Sex Differences in HIV RNA Level and CD4 Cell Percentage During Childhood

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Background. HIV-infected women have lower HIV RNA levels and higher CD4-cell counts than do men. This observation has been attributed to the immunomodulatory effects of sex steroid hormones, such as estrogen and progesterone. Limited data exist regarding potential sex differences in HIV RNA level and CD4 parameters among prepubertal children with untreated HIV infection.

Methods. We examined the relationship of sex to HIV RNA level and CD4 parameters among 670 perinatally HIV-infected, antiretroviral therapy–naive African children aged <18 years (median age, 4.8 years) using multivariate linear regression. In a subset of 188 children, we used longitudinal data to compare changes in HIV RNA levels and CD4 percentage over time. Levels of CD4 and CD8 T-cell activation (CD38+HLA-DR+) were also compared between boys and girls.

Results. Female children had lower HIV RNA levels (P = .0004) and higher CD4 percentages (P < .0001), compared to male children. Multivariate linear regression demonstrated an independent association of sex with both HIV RNA level and CD4 percentage after controlling for other covariates. Multilevel mixed-effects linear regression analysis of longitudinal HIV RNA level and CD4 parameter data showed that sex differences persisted across all observed ages. Levels of T-cell activation did not differ between the sexes.

Conclusions. Significant sex differences in HIV RNA levels and CD4 parameters are present in HIV-infected children before the onset of puberty. These data suggest that intrinsic genetic differences between male and female individuals, unrelated to sex steroid hormone levels, influence HIV RNA level and CD4 parameters in HIV-infected individuals.

Adult women infected with HIV exhibit significantly lower viral loads, compared with men [1–3]. Sex disparities have been described in the manifestation of infection from other viruses, such as hepatitis C virus and hepatitis B virus, [4–6] and in the incidence and severity of autoimmune diseases [7]. However, the molecular mechanisms underlying sexual dimorphism in immune function remain unknown. Previous attempts to explain sex differences in HIV infection and other infectious diseases have focused on the numerous immunodulatory effects of the major female sex steroid hormones estrogen and progesterone [8, 9]. Receptors for both estrogen and progesterone are expressed by most immune cell types, and levels of these hormones influence the expression of CCR5 by CD4 T cells and production of several cytokines [10, 11]. Exogenous administration of these hormones and their natural fluctuation during the ovulatory cycle have been shown to modulate innate and adaptive immune responses and may influence the rate of HIV replication [12]. The recent demonstration that production of interferon (IFN)–α by plasmacytoid dendritic cells in response to HIV-derived TLR ligands is higher in women than in
men and correlates with plasma progesterone levels has been suggested to be a potential biological mechanism for the observed sex differences in HIV load [13, 14].

Comparison of HIV RNA levels between prepubertal boys and girls provides a means to uncouple the impact of sex hormones from other sex-specific genetically determined effects. Two small studies in the United States and Europe described lower HIV RNA levels among girls than among boys [15, 16], but the majority of children in both cohorts were receiving combination antiretroviral therapy (ART). One study of ART-naive Kenyan infants detected no sex difference in viral load but included only a small number of participants [17]. Here, we assessed HIV RNA levels, CD4 parameters, and T-cell activation markers in a large cohort of HIV-infected children before the initiation of ART and found a significant sex disparity in both HIV RNA levels and CD4 percentage beginning in early childhood that was not associated with differences in indices of generalized T-cell activation.

METHODS

Study Participants
Data from 3 cohorts of HIV-infected ART-naive African children (<18 years of age) were analyzed. Cohorts 1 and 2 included children initiating HIV care at McCord Hospital in Durban, South Africa (May 2004–December 2008) and St. Mary’s Hospital in Marrianhill, South Africa (January 2007–December 2008), respectively. Cohort 3 consisted of 300 children enrolled from October 2005 through September 2006 in the Children with HIV and Malaria Project, an observational study based in Kampala, Uganda [18]. The participants in the study who did not meet criteria for initiation of ART at enrollment were followed up, with HIV RNA and CD4 levels measured every 12 weeks. In all cohorts, ART was initiated immediately for children determined to be eligible according to the country guidelines. This research was approved by the ethical review boards at each site, including McCord and St. Mary’s Hospitals, Massachusetts General Hospital, the Uganda National Council of Science and Technology, the Makerere University Research and Ethics Committee, and the University of California, San Francisco Committee on Human Research.

Quantitative CD4 and HIV RNA Parameters
CD4-cell counts and percentages were determined from fresh whole blood samples on a 4-color flow cytometer. Plasma HIV RNA levels were measured from fresh blood specimens. For cohort 3, all plasma HIV RNA levels were measured using the Roche Amplicor, version 1.5 (dynamic range, 400–750 000 copies/mL). For cohorts 1 and 2, the Roche Amplicor, version 1.5, assay was used for most viral load determinations, but for some, the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (dynamic range, 48–10 000 000 copies/mL) or the NucliSENS easyMAG (bioMérieux; dynamic range, 25–3 000 000 IU/mL) was used. Because the dynamic ranges of these assays differed, for combined analyses, we assigned all HIV RNA measurements >500 000 copies/mL as a value of 500 001 copies/mL, and we assigned measurements <400 copies/mL as a value of 399 copies/mL. Log-transformed HIV RNA levels were used for all analyses.

CD4 and CD8 Activation Markers
For the participants in cohort 3, the proportions of CD4+ and CD8+ lymphocytes coexpressing the activation markers CD38 and HLA-DR were measured in freshly isolated peripheral blood mononuclear cells with use of the following antibodies: CD3 APC, CD8 PerCp-Cy5.5, HLA-DR FITC, and CD38 PE (as described elsewhere [19]). A minimum of 30 000 CD3+ cells per sample were acquired using a 4-color flow cytometer (FACS Calibur; BD Biosciences). CD4 and CD8 activation were defined as the percentage of CD3+ CD8- and CD3+ CD8+ lymphocytes, respectively, that coexpressed CD38 and HLA-DR. Data were analyzed using FLOWJO software (TreeStar).

Statistical Analyses
The Fisher’s exact and Mann-Whitney U tests were used for bivariate comparisons. Multivariate linear regression models were developed to assess the relationship of sex to HIV RNA level and CD4 percentage, adjusting for age, CD4 percentage, absolute CD4 count, cohort, and terms for the interaction of sex with age. The final models were chosen using backward variable selection, including only predictors that had a P value of ≤.05 in the full model. Because the truncation of HIV RNA levels >5.7 log copies/mL could violate the distributional assumptions underlying the linear regression model, each regression was repeated using bootstrap-based inference with 1000 repetitions. Similar models were developed to assess the relationship of sex to CD4 percentage. Frequencies of CD38+HLA-DR+ cells among CD3+ CD8- and CD3+ CD8+ lymphocytes were compared between male and female children with use of a multivariate linear regression that included sex, age, HIV RNA level, CD4 percentage, and absolute CD4-cell count.

To explore the association of HIV RNA level with sex over time, a multilevel mixed-effects linear regression model accounting for repeated measures was developed using longitudinal data from cohort 3. Mean changes in HIV RNA level with age were examined graphically by plotting observed measurements for each individual as a function of age. Mean trends for female and male children were estimated using locally linear regression smooths to allow visual inspection of observed differences without specifying a parametric model. Differences in age-related changes in HIV RNA level between sexes were evaluated using linear mixed effect regression models, accounting for combined analyses, we assigned all HIV RNA measurements >500 000 copies/mL as a value of 500 001 copies/mL, and we assigned measurements <400 copies/mL as a value of 399 copies/mL. Log-transformed HIV RNA levels were used for all analyses.
for within-individual correlations in repeated outcome measures. Regression models allowing sex-specific intercepts and slopes were fitted using restricted maximum likelihood with unstructured covariances. In addition, we examined the possibility of nonlinearity in age-related trends by including polynomial terms in the models. We performed the same analyses to evaluate the relationship of CD4 percentage with sex and age.

All statistical tests were 2-tailed, with a P value < .05 considered to be statistically significant, and were performed using Stata statistical software (release 11.1; StataCorp) and R (version 2.11.1; R Foundation for Statistical Computing).

RESULTS

Cohort Characteristics
A total of 670 ART-naive HIV-infected African children aged 1 day to 18 years (median, 4.8 years) were studied. The cohorts were balanced with regard to sex but differed substantially with regard to age and level of immune compromise (Table 1) because of varying demographic characteristics and referral patterns. All children were black Africans. Consistent with the high HIV RNA levels known to occur during infancy and early childhood [20, 21], 36% of the children in our study had HIV RNA levels >5.7 log copies/mL. These children were significantly younger (median age, 1.56 vs 5.55 years; P < .0001), had lower CD4 cell percentages (median, 15 vs 17; P = .02), and were more likely to be male (40% vs 30%; P = .01).

Sex Differences in HIV RNA Levels
In cross-sectional analysis, HIV RNA levels were significantly lower in female than in male children (P = .001) (Table 2). This difference was most pronounced among older children and those with higher CD4 percentages and absolute counts (Table 3). In multivariable regression analysis, sex remained a significant predictor of HIV RNA level (P = .026) after adjustment for age (P < .001) and CD4 percentage (P < .001). When added to the multivariate model, the interaction term between sex and age did not achieve statistical significance (P = .37). Confidence intervals (CIs) and P values with use of bootstrap-based inference were similar. To more specifically restrict our dataset to the prepubertal period, we repeated the multivariate analysis including only children aged ≥10 years (n = 612) [20] and found that female sex remained significantly associated with lower HIV RNA level (P = .046).

Sex Differences in CD4 Parameters
In cross-sectional analysis, female children had significantly higher CD4 cell percentages than male children (median, 18% vs 15%; P < .0001, Figure 3) and a trend toward higher absolute CD4 cell counts (median, 602 vs 531 cells/µL; P = .053, Table 2). When stratified by age, CD4 cell percentages were significantly higher among female children ≥2 years of age (P < .0001), but no difference was seen among younger children (Table 4). When stratified by HIV RNA level, there was a pronounced sex difference in CD4 cell percentages among children with HIV RNA levels in the quantifiable range (<5.7 log copies/mL; P < .0001), but no difference was observed among children with HIV RNA levels exceeding the threshold for quantification (≥5.7 log copies/mL; P = .63). Female sex remained independently associated with CD4 cell percentage (P < .001), after adjusting for CD4 cell count (P < .001), age (P < .001), and HIV RNA level (P < .001) and cohort (P < .001) in multivariate linear regression modeling. There was a statistically significant interaction between sex and HIV RNA level (P = .006), with each 1 log copies/mL increase in HIV RNA level associated with a decrease of 0.6% in CD4 cell percentage among male children, but a 2.7% decrease among female children.

Longitudinal Analyses
To further evaluate the effect of sex on age-related changes in HIV RNA level, we developed a multilevel mixed-effects linear regression model with use of longitudinal data from cohort 3. A total of 188 HIV-infected children with a median of 10 HIV RNA measurements (range, 3–14) over a median of 756 days

Table 1. Cohort Characteristics

<table>
<thead>
<tr>
<th>Location</th>
<th>Cohort 1 Durban, South Africa</th>
<th>Cohort 2 Marianhill, South Africa</th>
<th>Cohort 3 Kampala, Uganda</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>88</td>
<td>317</td>
<td>265</td>
</tr>
<tr>
<td>Females</td>
<td>51%</td>
<td>47%</td>
<td>54%</td>
</tr>
<tr>
<td>Age (years)a</td>
<td>1.1 (0.5–7.2)</td>
<td>3.0 (0.8–7.3)</td>
<td>5.7 (3.8–7.7)</td>
</tr>
<tr>
<td>CD4 percentagea</td>
<td>17 (10–22)</td>
<td>13 (8–18)</td>
<td>21 (15–27)</td>
</tr>
<tr>
<td>CD4 count (cells/µL)a</td>
<td>405 (181–745)</td>
<td>427 (184–822)</td>
<td>700 (455–985)</td>
</tr>
<tr>
<td>HIV RNA Levelab</td>
<td>5.7 (4.9–5.7)</td>
<td>5.4 (4.9–5.7)</td>
<td>5.2 (4.6–5.6)</td>
</tr>
</tbody>
</table>

**NOTE.** Values are medians (interquartile range) unless otherwise listed.

a P < .001 by Kruskal-Wallis test.

b log₁₀ (copies/mL): values truncated the narrowest dynamic range of 2.6–5.7.
of follow-up were analyzed. We found that mean log viral loads were 2-fold higher among boys (mean difference, 0.31 log copies/mL; 95% CI, .16–.45 copies/mL; \( P = .001 \)) across all ages tested (Figure 1). Multilevel mixed-effects linear regression analysis of CD4 cell percentage similarly showed that female patients had higher CD4 cell percentages (mean difference, 2.31; 95% CI, .65–3.97; \( P = .006 \)) across all ages tested (Figure 2). Models allowing for different slopes for boys and girls or non-linearity in age-related changes did not significantly improve fits for either outcome.

**T Cell Immune Activation Levels**

T cell immune activation has been shown to be a strong independent predictor of HIV disease progression [22–25], and it has recently been reported that HIV-infected women exhibit higher levels of CD8 T-cell activation than do men after adjusting for viral load [13]. We compared T cell immune activation parameters among 100 female and 60 male ART-naive HIV-infected children (median age, 6.8 years) from cohort 3.

The median level of CD38 and HLA-DR coexpression on CD4 T lymphocytes was similar between boys (13%; interquartile range [IQR], 8%–17%) and girls (12%; IQR, 8%–16%; \( P = .63 \)). Levels of CD38 and HLA-DR coexpression on CD8+ T lymphocytes were also nearly equivalent, with 43% (IQR, 32%–52%) for male patients and 44% (IQR, 33%–52%) for girls (\( P = .93 \)). In multivariate linear regression, CD4 activation levels were strongly associated with CD4 cell percentage (\( P < .001 \)), but not sex (\( P = .74 \)), absolute CD4 cell count (\( P = .10 \)), age (\( P = .75 \)), or HIV RNA level (\( P = .10 \)). CD8 activation levels were associated with both CD4 percentage (\( P = .009 \)) and absolute CD4 cell count (\( P = .022 \)), but not sex (\( P = .31 \)), age (\( P = .63 \)), or HIV RNA level (\( P = .21 \)).

**World Health Organization (WHO) Pediatric HIV Treatment Guidelines**

The 2010 WHO guidelines recommend initiation of ART for all HIV-infected children \(<24\) months of age (regardless of CD4 parameters), for children \(24–59\) months of age with a CD4 cell percentage \(<25\) or absolute CD4 cell count \(<750\ cells/\mu L\), and for children \(>5\) years of age with an absolute CD4 cell count \(<350\ cells/\mu L\). [26]. Because of the observed sex difference in CD4 cell percentages, we compared the proportion of boys and girls in our cohort who would qualify for ART under the current CD4-based guidelines. Among children \(\geq24\) months of age, 109 (48%) of 225 of female children were eligible for ART, compared with 141 (63%) of 224 male children (\( P = .0023 \)).

**DISCUSSION**

Men and women are known to respond differently to a number of infections, but the relative contribution of sex hormones to this disparity has been unclear. Here, we revealed basic biologic differences in HIV load and CD4 cell dynamics between male and female individuals that are evident during early childhood, when levels of the major sex steroid hormones estrogen, progesterone, and testosterone do not appreciably differ between boys and girls [27]. These data suggest that intrinsic biologic differences in the immunologic response to HIV exist between boys and girls throughout childhood—a finding that has important implications for our understanding of the biology of

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**Table 2. Age, CD4 Cell Parameters, and HIV RNA Level by Sex**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>n = 338</th>
<th>332</th>
<th>( P ) value^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0 (1.3–7.7)</td>
<td>4.6 (1.6–7.1)</td>
<td>.34</td>
<td></td>
</tr>
<tr>
<td>602 (316–938)</td>
<td>531 (231–852)</td>
<td>.053</td>
<td></td>
</tr>
<tr>
<td>18 (11–25)</td>
<td>15 (9–21)</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>5.2 (4.5–5.7)</td>
<td>5.5 (4.9–5.7)</td>
<td>.0004</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. HIV RNA Levels Within Subgroups of Age, CD4 Cell Percentage, and CD4 Cell Count**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>n =</th>
<th>Females</th>
<th>Males</th>
<th>( P ) value^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>211</td>
<td>5.7 (5.4–5.7)</td>
<td>5.7 (5.6–5.7)</td>
<td>.23</td>
</tr>
<tr>
<td>2–6</td>
<td>202</td>
<td>5.2 (4.5–5.7)</td>
<td>5.4 (4.9–5.7)</td>
<td>.06</td>
</tr>
<tr>
<td>( \geq6 )</td>
<td>257</td>
<td>4.7 (4.3–5.2)</td>
<td>5.0 (4.6–5.5)</td>
<td>.001</td>
</tr>
<tr>
<td>CD4 Percentage^b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15</td>
<td>278</td>
<td>5.4 (4.9–5.7)</td>
<td>5.5 (4.9–5.7)</td>
<td>.80</td>
</tr>
<tr>
<td>15–24.9</td>
<td>251</td>
<td>5.4 (4.7–5.7)</td>
<td>5.5 (4.9–5.7)</td>
<td>.10</td>
</tr>
<tr>
<td>( \geq25 )</td>
<td>134</td>
<td>4.7 (4.2–5.4)</td>
<td>5.1 (4.8–5.7)</td>
<td>.004</td>
</tr>
<tr>
<td>CD4 Count (cells/\mu L)^c</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;350</td>
<td>201</td>
<td>5.2 (4.4–5.7)</td>
<td>5.3 (4.8–5.7)</td>
<td>.15</td>
</tr>
<tr>
<td>350–750</td>
<td>214</td>
<td>5.4 (4.6–5.7)</td>
<td>5.3 (4.7–5.7)</td>
<td>.80</td>
</tr>
<tr>
<td>( \geq750 )</td>
<td>232</td>
<td>5.2 (4.6–5.7)</td>
<td>5.7 (5.1–5.7)</td>
<td>.001</td>
</tr>
</tbody>
</table>

**NOTE.** Values are medians (IQR) of HIV RNA Log_{10}[copies/mL] unless otherwise listed.

^a By Mann-Whitney U Test.

^b Data not available for 7 children.

^c Data not available for 23 children.
antiviral immune response and potential implications for treatment guidelines.

Sex differences in HIV RNA levels among adults have been well established. During early HIV infection, plasma HIV RNA levels are ~0.5 log lower in adult women than in men, but this difference narrows as the disease progresses, becoming statistically insignificant when CD4 cell counts are ≤ 200 cells/mL [1–3, 28, 29]. CD4 cell counts are also higher among adult women with and without HIV infection [3]. Prior studies of HIV RNA levels and CD4 cell parameters among male and female children have been limited and have yielded conflicting results.

One study of children receiving ART found HIV RNA levels to be 0.38 log lower in girls than in boys, with no difference in CD4 cell count or percentage [15]. Another group reported that HIV RNA levels were higher in girls at the peak of viremia but consistently lower after 4 years of age [16]. Of note, this group also reported CD4 cell counts to be significantly lower among HIV-infected girls than boys [30], in contrast to our findings of higher CD4 cell percentages among girls. However, these studies included few or no children who were naive to combination ART [15, 16, 30]. The only prior study to focus on ART-naive children was limited to 38 infants and found no difference in

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>n=</th>
<th>Females</th>
<th>Males</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>210</td>
<td>15 (10–21)</td>
<td>17 (11–21)</td>
<td>.692</td>
</tr>
<tr>
<td>2–6</td>
<td>200</td>
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<td>14 (8–21)</td>
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<tr>
<td>≥6</td>
<td>253</td>
<td>19 (10–26)</td>
<td>15 (9–22)</td>
<td>.021</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>CD4 Count (cells/μL)</th>
<th>n=</th>
<th>Females</th>
<th>Males</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;350</td>
<td>197</td>
<td>9 (5–13)</td>
<td>8 (3–13)</td>
<td>.082</td>
</tr>
<tr>
<td>350–750</td>
<td>214</td>
<td>19 (13–25)</td>
<td>17 (13–21)</td>
<td>.045</td>
</tr>
<tr>
<td>≥750</td>
<td>232</td>
<td>23 (19–31)</td>
<td>21 (17–26)</td>
<td>.010</td>
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</table>

<table>
<thead>
<tr>
<th>HIV RNA Log10 (copies/mL)</th>
<th>n=</th>
<th>Females</th>
<th>Males</th>
<th>P value</th>
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<tr>
<td>&lt;5.0</td>
<td>220</td>
<td>22 (13–29)</td>
<td>15 (9–22)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>5.0–5.6</td>
<td>186</td>
<td>16 (11–23)</td>
<td>14 (10–22)</td>
<td>.109</td>
</tr>
<tr>
<td>≥5.7</td>
<td>232</td>
<td>15 (10–20)</td>
<td>15 (10–19)</td>
<td>.630</td>
</tr>
</tbody>
</table>

NOTE. Values are medians (IQR) unless otherwise listed.

a N = 663 children with CD4 percentage data.
b By Mann–Whitney U test.
c Data not available for 27 children.
d 36% had the truncated maximum value of 5.7 Log10(copies/mL).

Figure 1. Loess-smoothed regression lines showing the relationship of mean HIV RNA levels (log10 copies/mL) to age for male and female children followed longitudinally in cohort 3. HIV RNA levels were lower among female children across all observed ages.

Figure 2. Loess-smoothed regression lines showing the relationship of CD4 cell percentage to age for male and female children followed longitudinally in cohort 3. CD4 cell percentages were higher among female individuals across all observed ages.
HIV RNA levels between male and female individuals [17]. For our study, we drew data from a large cohort of children, none of whom had received prior ART.

Multiple factors likely contribute to sex differences in the course and clinical manifestations of infectious diseases, including behavioral and epidemiologic risk factors, socioeconomic disparities, differences in gene expression, and levels of sex steroid hormones. Our data suggest that sex hormones are unlikely to be the major driver of the sex disparity in HIV RNA levels. Circulating levels of estrogen, progesterone, and testosterone remain low throughout the prepubertal years in both girls and boys, with the exception of the transient mini-puberty of infancy, which resolves within 4–6 months after birth. This brief postnatal hormone surge is believed to drive sexual differentiation in the brain [31] and could similarly imprint sex-based differences on immune cell populations. Alternatively, genetic differences between male and female individuals may drive sexually dimorphic immune responses. Experiments comparing transgenic XY and XX mice with a common gonadal type indicate that the XX sex chromosome complement, rather than female steroid hormones, predisposes female mice to autoimmune disease [32]. Sex-based differences in gene regulation and tissue-specific gene expression have been well established to impact human disease susceptibility and severity [27, 33]. Of the ~1000 human X chromosome genes that lack a homologue on the Y chromosome, ~15% escape X inactivation to some degree [34], potentially resulting in gene dosage differences between male and female individuals. Of note, many genes with key immune functions, including those encoding TLR7, TLR8, IRAK, CD40L, and FoxP3, reside on the X chromosome and could be subject to gene dosage effects [8].

The mechanisms by which sex-biased gene expression might ultimately result in plasma HIV RNA differences are not clear, but could include differences in target cell availability or permissiveness to infection, as well as differences in cytokine production or other innate or adaptive immune mechanisms. CD8+ T cell activation levels were recently shown to differ by sex and correlate with plasma progesterone levels in adults [13]. Levels of CD38 expression by T cells are naturally high in infants and decrease with age, making interpretation in the context of HIV infection more challenging [35]. However, we did not find a sex difference in CD4 or CD8 activation levels in our prepubertal children, suggesting that sex hormone–mediated changes in T-cell activation do not underlie the sex differences in plasma HIV RNA levels in children.

These results have potential implications for pediatric HIV treatment guidelines. HIV RNA levels and CD4 cell parameters are currently the key laboratory measures used to guide initiation and monitoring of ART. In our study, fewer girls would be eligible for ART on the basis of WHO pediatric guidelines, and other studies have suggested similar discrepancies for adult women [1–3]. The clinical significance of this disparity in eligibility is unclear, but 3 studies involving children have noted higher mortality rates among girls initiating ART [15, 16, 36]. Further study is indicated to determine whether initiation of ART at higher CD4 cell counts in female children might be needed to optimize outcomes.

Our analysis has several limitations. HIV RNA levels were truncated at 5.7 log copies/mL in more than one-third of study participants, limiting our ability to accurately quantify the effect size of sex at high HIV RNA levels. This limitation would be expected to conservatively bias our analysis toward the null hypothesis of no difference and may have decreased our sensitivity to detect sex-based differences among the younger or more severely immunosuppressed children who have high HIV RNA levels. It is also possible that our study population was vulnerable to a healthy survivor bias. If female individuals were more likely to have received poor care and died, we would have enrolled a healthier cohort of female persons than male persons. However, in that case, one would predict our study population to be disproportionately male, when in fact, the overall proportion was 50%. Furthermore, the fact that sex differences were stable over time in the longitudinal data analysis makes the influence of such bias less likely. Another potential limitation of our analysis is that data were combined from 3 cohorts that differed by age, stage of disease, and HIV RNA assay used; however, each cohort was balanced with regard to sex, and inclusion of cohort in the multivariate models did not alter the results.
In summary, we found substantial differences in HIV RNA level and CD4 cell percentages between HIV-infected boys and girls throughout childhood and before the onset of puberty. These data suggest that sex-specific mechanisms other than steroid hormones are important in the immunologic development and control of HIV infection in children. Our findings have broad implications for the understanding of sex differences in immune function and highlight the need for further study of the molecular mechanisms underlying this sexual dimorphism.

Acknowledgments

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed in the Acknowledgements section.

References