN-γ levels were modeled as a function of IL-18 levels. Figure 2 shows raw data grouped by study week and with predicted IFN-γ levels represented as lines in subjects receiving ART (figure 2*A*) and not receiving ART (figure 2*B*). Higher IL-18 levels resulted in lower IFN-γ levels (figure 2*A* and 2*B*), with each $\log_e$ IL-18 increase predicting a -0.48 (95% confidence interval [CI], -0.86 to -0.10) change in $\log_e$ IFN-γ levels ($P = .015$). By contrast, whole blood stimulated with LPS for 24 h demonstrated a direct association between IL-18 and IFN-γ levels (subjects receiving ART, figure 2*C*; subjects not receiving ART, figure 2*D*). Each $\log_e$ increase in IL-18 level predicted a 0.82 (95% CI, 0.21–1.44) change in $\log_e$ IFN-γ level ($P = .010$).

Higher whole-blood IL-18 levels resulted in decreased CXCR4 expression (subjects receiving ART, figure 2*E*; subjects not receiving ART, figure 2*F*), with each $\log_e$ increase in IL-18 level predicting a -0.40 (95% CI, -0.59 to -0.22) change in $\log_e$ CXCR4 molecules per $CD4^+$ T cell ($P < .0001$). CXCR4 expression increased with time on study drug in subjects receiving ART (indicated as multiple lines in figure 2*E*) but not in subjects not receiving ART (figure 2*F*). IL-18 was not a statistically significant predictor of $CD4^+$ T cell count ($P > .27$), CCR5 expression ($P > .11$), or HIV-1 RNA level ($P > .09$).

Discussion. IL-18 and IFN-γ levels were measured in whole blood immediately after venipuncture and after 24 h of LPS stimulation in this longitudinal cohort study of 28 subjects with early HIV-1 infection. Receipt of ART was not a significant predictor in any of the models described above.

The mean IL-18 level was higher in freshly obtained blood from subjects with early HIV-1 infection, compared with that in healthy control subjects (figure 1), which suggests HIV-1–induced immune activation. Increased IL-18 levels in later stages of HIV-1 infection has been reported [10–13]. Because IL-18 induces IFN-γ [2], we expected IFN-γ levels to increase with increasing IL-18 levels. Unexpectedly, an inverse relationship between circulating IL-18 and IFN-γ levels was observed (figure 2*A* and 2*B*). Because IFN-γ is suppressed by HIV-1 envelope protein gp41 [14], IL-18 may be unable to induce IFN-γ in the presence of HIV-1 antigenemia. In addition, low cell-surface IL-18 receptor expression may have blunted the biological activity of IL-18 (IFN-γ production) [2]. In PBMCs stimulated for 2 days with IL-2 (5 ng/mL) and phytohemagglutinin (3.3 μg/mL), IL-18 α-receptor expression was higher than that in unstimulated cells (data not shown). This suggests increased responsiveness of cells to IL-18 in the presence of stimulation. Therefore, we reassessed the relationship between IL-18 and IFN-γ in whole blood stimulated with LPS. In contrast to unstimulated blood, the association between IL-18 and IFN-γ in LPS-stimulated blood was direct (figure 2*C* and 2*D*). LPS may have increased IL-18 receptor expression, resulting in increased IL-18 function and IFN-γ production.

Although a previous study of more-advanced HIV-1 infection showed a direct relationship between circulating IL-18 and HIV-1 levels [13], no relationship between IL-18 and virus production was observed in our early infection cohort. The small sample size may have accounted for this difference, or there may not be a direct relationship between IL-18 and HIV-1 levels during early infection. Although IL-18 did not predict CCR5 expression, an inverse relationship between whole-blood IL-18 levels and CXCR4 expression was observed (figure 2*E* and 2*F*). Because persons exposed to HIV-1 are usually infected with CCR5-using R5 HIV-1 strains [7], the reduced CXCR4 expression associated with increased IL-18 levels is noteworthy. Although this is only speculation, increased IL-18 levels during early HIV-1 exposure may favor initial infection with R5 HIV-1 virus by down-regulating the X4 virus CXCR4 coreceptor. Future studies will determine whether IL-18 plays a functional role in the down-regulation of CXCR4. Separate IL-18–related mechanisms may also account for initial infection with R5 HIV-1 strains. For example, in coinfection with X4 and R5 HIV-1 in macaques, CD8-mediated immunity selectively suppressed X4 viruses [8]. Because IL-18 is required for Th1-type immunity [2], IL-18 may participate in establishing the bottleneck that results in initial infection with R5 virus by enhancing immunity against X4 strains. Therefore, IL-18 may suppress HIV-1 by augmenting Th1-type immunity and reducing coreceptor expression. In late-stage HIV-1 infection, a depleted immune system may respond poorly to IL-18, facilitating the production of X4 virus. However, the involvement of IL-18 in HIV-1 infection remains speculative.

Because IL-18 suppresses in vitro HIV-1 replication and CD4 expression in PBMCs [6], is associated with reduced CXCR4 expression in circulating lymphocytes (figure 2*E* and 2*F*), and induces antiviral IFN-γ in vitro and in vivo [2], an antiretroviral role of IL-18 is suggested. IL-18–induced Th1-type immunity suggests that the administration of IL-18 may augment natural antiretroviral function. Th1-type cytokine therapy to treat HIV-1 infection has a precedent. IL-2 has been evaluated in phase 1 and 2 clinical trials as adjunctive therapy with antiretroviral drugs, and partial IL-2–induced immune reconstitution was documented [15]. Although the administration of IL-18 to treat

HIV-1 infection has a rationale, caution should be exercised because of the adverse effects that may result from its proinflammatory activities [2]. Before considering IL-18 to be therapy for HIV-1 disease or a marker of disease progression, further study is necessary.

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