Lack of Association between Human Immunodeficiency Virus Type 1 Antibody in Cervicovaginal Lavage Fluid and Plasma and Perinatal Transmission, in Thailand

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To determine the association between human immunodeficiency virus type 1 (HIV)–specific antibody and RNA levels in cervicovaginal lavage (CVL) samples and plasma, zidovudine treatment, and perinatal transmission, HIV subtype E gp160–specific IgG and IgA were serially measured in a subset of 74 HIV-infected women in a placebo-controlled trial of zidovudine, beginning at 36 weeks of gestation. HIV IgG was detected in 100% of plasma and 97% of CVL samples; HIV IgA was consistently detected in 62% of plasma and 31% of CVL samples. Antibody titers in CVL samples correlated better with the RNA level in CVL samples than with plasma antibody titers. Zidovudine did not affect antibody titers. Perinatal HIV transmission was not associated with antibody in CVL samples or plasma. HIV-specific antibody is present in the cervicovaginal canal of HIV-infected pregnant women; its correlation with the RNA level in CVL fluid suggests local antibody production. However, there was no evidence that these antibodies protected against perinatal HIV transmission.

Most perinatal transmission of human immunodeficiency virus type 1 (HIV) occurs during the intrapartum period, particularly in non–breast-feeding women [1, 2], and may be due to exposure of the newborn to infected blood or secretions while passing through the birth canal or to a compromise in the placental barrier before delivery. Recent studies have shown that high maternal virus levels in blood [3–5] and cervicovaginal lavage (CVL) samples [6] are associated with increased risk for perinatal transmission. Zidovudine can reduce the risk of transmission by reducing maternal virus levels [4, 6]. Nevertheless, some women with high levels of virus in plasma and cervicovaginal secretions do not transmit HIV to their infants [4–9]. This suggests that transmission may be inhibited in part by systemic or local protective mechanisms, such as HIV-specific antibody. Although the role of antibody in perinatal HIV transmission is unknown, mucosal antibody in other viral infections has been reported to prevent disease, decrease viral replication, or lessen the severity of disease [10, 11]. If found to be protective against perinatal HIV transmission, mucosal antibody could be enhanced by vaccine or passive antibody infusion and could be a supplement or an alternative to antiretroviral treatment. The presence of IgG and IgA antibody to HIV in cervicovaginal secretions of HIV-infected women has been reported in several studies [12–18]. However, the rates of antibody detection differ, and the relationship of antibody to HIV level and perinatal transmission has not been well characterized. In one study, HIV-specific secretory IgA (sIgA) in CVL specimens was associated with increased risk for perinatal transmission [16]. Another study suggested that mucosal HIV sIgA did not totally neutralize virus in culture and was not associated with transmission [17]. We conducted this study of HIV-infected pregnant women to determine whether HIV-specific antibody in CVL specimens was associated with plasma antibody, HIV RNA levels in CVL specimens, antenatal zidovudine treatment, and risk for perinatal transmission.
Table 1. Percentages of plasma and cervicovaginal lavage (CVL) specimens positive for human immunodeficiency virus (HIV) subtype E gp160–specific IgG and IgA, overall and by study visit.

<table>
<thead>
<tr>
<th>Time point</th>
<th>Plasma</th>
<th>CVL samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>IgA</td>
</tr>
<tr>
<td>All time points</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td>Any time point</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>Prenatal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All prenatal</td>
<td>214</td>
<td>92</td>
</tr>
<tr>
<td>36 Weeks’ gestation</td>
<td>74</td>
<td>92</td>
</tr>
<tr>
<td>37 Weeks’ gestation</td>
<td>72</td>
<td>93</td>
</tr>
<tr>
<td>38 Weeks’ gestation</td>
<td>68</td>
<td>72</td>
</tr>
<tr>
<td>1 Month postpartum</td>
<td>72</td>
<td>100</td>
</tr>
</tbody>
</table>

NOTE. P < .001 for each comparison of women positive for HIV IgA in plasma and CVL samples during pregnancy and 1 month postpartum.

Methods

Background and study population. This study was conducted at 2 large hospitals in Bangkok as a substudy of a randomized placebo-controlled efficacy trial of short-course oral antenatal zidovudine to prevent perinatal HIV transmission [4]. Treatment with zidovudine consisted of 300 mg orally twice daily from 36 weeks’ gestation until the onset of labor and 300 mg every 3 h from the onset of labor until delivery. All women were provided infant formula and counseled to not breast-feed. Infants were considered HIV-infected if any polymerase chain reaction (PCR) test was positive and were considered uninfected if their last available PCR test result was negative at >2 months of age. From May 1996 through December 1997, 397 HIV-infected pregnant women (198 in the zidovudine group and 199 in the placebo group) were enrolled and randomly assigned to the treatment or control group. The estimated perinatal HIV transmission rate was 9.4% in the zidovudine group and 18.9% in the placebo group, a 50.1% reduction in transmission risk (P = .006) [4].

Women consecutively enrolled between February and July 1997 were offered enrollment into this substudy [6]. Blood samples for measurement of HIV RNA and antibody were collected at 36 weeks’ gestation (before the start of study treatment), weekly until delivery, and at 1 month postpartum. CVL samples were collected at 36, 37, and 38 weeks’ gestation and at 1 month postpartum.

CVL sample collection. CVL samples were obtained according to published procedures of the Division of AIDS, National Institutes of Health, virology manual for HIV laboratories [19], with the modification that 3 mL (instead of 10 mL) of saline was used to flush the cervix and the vaginal wall. After the flush, as much of the fluid as possible was re-collected with a transfer pipette. Samples were transported on ice to the local laboratory and centrifuged at 750–1000 g at 4°C for 15 min. The supernatants and pellets were separately aliquoted and stored at −70°C.

HIV subtype E gp160–specific IgG and IgA measurements by EIA. The method used to measure HIV subtype E gp160–specific IgG and IgA in CVL samples and plasma has been described elsewhere [18, 20]. Briefly, recombinant vaccinia virus–expressed oligomeric gp160 from the HIV clade E strain TH023, a primary isolate of the R5 type, at a concentration of 0.6 μg/mL in PBS was coated overnight at 4°C onto round-bottom microtiter plates. For HIV IgA, the plates were blocked with bovine serum albumin (BSA)–casein for 1 h, washed 3 times, and incubated overnight with 2-fold dilutions of samples in BSA-casein at room temperature. The plates were then washed 4 times, incubated for 6 h at room temperature with horseradish peroxidase (HRP)–labeled rabbit anti-human IgA, and washed 4 more times, after which substrate (3,3′,5,5′-tetramethylbenzidine) was added. The reactions were stopped with 1 M H3PO4 after 15 min at 37°C. For HIV IgG, the plates were incubated with 2-fold dilutions of samples for 1 h at 37°C. The plates were then washed 3 times, incubated for 1 h at 37°C with HRP-conjugated goat anti-human IgG, and washed 3 more times, after which substrate (ABTS [2,2′-azino-di(3-ethylbenzthiazoline-6-sulfonate), 0.3 g/L in a glycine/citric acid buffer; Kirkegaard & Perry, Gaithersburg, MD) was added. The reactions were stopped with 0.5% SDS after 30 min at 37°C. Antibody end-point titers were determined from the maximum dilution of plasma or CVL sample at which the optical density signal was more than twice the mean ± 2 SD for low-risk HIV-seronegative controls. The seronegative controls were at low risk for HIV infection and came from a study published elsewhere [20].

HIV RNA testing by PCR. HIV RNA levels in plasma and CVL samples were determined by use of a quantitative reverse transcriptase–PCR assay (Amplicor HIV-1 Monitor test, version 1.5; Roche Diagnostic Systems, Branchburg, NJ). CVL supernatants were tested by the same procedures as used for plasma. The lower quantitation limit for this assay was 400 copies/mL.

Statistical analysis. Data were analyzed with SAS software, version 6.12 (SAS Institute, Cary, NC). For analyses that used reciprocal end-point titers as continuous variables, samples that tested negative at the starting dilution were assigned the highest potential titer below the starting dilution. For HIV IgG in plasma (starting dilution, 1:12,800), a reciprocal titer of 6400 was assigned. For HIV IgG in CVL fluid and HIV IgA in plasma (starting dilution, 1:100), a reciprocal titer of 50 was assigned. For HIV IgA in CVL fluid (starting dilution, 1:4), a reciprocal titer of 2 was assigned. For analyses with dichotomous variables, samples that tested negative at the starting dilution were considered negative. For analyses that used RNA measurements as continuous variables, values below the level of quantitation (400 copies/mL) were assigned a value of 200 copies/mL.

To evaluate differences in antibody titers by treatment group and by infant infection outcome, we analyzed log-transformed end-point antibody titers. Medians, means, and SDs of end-point titers for paired plasma and CVL samples were determined by treatment group and infant infection outcome. Mean antibody titers during late pregnancy for each woman were calculated as the mean of titers at 36, 37, and 38 weeks’ gestation. We compared treatment groups and infant HIV infection outcomes by use of Student’s t test for continuous variables and χ2 for categorical variables. Spearman rank-order correlation coefficients were used to correlate plasma and CVL antibody titers and to correlate antibody titers and HIV RNA levels. Two-sided P values <.05 were considered statistically significant.

Results

HIV gp160–specific antibody. Of 74 HIV-infected women, 36 were assigned to the zidovudine group and 38 to the placebo group. Results of plasma testing were positive for HIV IgG for
all women at all time points, for HIV IgA for 46 (62%) at all time points, and for HIV IgA at least once for 71 (96%; table 1). In CVL samples, 72 women (97%) were positive for HIV IgG at all time points, all were positive for HIV IgG at least once, 23 (31%) were positive for HIV IgA at all time points, and 71 (96%) were positive for HIV IgA at least once.

The percentages of women positive for HIV IgG in plasma and CVL samples were high (>90%) both during pregnancy and at 1 month postpartum. In contrast, the percentages of women positive for HIV IgA in plasma and CVL samples were significantly higher at 1 month postpartum than during pregnancy (table 1).

HIV antibody titers were similar during the late pregnancy visits (36, 37, and 38 weeks). For all women, there was no significant difference in plasma HIV IgA titers between pregnancy and postpartum specimens. However, mean titers of HIV IgG in postpartum plasma samples and both IgA and IgG in postpartum CVL samples were significantly higher than during pregnancy (table 2).

Correlation between HIV-specific antibodies in plasma and CVL samples. Overall, there was a significant correlation between HIV IgG in CVL samples and plasma (r = .42; P < .001). HIV IgA titers in CVL fluids during pregnancy were similarly correlated with antibody titers in plasma (r = .51; P < .001). However, there was no correlation between HIV IgA titers in CVL samples and plasma at 1 month postpartum (r = .07; P = .54).

Correlation between antibodies and HIV RNA levels in CVL samples. There was a modest but significant correlation between HIV-specific antibody titers and RNA levels in CVL samples (figure 1). HIV antibody titers in CVL samples were more closely correlated with RNA levels in CVL samples than were HIV antibody titers in plasma.

Antibody level and perinatal HIV transmission. Of 74 in-
Table 2. End-point titers of human immunodeficiency virus subtype E gp160–specific IgG and IgA in plasma and cervicovaginal lavage (CVL) specimens during pregnancy (36–38 weeks’ gestation) and at 1 month postpartum.

<table>
<thead>
<tr>
<th>Sample, antibody, time point</th>
<th>n</th>
<th>Geometric mean</th>
<th>Mean log</th>
<th>Median (range)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma IgG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy</td>
<td>74</td>
<td>252,000</td>
<td>5.4</td>
<td>341,000 (14,900–1,911,000)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Postpartum</td>
<td>72</td>
<td>511,000</td>
<td>5.7</td>
<td>819,200 (512,000–3,276,800)</td>
<td>.05</td>
</tr>
<tr>
<td>CVL fluids IgG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy</td>
<td>74</td>
<td>685</td>
<td>2.8</td>
<td>808 (50–21,000)</td>
<td>.14</td>
</tr>
<tr>
<td>Postpartum</td>
<td>72</td>
<td>970</td>
<td>3.0</td>
<td>800 (50–25,600)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Data for titers during pregnancy are mean values for 36, 37, and 38 weeks’ gestation for each woman.

* For comparison of mean log antibody titers during pregnancy and at 1 month postpartum.

Discussion

Transmission of HIV from mother to infant during labor and delivery may be influenced in part by local factors in the genital mucosa. We recently reported the association between high HIV RNA level in cervicovaginal secretions at 38 weeks’ gestation and increased risk for perinatal HIV transmission, as well as the ability of zidovudine administered late in pregnancy to reduce cervicovaginal HIV RNA levels [6]. In this analysis from the same study, we have demonstrated that HIV-specific antibodies are detectable in CVL fluids, although IgA is detectable less frequently than is IgG, and that HIV-specific antibody titers correlate with HIV RNA levels in the cervicovaginal canal.

Despite finding a significant correlation between both IgG and IgA titers and RNA levels in CVL samples, we did not find an association between perinatal transmission and plasma or CVL fluid antibody level. In other viral infections, mucosal antibody has been reported to decrease viral replication and to eliminate disease or lessen its severity [10, 11]. In a cohort of highly exposed but uninfected women, high HIV IgA levels were detected in cervicovaginal secretions [21]. It has been suggested that maternal neutralizing antibody is important in preventing perinatal HIV transmission [3]. One study with a small sample reported detection of HIV sIgA from women from whom HIV could be isolated in cervicovaginal secretions, a finding that suggested that the antibody was not neutralizing the virus [17]. Our finding that there was no difference in HIV antibody titers in plasma or CVL samples or in frequency of antibody detection in plasma and CVL samples between women who transmitted and women who did not transmit may be explained by a lack of substantial in vivo neutralization. In one study, increased risk of perinatal HIV transmission was associated with the presence in CVL fluids of HIV-specific sIgA but not IgG or IgM antibodies, but the HIV level in CVL samples was not evaluated [16]. Our finding that antibody titers and virus levels in CVL fluids are related and our finding elsewhere that a high HIV RNA level in CVL fluids increases the risk for perinatal transmission at both high and low levels of virus in plasma [6] suggest that this reported association between HIV-specific sIgA in CVL fluids and the increased risk for perinatal transmission may have been confounded by the virus level in CVL fluids.

Our results indicate that HIV env–specific responses in plasma and CVL samples were predominantly IgG, with weak to undetectable env-specific IgA responses. This finding supports the results of other studies [15, 18] and suggests that there may be an increase in or overproduction of HIV IgG compared with IgA.

We did not find changes in antibody titers as a result of short-course zidovudine treatment, although HIV RNA levels in plasma and CVL samples decreased as early as 1 week after the start of treatment [4, 5]. Antibody titers in plasma and CVL samples in both groups were similar and remained relatively constant. This finding is consistent with the reported lack of effect of antiretroviral treatment on HIV IgG or IgA responses.
Figure 2. Transmitting versus nontransmitting women: human immunodeficiency virus (HIV) subtype E gp160-specific IgA and IgG antibody in cervicovaginal lavage (CVL) samples and plasma. Shown are box plots (median, 25th and 75th percentiles) of antibody end-point titers for 12 women with HIV-infected infants (shaded boxes), compared with 62 women with uninfected infants (open boxes), at 36 (baseline), 37, and 38 weeks' (W) gestation and at 1 month postpartum (PP).

[18]. However, our negative finding could also be explained by the short treatment and observation period and relatively weak antiretroviral activity of zidovudine monotherapy. It is possible that potent combination antiretroviral therapy that decreases plasma HIV RNA to levels below quantitation might be associated with significant declines in HIV-specific antibody levels.

The correlation between HIV IgG and HIV IgA levels in plasma and CVL fluids suggests that antibody in CVL fluids derives primarily from plasma. However, the lack of correlation between HIV IgA titers in plasma and CVL fluids postpartum and the observation that IgA levels in CVL fluids are higher postpartum than during pregnancy also suggest that HIV IgA is produced locally in the female genital tract, such as in the mucosal lining of the uterine cavity, which is occupied during pregnancy. This agrees with observations that, although HIV IgA in CVL fluids reflects systemic transudation and secretory local production, most HIV IgG antibody at the mucosal surface of the genital tract is derived from serum and crosses to the mucosal surface by passive diffusion [10, 22, 23]. Furthermore, our findings that antibody levels (particularly IgA) in CVL samples correlate more strongly with virus levels in CVL samples than with antibody levels in plasma and that the virus level in CVL fluids increased at 1 month postpartum [6] suggest that antibody in the cervicovaginal canal also reflects a local immune response to virus in the cervicovaginal canal.

Our data have some limitations. Although we followed currently recommended procedures for CVL sample collection, no
standardized procedure allows the accurate measurement of the volume of cervicovaginal secretions; thus, the accuracy of quantitative measures of virus and antibody in CVL samples is limited. Nevertheless, our quantitative measures were consistent over time. In addition, because most locally produced IgA derives from the cervix and the uterine body, cervical sample collections by CVL may not be as sensitive as by wick in detecting IgA. Finally, although CVL specimens obtained during delivery might best reflect transmission risk factors, it would be difficult to obtain CVL specimens at delivery without contamination by blood or other fluids and without posing unnecessary risks. We assumed that specimens obtained at 38 weeks’ gestation are a good marker for mucosally derived antibody in the birth canal at delivery.

This study confirms that HIV-specific antibody is detectable in the cervicovaginal canal and that some HIV-specific antibody is likely produced locally by the cervicovaginal mucosa. Although plasma and cervicovaginal subtype E gp160–specific antibody levels do not seem to protect against perinatal transmission, it remains possible that antibody directed at specific epitopes or with different functional capacity plays a role in perinatal transmission [24]. Further investigation is needed to better define the relationship between mucosal antibody, virus level, and transmission.

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Acknowledgments

We thank T. Dondero, J. Karon, E. Lackritz, B. Parekh, R. Resesp, and R. J. Simonds (Centers for Disease Control and Prevention) and K. Nelson and the late M. L. Clements-Manoh (Johns Hopkins University) for their support and advice; R. El Habib (Pasteur-Merrieux- Connaught) for donation of recombinant vaccinia virus-expressed oligomeric gp160 from HIV type 1 (TH023); and E. Hollander and J. Lateef (Henry M. Jackson Foundation) for technical assistance. We gratefully acknowledge the dedicated field work of the project study nurses and的社会 workers: K. Neeyapun and B. Jetsawang (team leaders); and S. Bhengsi, S. Henchaichon, S. Jalan chavanapat, K. Klumthanom, R. Krajangthong, C. Prasert, W. Sanyanusin, W. Suwannapha, W. Triphanitchuk, S. Sorapipatanat, S. Suwannairet, and C. Yu vesevee. We also thank the HIV/AIDS Collaboration laboratory staff for specimen processing and virus load testing: T. Chaowanachan, P. Wasinrappee, N. Kaewpun t, O. Sukrispanich, A. Srisut, and W. Leelawiwat.

References