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Increased Circulating Interleukin-7 Levels in HIV-1–Infected Women

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Summary

Sex-based differences in CD4 T-cell (CD4) counts are well recognized, but the basis for these differences has not been identified. Conceivably, homeostatic factors may play a role in this process by regulating T-cell maintenance and repletion. Interleukin (IL)-7 is essential for normal T-cell production and homeostasis. We hypothesized that differences in IL-7 might contribute to sex-based differences in CD4 counts. Circulating IL-7 levels were analyzed in 299 HIV-1–infected women and men. Regression analysis estimated that IL-7 levels were 40% higher in women than in men ($P = 0.0032$) after controlling for CD4 count, age, and race. Given the important role of IL-7 in T-cell development and homeostasis, these findings suggest that higher IL-7 levels may contribute to higher CD4 counts in women.

Keywords

interleukin-7; sexual dimorphism; CD4-positive T cells; cytokines; sex differences

Measurements of CD4⁺ T-cell (CD4) counts are used to assess prognosis and to guide therapeutic interventions in HIV-1–infected individuals. Absolute CD4 counts in HIV-1–uninfected women are approximately 75 to 125 cells/ μ L higher than those in HIV-1–uninfected men,¹ and these sex-based differences have been shown to persist for 5 or more years after HIV-1 seroconversion.^{2,3} The basis for these differences has not been

determined, although sex steroids and gonadotropins seem to influence CD4 counts via interactions that are not yet fully understood.⁴

CD4 counts in HIV-1–infected and –uninfected individuals may be governed by homeostatic mechanisms that regulate T-cell production or steady-state T-cell counts. In a recent analysis of thymic function in mice, female mice were found to have a slower rate of thymic involution than age-matched male mice.⁵ Similarly, an analysis of human thymopoiesis in healthy adults demonstrated that thymic output is better preserved in women than in men.⁶ Thus, regulators of T-cell production or homeostasis may influence sex-based differences in CD4 counts.

Interleukin (IL)-7 is a lymphopoietic cytokine that is crucial for normal thymopoiesis^{7,8} and homeostatic T-cell expansion^{9,10} in mice. IL-7 also seems to play an important role in human T-cell production¹¹ and homeostasis.^{12,13} Increases in circulating levels and production of IL-7 have been observed in lymphopenic humans,^{12–14} and it has been postulated that such increases occur as part of a homeostatic response to enhance T-cell production and expansion. Given the importance of IL-7 in homeostasis and T-cell production, we hypothesized that sex-based differences in IL-7 levels might contribute to differences in CD4 counts between HIV-1–infected women and men.

METHODS

Study Design

A cross-sectional study compared IL-7 levels and CD4 counts in 233 HIV-1–infected women and 66 HIV-1–infected men. Participants were naive to highly active antiretroviral therapy (HAART). Complete information on CD4 counts, age, race, and IL-7 level was required for inclusion. No entry criteria were set for duration of infection, disease stage, or other immunologic or virologic parameters. Blood specimens and clinical data for the female cohort were obtained from the Women's Interagency HIV Study (WIHS), a multisite cohort study of HIV-1 infection in women recruited from October 1994 through November 1995.¹⁵ Blood specimens and clinical data for the male cohort were obtained from study volunteers at Stanford University between 1993 and 1999 and were previously reported within a larger study.¹² A subset of the original male cohort, consisting of 66 HAART-naive men who met the previously cited inclusion criteria, was selected for this analysis. Institutional review board approval was obtained from all study sites, and the research was conducted with the understanding and consent of each participant.

Interleukin-7 Measurements

IL-7 immunoassays (R&D Systems, Minneapolis, MN) were performed in duplicate on acid citrate dextrose plasma specimens stored at -70°C or lower.

Data Analysis

Mann-Whitney U and χ^2 tests were used to compare numeric and categorical variables between men and women. Multiple regression was used to model IL-7 levels in terms of sex, CD4 count, age, and race, applying logarithmic transformations to IL-7 levels and CD4 counts to eliminate skewness. All statistical analyses were performed using SAS Software, version 8.2 (SAS Institute, Cary, NC).

RESULTS

Cohort characteristics are displayed in Table 1. The male cohort was older, with lower CD4 counts and significantly higher HIV-1 RNA levels, than the female cohort. Race distribution

differed between the cohorts; the men were predominantly white, whereas most of the women were black or Hispanic. Median IL-7 levels were higher in women (3.4 vs. 2.2 pg/mL; $P = 0.007$). A multivariate regression model was used to analyze potential predictors of IL-7 levels, including age ($P = 0.14$), sex ($P = 0.0032$), race (black [$P = 0.52$], Hispanic [$P = 0.16$], and other [$P = 0.062$] vs. white [reference]), $\log_{10}$ CD4 count ($P < 0.0001$), and a quadratic term for $\log_{10}$ CD4 count ($P = 0.0008$).

There were inverse nonlinear associations between $\log_{10}$ IL-7 levels and $\log_{10}$ CD4 counts in separate models for women and men (Fig. 1). The separate curves did not seem to fit better than a pooled model with a common quadratic CD4 effect plus a sex effect ($P = 0.96$, likelihood ratio test). Multivariate regression analysis demonstrated that at a given CD4 count, circulating IL-7 levels were 40% higher in women than in men when controlling for age and race (confidence interval: 12% to 75%; $P = 0.0032$). There was some indication that the sex differences might be greater among persons with a CD4 count >200 cells/ μ L (women 51% higher) than among those with a CD4 count <200 cells/ μ L (women 24% higher), but this interaction did not reach statistical significance ($P = 0.15$). Among persons with a CD4 count <50 cells/ μ L, where a ceiling effect limiting the sex difference would seem most likely, the estimated effect of sex was identical to the overall estimate (women 40% higher), but this had high uncertainty because of the small sample in this range. The estimated sex difference among those with a CD4 count >350 cells/ μ L was also similar at 44%. When viral load was added to the primary model of IL-7 levels, its estimated effect was a 7% increase per $\log_{10}$ viral load increase (confidence interval: -22% to 16%; $P = 0.12$). This addition reduced the estimated sex effect to 23% (confidence interval: -4% to 58%; $P = 0.10$).

DISCUSSION

We sought to estimate sex differences in circulating IL-7 levels controlled for CD4 count. We also investigated whether the previously reported relation between circulating IL-7 levels and CD4 counts, established in a predominantly HIV-1-infected male cohort,¹² is present as well in HIV-1-infected women. Theoretically, increased CD4 counts in women might be attributable to higher levels of IL-7 or to alterations in T-cell homeostasis attributable to sex-based differences in the relation between IL-7 and CD4 counts. In a cross-sectional analysis of 299 HIV-1-infected persons, we found a similar inverse relation between IL-7 and CD4 counts in women and men. We also found that circulating levels of IL-7 averaged 40% higher in women, and we reason it is likely that increased levels of circulating IL-7 reflect higher local concentrations of IL-7 in peripheral or central immune tissues. Because IL-7 facilitates T-cell development and proliferation,⁷⁻¹³ increased circulating IL-7 levels may contribute to the higher CD4 counts found in women. In addition, and despite some evidence to the contrary,^{16,17} IL-7 may enhance HIV-1 infection, replication, and cytopathicity in vivo.¹⁸⁻²¹ Thus, it is possible that higher levels of IL-7 in women might contribute to faster disease progression at a given viral set point.²²⁻²⁴

Certain limitations of this study warrant consideration. We cannot exclude the possibility that a difference in the duration of infection between these cohorts contributed to the observed differences, because reliable data on the duration of infection were not available. We did, however, control for CD4 count, which is commonly used as an indicator of disease progression. Although controlling for viral load reduced the estimated effect of sex on log IL-7 and increased its P value to 0.10, the evidence for a viral load effect on IL-7 was not strong ($P = 0.12$), and plausible mechanisms for such an effect are not apparent, other than via CD4 count, which is already controlled for in the model. A longitudinal analysis of men and women from the point of infection could provide more definitive conclusions than a cross-sectional analysis with regard to the contribution of IL-7 to sex-based differences in

HIV disease. In fact, given the well-documented differences in immune function between women and men,^{25,26} we believe that a prospective longitudinal cohort of HIV-1-infected women and men is warranted to examine sex-based differences in many aspects of HIV immunopathogenesis.

Whereas the data reported here do not confirm a role for IL-7 in sex-based differences in CD4 counts in HIV-1-seronegative individuals, a role is possible, because the estimated effect of sex on IL-7 seemed similar to the overall estimate when restricted to those with a CD4 count >350 cells/ μ L. Additional sex-based differences, including differences in sex steroids, may contribute to differences in the homeostatic regulation of CD4 counts via mechanisms independent of IL-7 or by influencing the regulation of IL-7. Continued research is indicated to determine the cause of sex-based differences in IL-7 levels and to define factors responsible for sexual dimorphism in lymphocyte populations and immune function.

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FIGURE 1.

Circulating IL-7 levels are higher in women than in men. Quadratic models were fitted to describe the association between IL-7 and CD4 cells, with separate curves fitted for women (o; ---) and men (x; —). Despite the similarity in shape, there is a significant shift, such that at a given CD4 count, IL-7 levels are 40% higher in women than in men ($P = 0.0032$).

TABLE 1

Cohort Characteristics

	Women (N = 233)		Men (N = 66)		P
	Median	Range	Median	Range	
Age (y)	38	22–62	41	27–68	0.009
CD4 count (cells/ μ L)	280	1–1220	246	4–644	0.25
CD4 % (of lymphocytes)	18.0	0–47.5	16.2	1.2–42.5	0.17
HIV-1 RNA (copies/mL)	23,000	80–6,010,000	59,475	5128–457,050	0.0004
IL-7 level (pg/mL)	3.4	0.5–25.3	2.2	0.4–19.5	0.007
Race					
Black		49%		12%	<0.0001
White		16%		80%	
Hispanic		32%		2%	
Other		3%		6%	