CD8 T cell function, lymphocyte surface phenotype, serum markers of immunologic activation, and viral burden were assessed in 75 human immunodeficiency virus (HIV)-infected pregnant women, including 9 who transmitted infection to their infants. Serial studies during and after pregnancy showed no significant differences in levels of cell-surface or serum activation molecules in transmitting compared to nontransmitting mothers, with the exception of a postpartum increase in tumor necrosis factor α in transmitting women. The transmitting women had a median plasma viral load of 65,516 RNA copies/mL at delivery versus 5139 in nontransmitting women. During the third trimester, the CD8 cells of 81% of the nontransmitting and 44% of the transmitting mothers suppressed HIV production in vitro by >50%. Women with <50% suppression had a 3.4 times greater risk of transmitting HIV to their infants. CD8 suppression and viral load were interrelated, but when either CD4 percent or AZT use was controlled for, suppression was still significant.

Transmission of human immunodeficiency virus (HIV) from mother to infant may occur in utero, during labor and delivery, or via breast milk. Maternal transmission is the principal route of HIV infection in young children worldwide and occurs at a rate estimated to be between 15% and 40% in the absence of antiviral therapy [1].

The biologic factors involved in the transmission of HIV infection from mother to infant are multiple, complex, and still poorly understood [reviewed in 1]. We and others [2–5] have shown that high maternal viral burden is a risk factor for vertical transmission. Viral burden in the periphery is a reflection of the dynamic and complex interaction of HIV and the host immune response, which is capable of both controlling and enhancing viral replication [reviewed in 6, 7]. Better understanding of the immune response in HIV-infected pregnant women will help elucidate mechanisms of pathogenesis and facilitate the design of optimal strategies for reducing mother-to-infant transmission.

The CD8 T cell-mediated response to HIV, particularly the ability to suppress virus in vitro, is considered to be critical in controlling HIV infection and has been associated with a prolonged asymptomatic state [8, 9]. However, other indicators of activated immunity in HIV-infected individuals are surrogates for pathogenesis rather than protection. Serum β2 microglobulin and neopterin and elevated expression of the CD38 molecule on T cells have been associated with a poor prognosis [10]. The cytokine tumor necrosis factor alpha (TNFα), produced by activated monocytes in HIV-infected individuals, can directly up-regulate HIV replication [7].

We characterized protective and pathogenic aspects of the immune response of HIV-infected pregnant women to determine whether certain immunologic profiles were associated with vertical transmission. We report here an increased risk of vertical transmission in mothers with low CD8 cell function during the third trimester of pregnancy. Transmitting women also had a higher median viral burden.

Methods

Subjects. HIV-infected pregnant women were enrolled in the study during 1991–1995. Subjects were recruited through the UCLA Maternal Child Immunology Clinic, UCLA Pediatric AIDS Clinical Trials Unit, and the clinics of Harbor-UCLA and Long Beach Memorial Hospitals. One hundred and twelve HIV-infected pregnant women were recruited for the study, and the overall transmission rate for the cohort was 12%. Data on 75 women who had 2 or more samples, including 9 who transmitted infection to their
infants, are included in this analysis, although not all subjects are included in every analysis or at every time point.

Infection status of the mothers was determined by p24 ELISA with confirmatory Western blot. Transmission of infection was defined as 2 or more positive cell coculture or polymerase chain reaction (PCR) results in the infants during the first month of life; uninfected status was confirmed by the loss of bands on Western blot at 15 months of age.

CD8 cell function was assessed during the third trimester. Samples for other immunologic tests on the women were obtained once each trimester (there were insufficient data for analysis from the first trimester) and at delivery, 6 weeks, and 3, 6, and 12 months postpartum. Data from 1 transmitting mother with primary infection during pregnancy were not pooled with the other transmitting mothers’ data for determination of the median values for the immunologic markers because the immune response during primary infection is different from that seen in established infection [11].

Immunophenotyping. Lymphocyte surface antigen expression was characterized by standard techniques using fluorochrome-labeled monoclonal antibodies (mabs) and fluorescence detection by laser flow cytometry [12]. The mabs were used in 2- or 3-color combinations, the first fluorochrome label being fluorescein isothiocyanate, the second label being phycoerythrin, and the third label being peridinin chlorophyll protein (Becton Dickinson Immunocytometry Systems, Mountain View, CA). The mab combinations included CD3/CD4, CD3/CD8, CD3/CD19, CD3/CD16 + 56 for the major T cell subsets, B and NK cells, and HLA-DR/CD38/CD8 for activated CD8 cells, and CD8/CD11a(S6F1)/CD3 for activated CD8 cells associated with cytotoxic function.

CD8 cell-mediated suppression of HIV production. The ability of CD8 T cells to suppress virus production in autologous CD4 cells was assessed using our previously published technique [13]. Briefly, peripheral blood mononuclear cells were obtained by Ficoll-hypaque density gradient centrifugation. CD4 and CD8 T cells were isolated by adherence to anti-CD4 or anti-CD8 mab-coated flasks (MicroCELLector; Applied Immune Sciences, Santa Clara, CA). The CD4 cell fraction was incubated for 3 days at 37°C in complete RPMI 1640 medium (CM) containing 10% human AB serum, 200 ng/mL of CD3 mab (OKT3; Ortho Diagnostics, Raritan, NJ), 40 U/mL recombinant IL-2 (Cellular Products, Buffalo, NY), and 10% human IL-2 (Bio-Tech Imaging, Frederick, MD). The CD8 cell fraction was incubated for 3 days at 37°C in CM without the CD3 mab or human IL-2. After 3 days of culture, the cell fractions were collected from each flask, checked for viability using trypan blue staining, and counted. The purified CD4 and CD8 cell fractions were cultured either alone as the positive control (CD4 cells) or negative control (CD8 cells) for p24 production or cocultured at a CD8:CD4 ratio of 1:1 in triplicate wells of a 96-well microplate. Supernatant culture fluids were removed after 10 days and assessed for HIV p24 antigen by commercial ELISA (Coulter, Hialeah, FL). Results were considered positive if a ratio of 1:1 CD4:CD8 cells reduced p24 levels by >50% compared with levels of p24 antigen in cultures of CD4 cells alone.

Soluble activation molecules and cytokines. Levels of the immune activation molecules neopterin and β2 microglobulin and the cytokine TNFα were assessed in serum of HIV-infected pregnant women using commercial kits routinely used in the laboratory. Neopterin was determined using the IMMUnTest Neopterin radioimmunoassay (Henning Berlin GMBH, Berlin, Germany), β2 microglobulin was determined using an automated microparticle enzyme immunoassay system (IMx; Abbott, Abbott Park, IL), and TNFα levels were determined using the Innotest-hTNFα, ELISA (Innogenetics, Ghent, Belgium).

Plasma viral RNA levels. Quantitative HIV RNA PCR was performed as we have described in detail previously [2] using the Amplicor HIV Monitor assay (Roche Diagnostics, Nutley, NJ).

Statistical analysis. Median and interquartile determinations were made for all laboratory test results. Differences between groups were assessed using the Wilcoxon rank sum test. The relative risk was calculated to express the association between defective CD8 cell function (categorized dichotomously as described) and risk of transmission using Stata (version 5.0, 1997; Stata, College Station, TX). Logistic regression (LogXact, version 2.0, 1996; Cytel, Cambridge, MA) was used to assess the association between risk of transmission and low CD8 cell function when controlling for RNA viral load, AZT use, or CD4 percentage.

Results

Study subjects. The demographics of the HIV-positive mothers reflected the general distribution of the seroprevalent female populations in Los Angeles, with 41% Hispanic, 32% black, 23% white, and 4% other. The majority of the women had sex with an infected partner as their only risk factor; 6% used cocaine or intravenous drugs during pregnancy. Only 1 woman had a diagnosis of AIDS during pregnancy; 13% had CDC class B symptoms [14]. The characteristics of the transmitting and nontransmitting women at the third trimester/delivery are summarized in table 1. Seventy-three percent of the nontransmitting women received AZT at some time during pregnancy, including treatment at labor and delivery only. Four of the 9 transmitting women (44%) received AZT during pregnancy, although 1 was noncompliant and did not receive the full dose and a second developed primary HIV infection during the second trimester and had rising viral titers despite treatment.

Expression of cell surface antigens. Immunophenotyping was performed on serial samples during and after pregnancy. The percents of the major cell subsets did not differ significantly between the transmitting and nontransmitting women throughout pregnancy and postpartum (P > .08 at all points tested). However, CD4 cell percents tended to be higher and CD8 percents lower overall in the transmitting women (figures 1 and 2; data not shown for CD19 B cells and CD56 + 16 NK cells). There were no significant differences between the proportions of HLA-DR + CD38 + CD8 + cells (P > .2 at all points tested, figure 3) or CD8 + S6F1 + CD3 + cells (P > .2 at all points tested, figure 4) in women who transmitted infection and those who did not, although the latter subset tended to be lower in transmitting women. Percentages of the various T cell subsets for healthy, HIV-negative nonpregnant women are shown in each figure for comparison. We have shown previously [15] that percentages of these T cell subsets are not significantly different in healthy, HIV-negative pregnant versus nonpregnant women.
Table 1. Description of transmitting and nontransmitting mothers.

<table>
<thead>
<tr>
<th>Parameter tested</th>
<th>HIV-positive pregnant women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transmitters (n=9)</td>
</tr>
<tr>
<td>% CD4b</td>
<td>34 (12–48)</td>
</tr>
<tr>
<td>Plasma RNA copy number</td>
<td>65,516 (28,999–148,860)</td>
</tr>
<tr>
<td>Race:</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>3</td>
</tr>
<tr>
<td>Black</td>
<td>2</td>
</tr>
<tr>
<td>White</td>
<td>3</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
</tr>
<tr>
<td>AZT use:</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4 (44%)</td>
</tr>
<tr>
<td>No</td>
<td>5 (56%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>12 (18%)</td>
</tr>
<tr>
<td>CD8 cell suppression</td>
<td>44% (4/9)</td>
</tr>
</tbody>
</table>

a Values at 3d trimester/delivery.
b Median values (5th–95th percentile).
c Excluding unknowns.
d 36 of 66 women were HIV culture-positive and could be evaluated.

CD8 cell-mediated suppression of HIV production. Evaluation of CD8 suppressive function was performed during the third trimester. Of the 66 nontransmitting mothers, 30 were negative for virus production in culture and thus could not be evaluated for CD8 cell-mediated suppression. The remaining 36 (53%) had detectable HIV p24 antigen produced in their CD4 cell cultures; 29 of the 36 (81%) evaluable subjects showed >50% suppression of HIV in cultures containing CD8 cells as compared with cultures of CD4 cells alone.

All 9 of the transmitting mothers had p24 antigen detectable in their CD4 cell cultures and could be evaluated for CD8 cell-mediated suppression. Four of the 9 (44%) transmitting women showed >50% HIV suppression (table 1). Relative risk determined for mothers with evaluable CD8 cell-mediated function indicated that those with <50% suppression had a 3.4 times greater risk of transmission (95% confidence interval [CI], 1.1–10.7) than those able to suppress HIV in vitro.

It is of note that the 30 nontransmitting women who could not be evaluated for CD8-mediated suppression because of negative CD4 cell cultures were not significantly different from those 36 who could be assessed with respect to CD4 percent, ethnicity, AZT therapy, or illicit drug use. Not surprisingly, however, the 36 women from whom virus could be cultured did have significantly higher median plasma RNA copy number than the virus-negative women (P<.007).

Since the rate of vertical transmission of HIV has been previously shown to be associated with AZT use, percent CD4, and viral load [1–5], multivariate analysis was used to determine whether the association between low CD8 cell function and transmission was independent of these variables. Low CD8 cell-

Figure 1. Median CD4 cell percentages for human immunodeficiency virus (HIV)-infected mothers who did (circles) or did not (triangles) transmit infection to their infants. Time points for testing on the x axis include the trimesters (tri) during pregnancy, delivery, and postpartum (pp). The interquartile range is indicated by the bars. Median value for healthy, HIV-seronegative, nonpregnant women is indicated by the dotted line. The numbers of subjects evaluated at each time point are given for the transmitting women and the nontransmitting women.
mediated suppression was a significant predictor of transmission (odds ratio [OR] = 10.7, P < .002) when controlling for AZT use (OR = 0.04, P < .001) and CD4 percent >25 (OR = 1.04, P = .07). Low CD8 cell function was not a significant predictor of transmission (P = .85) when controlling for the log of plasma RNA levels (P = .001). That is, CD8 suppression does not appear to be an independent predictor when controlling for viral load.

**Soluble activation molecules and cytokines.** Levels of the serum activation molecule β2 microglobulin were not significantly different between the transmitting and nontransmitting women at any time point tested (data not shown), although they were significantly higher (P < .001) in both groups of HIV-positive women (median value at delivery = 1.9 mg/mL) than in HIV-negative women (median value 1.0 mg/mL). Similarly, levels of serum neopterin were not significantly different be-
between the transmitting and nontransmitting women (data not shown), they were although significantly higher ($P < .001$) in the HIV-positive women (median at delivery = 13 nmol/L) than in HIV-negative controls (median = 4.9 nmol/L). The only serum marker that was significantly different between transmitting and nontransmitting women was the cytokine TNFα, which was increased postpartum in transmitting women. The difference was significant ($P < .03$) at 3 months postpartum (figure 5). It is of note that our previous study of healthy, uninfected pregnant women [15] showed that serum neopterin and β2 microglobulin (but not TNFα) increase during normal pregnancy over levels seen in healthy, nonpregnant females but not to the levels seen in HIV-infected pregnant women.

**Viral burden.** Plasma RNA levels were significantly higher ($P < .001$) at delivery in transmitting women compared with nontransmitters. Median values and 95th CI are shown in table 1.
Discussion

A vigorous cell-mediated immune response is thought to be critical to the control of HIV infection [reviewed in 6, 7]. In particular, the ability of CD8 cells to suppress viral production in vitro has been associated in HIV-infected homosexual men with low viral burden and asymptomatic disease [9]. The study presented here adds another dimension to the role of CD8 T cell-mediated suppression of HIV as a correlate of protective immunity in HIV infection, indicating that HIV-positive mothers whose CD8 T cells cannot suppress HIV production in vitro by at least 50% have a more than 3-fold greater chance of transmitting infection to their infants.

The transmission risk associated with <50% suppression of HIV may, in fact, be a conservative estimate. We have recently modified the assay system so that individuals whose CD8 cell cultures do not produce detectable autologous virus (such as those nontransmitting women who were virus-negative and unevaluable in the present study) can be assessed using CD4 cells exogenously infected with a pretittered amount of the NL4-3 strain of HIV-1. Recent data obtained in a group of HIV-infected asymptomatic homosexual men indicate that individuals with undetectable autologous virus production invariably show good (>70%) HIV suppression using this modified assay [16]. Unfortunately, however, we do not have the samples to retrospectively retest the subjects included in the present study. If we considered that the virus-negative mothers might be positive for suppressive function, the risk of transmission associated with reduced function would be considerably greater.

The interaction of CD8 cell-mediated viral suppression with other reported predictors of transmission must be considered in interpreting the results of our study. Not surprisingly, viral load was a confounding variable, indicating that CD8 cell function and levels of HIV in the periphery are interrelated. This suggests that when the immune system is able to control viral replication and to reduce viral burden, transmission is less likely to occur. However, as a predictor of transmission, CD8 suppression was independent of AZT use and CD4 > 25%, both of which have been associated with reduced risk of transmission [1-5]. In fact, the risk of transmission associated with low CD8 cell suppression was even greater when controlling for these variables. Although AZT is able to reduce the risk of transmission presumably at least in part by reducing viral burden [17, 18], it was not a confounding variable. This may be due to the fact that during the time frame of this study, AZT was not universally recommended during pregnancy. Thus, AZT was given predominantly to those with highest viral loads (and lowest CD4 cell counts) and often was only administered during labor and delivery, possibly reducing its impact in our study.

The other immunologic parameters studied herein were not significantly different between transmitting and nontransmitting women during pregnancy. This may be due to a reduced power to detect differences because of the small number of transmitting women. Previous studies of immunologic risk factors for maternal transmission are few and have not produced consistent findings. Low absolute numbers of maternal CD4 cells have been associated with vertical transmission [1, 2]. However, Borkowsky et al. [3] found, as we did, no significant differences in the percentages of CD4 or CD8 cells between transmitting and nontransmitting women. A recent report from the Women and Infants Transmission Study [19] in which 475 mother-infant pairs (with 94 infected infants) were studied indicated that low CD4 cell percent, high total CD8 cell percent, and CD8+/CD38+ and CD8+/HLA-DR+ cell percent were associated with transmission. However, the association held up only in those 42% of women whose HIV cultures were not persistently positive. In our study, plasma HIV RNA (which was detectable in all the HIV-infected women) rather than quantitative coculture was used as a measurement of viral load.

Although useful prognostic indicators in HIV infection [10], serum activation molecules have shown variable usefulness as markers for maternal transmission. A study of 27 transmitters and 83 nontransmitters in Africa found, as we did, no differences in β2 microglobulin levels between the groups [20]. However, a Swiss study found significantly elevated serum neopterin in transmitting women [21]. Serum TNFα has not, to our knowledge, been assessed in regard to maternal transmission. The increase in TNFα levels in the transmitting mothers may be related to their higher viral titers, as plasma RNA level and TNFα have been shown to correlate [22]. The timing of the rise in TNFα levels may reflect peripartum increases in viral titers seen in 5 of the transmitting women (data not shown).

In summary, our findings suggest that if viral load is reduced or controlled by an effective immune response, transmission of HIV from mother to baby is less likely to occur. Thus, the most effective strategies for reducing maternal transmission of HIV should be directed at both reducing viral load through highly active antiretroviral therapy and augmenting protective host responses such as CD8 T cell-mediated immunity.

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