Placental Abnormalities Associated with Human Immunodeficiency Virus Type 1 Infection and Perinatal Transmission in Bangkok, Thailand

David A. Schwartz,1,a Suthi Sungkarat,3
Nathan Shaffer,2,a Jirasak Laosakkitiboran,4
Wendy Supapol,1 Pichai Charoenpanich,3
Tuenjal Chuangsawanich,4
and Timothy D. Mastro2,5

1Departments of Pathology and Medicine, Division of Infectious Diseases, Emory University School of Medicine, and 2Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia; Departments of 3Obstetrics and Gynecology and 4Pathology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, and 5HIV/AIDS Collaboration, Nonthaburi, Thailand

The effects of human immunodeficiency virus (HIV) type 1 on the placenta and the role of the placenta in mother-to-child HIV-1 transmission are not well understood. Placentas from 78 HIV-infected and 158 HIV-uninfected women were examined as part of a prospective perinatal HIV transmission study in Bangkok. HIV-infected women were more likely than HIV-uninfected women to have chorioamnionitis (odds ratio [OR], 2.1; \( P = .03 \)), placental membrane inflammation (PMI; OR, 2.7; \( P = .02 \)), and deciduitis (OR, 2.3; \( P = .03 \)) and less likely to have villitis (OR, 0.3; \( P = .02 \)). However, among HIV-infected women, fewer women who transmitted infection to their child had chorioamnionitis (relative risk [RR], 0.2; \( P = .03 \)), funisitis (RR, 0.4; \( P = .1 \)), or PMI (RR undefined; \( P = .03 \)). These findings suggest that, in this population, HIV-infected women are at increased risk for placental membrane inflammatory lesions, but that placental inflammatory lesions are not associated with increased perinatal HIV transmission.

Recent studies suggest that most perinatal human immunodeficiency virus (HIV) type 1 infections occur at or near birth [1–4]. This contrasts with earlier hypotheses that most transmission occurs in utero through transplacental transmission [5, 6]. Although it is presumed that the placenta plays a major role in protecting against perinatal HIV transmission, the effects of HIV on the placenta are not well understood.

In view of several reports from Africa that chorioamnionitis is a risk factor for perinatal HIV transmission [7–10], there is heightened interest in determining whether there are differences in the spectrum of placental lesions, particularly inflammatory lesions, between HIV-infected and -uninfected women and whether specific lesions are related to an increased risk of perinatal HIV transmission. Currently, there are no clear answers to these questions. Findings on the effects of HIV on the placenta differ. Studies suggest both increased [11] and decreased [12] placental weight, decreased prevalence of villitis [10], and increased prevalence of chorionitis [13], chorioamnionitis, funisitis, placental membrane inflammation (PMI) [7], and villous immaturity and allantois vasculopathy [14] in placentas from HIV-infected women. However, other studies suggest no or few placental differences associated with HIV [13, 15].

Most large studies of perinatal HIV transmission have not evaluated placental pathology [16–22]. Among the few published studies that include placental pathology, several studies in Africa [7–10], India [23], and the United States [24] report increased transmission risk associated with inflammatory lesions of the membranes and cord (chorioamnionitis and funisitis). However, these findings were not confirmed in a separate analysis from Kenya [15] or in a large unpublished US study [25]. Different findings may be due to differences in study populations, number of placentas examined, diagnostic criteria and expertise, thoroughness of placental sampling, and variability in the definition of infant infection. Findings on transmission in developing countries may also be subject to misclassification, due to difficulty in distinguishing intrapartum and early postnatal transmission (via breast-feeding). Antibody-based and molecular studies of placentas from HIV-infected women have yielded markedly different results and have not clarified the pathogenic mechanisms or timing of perinatal HIV transmission [13, 14, 26–29]. In Thailand, we addressed these questions in a cohort of HIV-infected women who were enrolled ante-natally and who did not breast-feed and in a control group of...
noninfected women. We used standardized diagnostic criteria and focused our investigation on inflammatory conditions of the placenta.

**Subjects and Methods**

**Study participants.** The placental pathology study was conducted as a substudy of the Bangkok Collaborative Perinatal HIV Transmission Study, which enrolled pregnant women residing in the local catchment areas of the 2 largest antenatal clinics in Bangkok from November 1992 through March 1994. The methods and main findings regarding enrollment and the characteristics of the women, HIV transmission rate, and risk factors have been described elsewhere [30, 31]. None of the women received zidovudine. In accordance with local guidelines, the HIV-infected women did not breast-feed. The perinatal HIV transmission rate for the full cohort of 281 women with known outcomes was 24.2% (95% confidence interval [CI], 19%-29%). At one study hospital from October 1993 through March 1994, a consecutive sample of 78 of 81 HIV-infected women enrolled in the main transmission study were enrolled in the placenta substudy. For logistic reasons, placentas from 3 women were not collected. During this period, 155 HIV-infected women gave birth at the study hospital. All study women had laboratory testing (including CD4/CD8 lymphocyte counts, quantitative plasma virus load, and HIV-1 subtyping [31]), and their clinical histories were obtained.

Infants born to HIV-infected mothers were followed up every 2-3 months. Venous blood samples obtained at birth and at 2 and 6 months were tested for proviral HIV DNA by polymerase chain reaction (PCR). Infants were considered to be HIV infected if they had 2 positive DNA PCR test results or 1 positive PCR test result and a 1993 CDC AIDS-defining condition. Infants were defined as uninfected if they tested PCR negative on 2 samples, including 1 sample obtained at age ≥6 months, or if they seroreverted to HIV-negative status on antibody testing. Infants who did not meet these criteria were considered to have unknown infection status. Mothers of infected infants were considered to be “transmitters”; mothers of uninfected infants were considered to be “nontransmitters.”

For the placental pathology substudy, after an HIV-infected study woman gave birth, the next 2 available HIV-negative women who gave birth were enrolled as control subjects. During this period, ~9000 HIV-negative women gave birth. The 158 HIV-negative women were enrolled systematically, on the basis of time of delivery. Placentas from HIV-negative women were handled identically to those of HIV-infected women.

**Placental gross examination and processing.** Immediately after delivery, placentas were placed in buffered formalin and transferred to the Department of Obstetrics and Gynecology pathology laboratory. To ensure uniformity, one obstetrician trained in placental pathology (S.S.) processed all study placentas without knowing HIV status. Specific placental pathology submission forms were used. Most specimens were fixed for <24 h, although placentas delivered on weekends were fixed longer.

We used universal precautions and systematically examined and sampled all placentas in an identical manner, according to a standardized protocol. The specimens were measured, and extra- placental membranes, umbilical cord, and adherent blood clots were removed before the placental body was weighed. The placenta was sectioned serially at 1-cm intervals, to identify gross lesions. Findings were recorded by the same investigator in Bangkok on a placental pathology work sheet adapted from work sheets designed for the placental pathology component of the National Institutes of Health Women and Infants Transmission Study [32].

At least 11 sections were examined histologically: 4 sections of extra-placental membrane roll, with sampling of all 4 quadrants, and the region of membrane rupture; 2 sections of umbilical cord, including the proximal and distal regions of the cord; and 5 sections of the placental body, including the mid-disc maternal and fetal surfaces, tissue at and adjacent to the umbilical cord insertion, and the marginal placenta. In addition, grossly visible lesions (e.g., infarcts, abscesses, or hemorrhages) were sampled. Sections were processed for light microscopy, by standard histologic methods, at the study hospital. Slides were stained with hematoxylin-eosin and were shipped with corresponding pathology work sheets to Atlanta for final microscopic diagnosis. Paraffin blocks of placental tissue were saved for reference and future studies.

**Placental microscopic diagnosis.** All placental tissue slides were examined by one pathologist (D.A.S.) with placental and infectious disease expertise. Slides and coded placental pathology work sheets were evaluated without knowledge of HIV status or transmission outcome. The pathologist had access to the following information: maternal age, gestational age, pregnancy complications, mode of delivery, placental weight, fixation time, and gross placental features.

Microscopic findings were entered on a precoded form designed for thorough classification of inflammatory conditions of the placenta [32]. Funisitis (inflammation of the umbilical cord) was graded as mild, moderate, or severe; the predominant inflammatory cell type was noted; structural involvement was identified (vein, artery, and Wharton’s jelly), and associated findings (e.g., meconium-associated myonecrosis, microabscesses, vascular necrosis, or thrombosis) were described. Chorioamnionitis was semi-quantitatively classified as low grade (mild-to-moderate; average of <10 inflammatory cells per ×40 field in areas of involvement) or high grade (severe; average of >10 inflammatory cells per ×40 field in areas of involvement), on the basis of the number of inflammatory cells infiltrating the chorioamnionic plate. The anatomic distribution of chorioamnionitis (i.e., extra-placental membranes, placental disk, or both), predominant inflammatory cell type (e.g., neutrophils or lymphocytes), and associated findings (e.g., hemosiderin or meconium deposition, necrotizing inflammation, microabscesses, or amniotic macrophage hyperplasia) were noted. PMI was defined as the simultaneous occurrence of both chorioamnionitis and funisitis.

In the placental body, villitis (inflammation of the chorionic villi) was characterized by the predominant inflammatory cell type, the anatomic extent and localization (focal, multifocal, diffuse, or basal), and the presence of associated necrosis (necrotizing villitis). Other precoded placental body diagnoses included intervillitis, villous stromal fibrosis, edema, intravillous hemorrhage, infarction, hemorrhagic endovasculitis, the presence of circulating immature fetal erythroid cells, and maternal (intervillous) space abnormalities (i.e., leukocytosis, excessive fibrin, thrombosis, or abscesses). In any region of the placenta, the presence of infectious agents (e.g., viral inclusions, fungi, or bacteria) was noted.

**Data analysis.** Completed pathology work sheets were double
entered, verified, and merged with the main perinatal study database. Descriptive statistics and frequencies for the pathology data were generated. Statistical comparisons between HIV-infected and -uninfected women and HIV-infected transmitting and nontransmitting women were made by using the Wilcoxon rank sum test for continuous variables; odds ratios (ORs), relative risks (RRs), and 95% CIs were calculated for discrete variables. We used Pearson’s χ² and 2-tailed Fisher’s exact tests to test for statistical differences (P ≤ .05).

Results

Placentas from HIV-infected and -uninfected women. Placentas from 78 HIV-infected and 158 HIV-uninfected control women were studied. HIV-infected and -uninfected women did not differ in maternal age, gestational age, or rate of cesarean section (table 1). Only 1 woman had prolonged rupture of membranes (PROM; >24 h). There was 1 stillbirth. Primiparity, having ≥ 1 abortions, and VDRL positivity were associated with HIV infection.

The key placental pathology findings among HIV-infected and -uninfected women are shown in table 2. Mean placental weight was greater for HIV-infected mothers. To determine whether the association of decreased placental weight with HIV was confounded by prematurity or birth weight, their interaction was analyzed by logistic regression. HIV status (P < .001) and birth weight (P < .001) were independently associated with lower placental weight.

Significantly more HIV-infected than -uninfected women had chorioamnionitis (OR, 2.1), PMI (OR, 2.7), and plasma cellular deciduitis (OR, 2.3; table 2). In placentas with chorioamnionitis, high-grade chorioamnionitis was more prevalent in HIV-infected than in -uninfected women (OR, 7.3). Also, in placentas with chorioamnionitis, acute (polymorphonuclear) chorioamnionitis tended to be more common than chronic (lymphocytic) chorioamnionitis in HIV-infected than in -uninfected women (OR, 6.3), but this was not statistically significant. More uninfected women had villitis of all histologic types. Among HIV-infected women, the absence of villitis was not correlated with low CD4 cell count (P = .8). There were no other significant differences between placentas from HIV-infected and -uninfected women.

Placentas from transmitting and nontransmitting HIV-infected mothers. Definitive HIV infection status was known for 75 infants (96%) born to HIV-infected mothers in the pathology substudy. Of these, 17 (22.7%) were HIV infected. For mothers who transmitted infection, the frequency of several inflammatory lesions was decreased: chorioamnionitis (RR, 0.2; P = .03); PMI (RR undefined; P = .03); either villitis or chorioamnionitis (RR, 0.2; P = .03); and either villitis or PMI (RR undefined; P = .03; table 3). A trend toward decreased prevalence of funisitis in transmitting mothers was also noted.

No histopathologic findings occurred with increased frequency in the placentas of transmitting mothers. However, compared with placentas of nontransmitting mothers, placentas of transmitting mothers showed a trend toward a higher frequency of plasma cellular deciduitis and abnormal villous maturity. Only 1 of 17 HIV-transmitting mothers had a placenta with chorioamnionitis; this was low grade and neutrophilic. Among 18 nontransmitting mothers with chorioamnionitis and a grade determination (1 was missing), 10 (56%) were high grade, and 17 (94%) were predominantly neutrophilic. No associations were noted between perinatal HIV transmission and intravillous hemorrhage, infarction, or villitis.

Other covariates. We found no significant differences or trends between placentas from mothers presumed to have transmitted in utero (n = 4) and intrapartum (n = 12; 1 infected child could not be classified with regard to timing) [4]. However, there were few pathologic lesions among transmitting women, and the number of in utero and intrapartum infections in this subset was small. The 1 transmitting woman with chorioamnionitis transmitted intrapartum. Among HIV-infected women, maternal HIV plasma load was not related to chorioamnionitis (median RNA copies/mL, 11,000 vs. 18,000 for women with or without chorioamnionitis, respectively; P = .5) or other placental findings. Maternal HIV-1 subtype distribution (97% subtype E and 3% subtype B) was similar to that of the full perinatal cohort. Because of the high predominance of subtype E, it was not possible to determine whether there might be differences in placental pathology associated with viral subtype. None of the HIV-infected mothers had PROM (>24 h), so an effect on placental pathology could not be evaluated. Duration of ruptured membranes (<24 h) was not related to placental pathology. The prevalence of sexually transmitted diseases (STDs) was relatively low (table 1) [33]. Positive antenatal syph-
Table 2. Selected histopathologic findings in placentas of human immunodeficiency virus (HIV)-infected and -uninfected mothers.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HIV infected (n = 78)</th>
<th>HIV uninfected (n = 158)</th>
<th>Odds ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean placental weight (range), g</td>
<td>459 (280–700)</td>
<td>505 (300–890)</td>
<td>2.7 (1.1–6.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PMI</td>
<td>13 (17)</td>
<td>11 (7)</td>
<td>0.3 (0.1–0.9)</td>
<td>.02</td>
</tr>
<tr>
<td>Villitis</td>
<td>3 (4)</td>
<td>21 (13)</td>
<td>2.1 (1.0–4.2)</td>
<td>.03</td>
</tr>
<tr>
<td>Chorioamnionitis</td>
<td>21 (27)</td>
<td>24 (15)</td>
<td>7.3 (1.1–78)</td>
<td>.03</td>
</tr>
<tr>
<td>High grade</td>
<td>8 (40)</td>
<td>2 (8)</td>
<td>7.3 (1.1–78)</td>
<td>.03</td>
</tr>
<tr>
<td>Acute</td>
<td>19 (95)</td>
<td>18 (75)</td>
<td>6.3 (0.6–306)</td>
<td>.1</td>
</tr>
<tr>
<td>Plasmacellular deciduitis</td>
<td>16 (21)</td>
<td>16 (10)</td>
<td>2.3 (1.0–5.2)</td>
<td>.03</td>
</tr>
<tr>
<td>Hofbauer cell hyperplasia</td>
<td>11 (14)</td>
<td>16 (10)</td>
<td>1.5 (0.6–3.6)</td>
<td>.4</td>
</tr>
<tr>
<td>Funisitis</td>
<td>20 (26)</td>
<td>34 (22)</td>
<td>1.3 (0.6–2.5)</td>
<td>.5</td>
</tr>
<tr>
<td>PMI and villitis</td>
<td>1 (1)</td>
<td>1 (&lt;1)</td>
<td>2.0 (0.0–161)</td>
<td>.6</td>
</tr>
<tr>
<td>Villitis or chorioamnionitis</td>
<td>23 (29)</td>
<td>42 (27)</td>
<td>1.2 (0.6–2.2)</td>
<td>.6</td>
</tr>
<tr>
<td>Infection</td>
<td>6 (8)</td>
<td>13 (8)</td>
<td>0.9 (0.3–0.9)</td>
<td>.9</td>
</tr>
<tr>
<td>PMI or villitis</td>
<td>15 (19)</td>
<td>31 (20)</td>
<td>1.0 (0.5–2.0)</td>
<td>.9</td>
</tr>
<tr>
<td>Villitis and chorioamnionitis</td>
<td>1 (1)</td>
<td>3 (2)</td>
<td>0.7 (0.0–8.5)</td>
<td>1.0</td>
</tr>
<tr>
<td>Intervillitis</td>
<td>0</td>
<td>2 (1)</td>
<td>0.0 (0.0–10.8)</td>
<td>1.0</td>
</tr>
<tr>
<td>Villous stromal edema</td>
<td>31 (40)</td>
<td>58 (37)</td>
<td>1.1 (0.6–2.1)</td>
<td>.7</td>
</tr>
<tr>
<td>Decidual necrosis</td>
<td>6 (8)</td>
<td>7 (4)</td>
<td>1.8 (0.5–6.5)</td>
<td>.4</td>
</tr>
<tr>
<td>Intravillous hemorrhage</td>
<td>15 (19)</td>
<td>25 (16)</td>
<td>1.3 (0.6–2.7)</td>
<td>.5</td>
</tr>
<tr>
<td>Decidual vasculopathy</td>
<td>0</td>
<td>2 (1)</td>
<td>0.0 (0.0–10.8)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%), except where noted. CI, confidence interval; PMI, placental membrane inflammation (simultaneous chorioamnionitis and funisitis).

Discussion

This study addressed 2 questions: Are placental lesions associated with maternal HIV infection, and are placental lesions associated with perinatal HIV transmission? If specific placental lesions are related to perinatal transmission, this might suggest a mechanism for transmission and a possible strategy for interventions. Our results indicate that HIV-infected women are at increased risk for chorioamnionitis and PMI. However, these inflammatory lesions were not associated with an increased risk of perinatal transmission in our cohort. Surprisingly, we found an inverse relationship between chorioamnionitis and perinatal transmission.

Potential differences in placental pathology between HIV-infected and -uninfected women have not been well characterized. In our study, HIV-infected women were at increased risk for chorioamnionitis, PMI, and plasma cellular deciduitis but at decreased risk for villitis. The different associations of these inflammatory lesions may be related to differences in involvement of maternal or fetal cells. Unlike villitis, inflammation of the membranes and umbilical cord is thought to be mediated by fetal leukocytes [34]. Because chorioamnionitis and funisitis (PMI) are almost always associated with ascending maternal genital tract infections, their increased prevalence among HIV-infected women likely results from HIV-associated factors (e.g., sexual exposure, vaginitis, and STDs). We did not investigate the microbiologic causes of chorioamnionitis, and the cause was not determined in the African studies. Acute chorioamnionitis is also associated with PROM, a risk factor for intrapartum HIV transmission [1, 35]. However, because no HIV-infected women in our study had PROM, we could not study this association.

Our finding that villitis occurred less often in HIV-infected women may be related to HIV-associated immunosuppression and suggests a diminished capacity to develop an inflammatory response. Because the inflammatory cells associated with villitis are believed to be maternal in origin [36, 37], our data support the hypothesis that HIV-infected women may have a decreased ability to form villitis-type placental lesions. In Uganda and the United States, the frequency of villitis in HIV-exposed placentas was also decreased [10, 25]. Villitis, estimated to be 7%–18% in well-sampled placentas from geographically diverse cohorts of pregnant women, is used by pathologists as a marker to assess adequacy of sampling and diagnosis in control populations [38–40]. The villitis observed in our study is termed villitis of unknown etiology. It is not associated with a single infectious agent but is associated with increased perinatal morbidity and recurrence in future pregnancies.

On the basis of several early reports from Africa [7–10], we hypothesized that chorioamnionitis would be associated with perinatal HIV transmission, particularly intrapartum transmission. Recently, a US study found chorioamnionitis to be a risk factor for transmission in the presence of zidovudine [24], and it was suggested that chorioamnionitis, if associated with PROM and preterm birth, may be an important underlying risk factor for perinatal HIV transmission [35]. However, we found the opposite association between chorioamnionitis and perinatal HIV transmission. In our study, there were significantly fewer inflammatory lesions of the placental membranes and umbilical cord...
placental membrane inflammation (simultaneous chorioamnionitis and funisitis); ties in the placenta itself might be related more directly to intrapartum transmission risk, whereas abnormalities, it might be hypothesized that PMI (or chorioamnionitis), HIV transmission.

If placental abnormalities are related to perinatal transmission, it might be hypothesized that PMI (or chorioamnionitis), which often occurs just before delivery, might be related more directly to intrapartum transmission risk, whereas abnormalities in the placenta itself might be related more directly to utero transmission. However, because of the low level of placental pathology among transmitting women in our study and the small number of both in utero and intrapartum transmission events, we were limited in our ability to address this. In populations in which placental abnormalities appear to be related to transmission, the relationship of specific lesions with time of transmission should be examined.

Some previous studies of HIV-related placental pathology may have been limited by study design or technique. The lack of an uninfected comparison group, although not interfering with the analysis of risk factors for perinatal transmission, prevents the examination of pathologic differences between HIV-exposed and -unexposed placentas and precludes the ability to establish background rates for various lesions. In some studies, sampling of the placenta was limited and suboptimal, or the rate of villitis was so low that the adequacy of microscopic interpretation may be questioned. Our study design, which was intended to optimize the clinical, laboratory, and pathologic parameters necessary to evaluate the effect of HIV on the placenta and the role of placental lesions in perinatal transmission, represents one of the most thorough samplings of placentas reported in the perinatal HIV literature.

Our findings do not diminish the importance of the placenta in providing a barrier to perinatal HIV infection. However, our data suggest that it is premature to assign placental pathologic lesions a definite role as risk factors or indicators for perinatal HIV transmission. Additional well-designed studies in other populations, with uniform pathologic diagnostic criteria, are needed to help clarify the effect of HIV on the placenta and the potential role of placental lesions in perinatal HIV transmission.

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References


