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1999

MIMS EPrint: **2006.63**

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ISSN 1749-9097

## The Ariel Project: A Prospective Cohort Study of Maternal-Child Transmission of Human Immunodeficiency Virus Type 1 in the Era of Maternal Antiretroviral Therapy

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**In a prospective cohort study, clinical and biologic factors that contribute to maternal-child transmission of human immunodeficiency virus type 1 (HIV-1) were studied. HIV-infected pregnant women and their infants were evaluated prospectively according to a standardized protocol. Of 204 evaluable women, 81% received zidovudine during their pregnancy. The infection rate among the 209 evaluable infants was 9.1%. By univariate analysis, histologic chorioamnionitis, prolonged rupture of membranes, and a history of genital warts were significantly associated with transmission. Additional factors associated with transmission that approached significance included a higher maternal virus load at delivery and the presence of cocaine in the urine. In a logistic regression model, histologic chorioamnionitis was the only independent predictor of transmission. Despite a significantly higher transmission rate at one site, no unique viral genotype was found at any site. Thus, chorioamnionitis was found to be the major risk factor for transmission among women receiving zidovudine.**

Pediatric human immunodeficiency virus type 1 (HIV-1) infections result almost exclusively from maternal-to-child transmission, with transmission occurring in utero during gestation, intrapartum with exposure to maternal blood and secretions, and postpartum through breast-feeding. However, most transmission is believed to occur late in gestation or during delivery [1–3]. The infection rate for non-breast-fed infants in the absence of antiretroviral therapy is generally ~25%, ranging from 15% to 50% in different studies [1]. Risk factors for transmission

in addition to breast-feeding include preterm delivery and low birth weight; prolonged rupture of membranes; maternal AIDS or advanced HIV-1 infection; maternal intravenous drug use; birth order, with the firstborn of twins at greater risk; chorioamnionitis and genital infections during pregnancy; vaginal delivery; and bloody amniotic fluid [4–10]. Interventions that attempt to reduce the transmission rate by reducing exposure of the infant to maternal blood remain unproven [11].

The Pediatric AIDS Clinical Trials Group (ACTG) 076 study demonstrated that the administration of zidovudine to HIV-infected pregnant women and their infants reduced the rate of transmission by two-thirds, from 22.6% to 7.6% [12]. Consequently, in April of 1994, the US Public Health Service recommended the administration of zidovudine to all HIV-infected pregnant women and their infants [13, 14]. Recently, the ACTG 185 study found a transmission rate of 4.8% with the use of zidovudine according to these recommendations [15].

The Ariel Project is a multicenter, prospective study of the pathogenesis of maternal-child transmission of HIV. The objective of the study is to identify biologic and clinical factors associated with transmission, or lack of transmission, of HIV from mother to child. The project began enrolling women in 1993, before the results of the ACTG 076 study were available. However, when the results of ACTG 076 became available in the spring of 1994, most women had the opportunity to receive zidovudine therapy. Thus, the Ariel Project is the first major

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Received 30 January 1998; revised 17 August 1998.

Presented in part: 34th Annual Meeting of the Infectious Diseases Society of America, New Orleans, 18–20 September 1996 (abstract 116).

Written informed consent was obtained from all participants or their parents or guardians. When possible, consent was also obtained from the father of the fetus. Human experimentation guidelines of the US Department of Health and Human Services were followed in the conduct of the study. Institutional review committee approval of the protocol was obtained at each clinical site.

Financial support: Pediatric AIDS Foundation; B.T.K., K.L., and R.A.K. are Elizabeth Glaser Scientists of the Pediatric AIDS Foundation.

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The Journal of Infectious Diseases 1999;179:319–28

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0022-1899/99/7902-0004\$02.00

study of maternal-child transmission of HIV-1 with the use of preventive zidovudine treatment. In this report, we describe the clinical characteristics of the cohort and examine risk factors for transmission.

## Methods

*Study design.* The Ariel Project was conducted at 7 clinical sites in the United States and was open for enrollment from April 1993 to March 1995. HIV-infected pregnant women were eligible to participate if they were at <36 weeks of gestation at entry. Women could be receiving antiretroviral therapy at entry. We excluded women if they planned to breast-feed or if they planned to receive immune therapy during pregnancy, including HIV-1 vaccines, HIV-1 hyperimmune globulin, monoclonal antibodies, and CD4 IgG. Immunization of the infant with an HIV-1 vaccine was allowed.

Maternal study visits occurred at 12–18, 25, and 34 weeks of gestation, at delivery, and at 2 and 6 months postpartum. At the initial study visit, we obtained a complete medical and obstetric history, including information on previous pregnancies, sexually transmitted diseases, and the use of illicit drugs, alcohol, and medications. A complete physical examination was done, including a gynecologic exam. Women were classified according to the 1993 revised classification system for HIV-1 infection of adolescents and adults [16]. A fetal ultrasonogram was done at entry to determine the gestational age of the fetus if one had not been done previously.

At each subsequent study visit, we obtained an interval history, did a physical examination, and collected 20 mL of blood in heparinized tubes for immunologic and virologic testing. We also obtained a cervicovaginal lavage sample by flushing the cervix with 1 mL of bacteriostatic normal saline through a catheter and aspirating the fluid. The blood and vaginal samples were shipped at room temperature by an overnight courier to the Aaron Diamond AIDS Research Center. Starting in September of 1994, we collected maternal urine samples at entry and at delivery to test for the presence of illicit drugs. At delivery, before delivery of the placenta, we collected a sample of umbilical cord venous blood. The surface of the cord was carefully cleaned with povidone iodine to minimize contamination with maternal blood.

Infant study visits occurred at delivery, at 24 h of age, and at 1, 2, 4, 6, 9, 12, 18, and 24 months of age. At each visit, we obtained an interval history, did a physical examination, and collected 5 mL of blood in heparinized tubes for immunologic and virologic testing. Blood was shipped as noted above.

We defined infants as HIV-infected if a peripheral blood sample was positive for HIV-1 by culture and a second sample from a separate blood draw was positive by either culture or HIV-1 DNA polymerase chain reaction (PCR) testing. Uninfected infants had at least two peripheral blood samples that were negative for HIV-1 by both culture and DNA PCR, with 1 of the 2 samples obtained at no earlier than 14 weeks of age. We did HIV-1 antibody testing on the infants at 12 and 18 months of age to confirm their HIV-1 infection status. We defined infants with a confirmed infection as having an early infection if a peripheral blood sample drawn within 24 h of birth was positive for HIV-1 by culture or DNA PCR testing [17]. Likewise, infected infants were defined as having a late infection if a peripheral blood sample drawn within 24 h of

birth was negative by culture or DNA PCR testing. Infected infants who did not have a blood sample obtained within the first 24 h after birth were not further classified. Results from cord blood samples were not used for the determination of infection status nor for the timing of infection.

*Laboratory testing.* We detected HIV-1 proviral DNA in peripheral blood mononuclear cells (PBMC) by use of an HIV-1 DNA PCR assay (Amplicor; Roche Molecular Systems, Somerville, NJ) according to the manufacturer's instructions. Each sample was tested in duplicate, and the result was considered indeterminate if 1 duplicate sample was positive and the other negative. Blood collection and processing, isolation of HIV-1 from PBMC, and quantitative measurement of plasma HIV-1 viremia were done as previously described [18]. Determination of lymphocyte subsets (CD4, CD8) was done in the clinical laboratory at each site.

*Vaginal samples.* Cervicovaginal lavage samples were separated into cells and supernatant, and each fraction was cultured for HIV-1. The cell fraction was tested for the presence of HIV-1 DNA by PCR as previously described [19].

*Placental histology.* After delivery, the placenta, fetal membranes, and umbilical cord were either processed locally for routine histology or shipped by overnight courier to Texas Children's Hospital (Houston) for processing. Multiple full-thickness sections of the placenta, fetal membranes, and umbilical cord were reviewed by a single pathologist (E.P.), and the degree of histologic chorioamnionitis was assessed according to the following classification: 0, no inflammation; 1, acute chorionitis ( $\geq 5$  neutrophils/high-power field [hpf] restricted to the chorion, usually mild and seen in the free membranes); 2, mild acute chorioamnionitis (aggregate of 5–10 neutrophils/hpf involving the full thickness of the membranes); 3, moderate or severe acute chorioamnionitis (aggregates of >10 neutrophils/hpf involving the full thickness of the membranes); 4, chronic chorionitis (lymphocytic or histiocytic inflammation of any degree, usually within the chorion, and almost exclusively found on the free membranes).

In the analysis, grades 2 and 3 were considered to represent true acute chorioamnionitis, whereas the other grades, with less than full-thickness inflammation, were classified as the absence of significant inflammation. The most severe grade of inflammation was recorded, whether present within the free membranes of the amniotic sac or on the attached membranes of the placental disc.

*DNA sequencing.* We amplified *env* genes directly from uncultured and cultured PBMC DNA at end-point dilution, inserted them into the vector pGEM T (Promega, Madison, WI) by the principles of TA cloning, and sequenced them as described previously [20]. To ensure the integrity of both the PCR product DNA and the virus isolates, we used stringent quality control measures at all times [21]. The viral sequences, including a set of contemporary control sequences obtained from early seroconverters in the United States, were compared by use of a neighbor-joining phylogenetic tree-building method (PHYLIP) [22, 23]. Inpatient sets formed distinct clades, and there was no evidence for contamination of PCR product DNA [24, 25].

*Statistical analysis.* We used S-PLUS version 3.3 (MathSoft, Cambridge, MA) for most statistical calculations. We used a one-sided Fisher's exact test to compare proportions when a particular hypothesis was being tested. We estimated risk ratios and 95%

confidence intervals of the risk ratios with method “C” of Katz et al. [26].

A logistic regression model for the risk of transmission was developed, using the following variables: RNA, the geometric mean of all plasma RNA concentrations during pregnancy and delivery for each mother, excluding values below the threshold of detection; T4, the CD4 percent at delivery; AZT, zidovudine use by the mother, divided into three categories of no zidovudine use, initiating zidovudine use during pregnancy, and continuing zidovudine use from prior to pregnancy; DuRpMem, the duration of rupture of membranes; chorio, the histologic classification of chorioamnionitis as described above.

The logistic regression looks for explanatory variables of  $\log[P/(1 - P)]$ , where  $P$  is the probability of transmission:  $\log[P/(1 - P)] = \beta_0 + \beta_1 * \log(\text{RNA}) + \beta_2 * \log(\text{T4}) + \beta_3 * (\text{AZT}) + \beta_4 * (\text{DuRpMem}) + \beta_5 * (\text{chorio})$ .

**Results**

Between January of 1993 and March of 1995, 244 women enrolled in the study. Twenty-one women withdrew before delivery, leaving 223 women who delivered while on study. Delivery records were available for 216 infants, including 5 sets of twins. Four mother-infant pairs (all singleton infants) were lost to follow-up after delivery, and 3 infants died before their infection status could be determined, leaving 209 infants with confirmed infection status. The 204 mothers of these infants were considered evaluable.

The evaluable women had a median gestational age of 23.3 weeks at entry, and 98% were enrolled before 37 weeks’ gestation. Most were African-American (68%) or Hispanic (22%), and 68% had a previous pregnancy. Twenty-three percent of women with a previous pregnancy had a prior infected child. The median CD4 cell count at entry was 461/mm<sup>3</sup>. At delivery, 88% of women were CDC category A, 10% category B, and 2% category C. The rate of cesarean section was 20%, representative of that of the United States.

Seven women were coenrolled in the ACTG 076 protocol (5 in the treatment arm and 2 in the placebo arm) [27]. With the unblinding of 076 in February of 1994, 1 of the woman receiving placebo was changed to zidovudine for the final 4 weeks of pregnancy. In April of 1994, the Public Health Service recommended that all HIV-1–infected pregnant women and their infants receive zidovudine according to the ACTG 076 protocol [14].

Consequently, 81% of evaluable women received antiretroviral therapy during their pregnancy; of these, 82% initiated therapy during the pregnancy. All women received zidovudine and 4 also received zalcitabine or didanosine. Of the women who began zidovudine during the pregnancy, 64% started therapy before 21 weeks’ gestation, 25% between 21 and 28 weeks’ gestation, and 11% after 28 weeks’ gestation. Of the women who received prenatal zidovudine, 67% also received an intravenous infusion during delivery. Sixty-seven percent of all ev-

aluable infants received zidovudine after delivery. One uninfected infant participated in ACTG 230, a phase I trial of an HIV-1 glycoprotein vaccine.

Illicit drug use during the pregnancy was reported by 13% of the evaluable women, with 8.3% reporting cocaine or crack, 2.5% heroin or methadone, 2.5% marijuana, and 1.5% opiates. Results of a urine drug screen obtained at delivery were available from 61 women. For 56, there was agreement between the history of drug use and the drug screen (45 negative and 11 positive). Among the 5 women with discordant results, 2 women who reported marijuana use had negative urine tests, 2 who reported no drug use had positive tests (1 for marijuana and 1 for cocaine), and 1 who reported cocaine was positive for both cocaine and heroin.

*HIV-1 infection status of the infants.* Of the 209 infants with known infection status (including the 5 sets of twins), 19 were HIV-1–infected, giving an infection rate of 9.1% (95% confidence interval, 5.2%–13.0%). One set of twins was discordant, with the second-born twin infected; their mother was classified as a transmitting mother. The remaining 4 sets of twins were both uninfected. Four infected infants had early infections detectable by 24 h of age and 11 had late infections; the remaining 4 infected infants did not have an early sample to further classify their infections.

At the study visit at which each of the 19 infected infants first had a positive virologic test, HIV-1 DNA PCR was positive in 14 (74%) of 19, and HIV-1 PBMC culture was positive in 17 (89%) of 19. Twelve infants were positive by both tests at this visit, 5 were positive only by PBMC culture, and 2 were positive only by DNA PCR. Nine infected infants had plasma cultures done at the first positive visit, and 5 (56%) were positive. Likewise, 11 had quantitative RNA PCR testing done, and all were positive.

The sensitivity and specificity of the virologic assays in evaluating the infants is shown in table 1. Cord blood samples were excluded. To determine the sensitivity, blood samples from the 19 infected infants were considered to have “detectable virus” if they were obtained at or following the first visit at which either the HIV-1 culture or DNA PCR was positive. Only 4 (4.8%) of 83 samples with “detectable virus” were negative by

**Table 1.** Performance of virologic tests in detecting HIV-1 infection in exposed infants.

	Sensitivity <sup>a</sup>	Specificity <sup>b</sup>
HIV-1 DNA PCR <sup>c</sup>	68/83 (81.9)	1035/1066 (97.1)
Quantitative RNA PCR	73/74 (99.6)	11/11 (100)
PBMC culture	81/95 (85.3)	1230/1235 (99.6)
Plasma culture	9/26 (35)	332/332 (100)

NOTE. PCR, polymerase chain reaction; PBMC, peripheral blood mononuclear cells.

<sup>a</sup> No. of samples positive/total (%), using samples from infected infants with “detectable virus” as defined in text.

<sup>b</sup> No. of samples negative/total (%), using all available samples from uninfected infants, cord blood excluded.

<sup>c</sup> Indeterminate results were considered negative.

**Table 2.** Characteristics of transmitting and nontransmitting mothers.

	Transmitting	Nontransmitting	<i>P</i> <sup>a</sup>
Number	19	185	
Age at entry, years	24 (21–27)	25 (21–27)	.351
Gestational age at entry, weeks	26 (16–27)	24 (18–29)	.309
Gestational age at delivery, weeks	38 (37–39)	39 (38–40)	.177
Duration of rupture of membranes, h	9.5 (0.7–15)	3.0 (0.8–8)	.038
CD4 cell count at delivery, cells/mm <sup>3</sup>	372 (277–579)	453 (252–642)	.319
CD4% at delivery	21 (17–33)	27 (19–35)	.197
Plasma virus load at delivery, copies/mL			
All	6050 (2675–26,750)	3350 (705–9850)	.066
No zidovudine use	10,310 (7140–14,980)	1658 (710–9493)	.085
Zidovudine use	6026 (1594–10,700)	3754 (1126–9604)	.189

NOTE. Data are median (25th–75th percentile); for plasma virus load, geometric mean of values at prenatal visits and delivery for each mother was calculated, and data are median and range of means.

<sup>a</sup> Wilcoxon rank sum test.

both PBMC culture and DNA PCR. Because of the low sensitivity of plasma cultures, they were discontinued in the later phase of the study.

Infants with false-positive tests were extensively evaluated with serial cultures and PCR testing, and all were confirmed to be uninfected. In addition, retesting of duplicate samples by DNA PCR and comparison of *env* and/or *gag* gene sequences with those of the maternal virus isolate was done on 30 positive samples from 21 uninfected infants. In each, the positive result could not be confirmed, or the maternal and infant sequences differed substantially [28].

One hundred sixty (84%) of the uninfected children were documented to be HIV-1–seronegative on follow-up. Of the 117 uninfected children with serologic testing done at 1 year of age, 66 (56%) had a positive or indeterminate result. By 18 months of age, only 20 (14%) of the 144 uninfected children tested had a positive or indeterminate serologic test. All of the 23 uninfected children tested at 2 years of age were seronegative. Children were often not retested once they were seronegative.

**Cord blood.** Cord blood samples were not used to determine the infection status of the infants. However, cord blood was available from 198 infants, including 15 infected infants. Cultures of cord blood PBMC were positive in 3 (20%) of 15 infected infants and 2 (1.2%) of 168 uninfected infants. The utility of a cord blood culture in predicting the infection status of the infant is limited (sensitivity, 20%; specificity, 98.8%; positive predictive value, 60%; negative predictive value, 93.3%). Cord blood plasma cultures were positive in none of 8 infected infants and 2 (2.3%) of 86 uninfected infants.

**Vaginal samples.** Cells from cervicovaginal lavage samples were culture-positive in 6 of the 166 women tested. Likewise, the cervicovaginal lavage supernatant was culture-positive in 4 of 138 women. The result of the cervicovaginal lavage cultures did not correlate with the infection status of the infant. A total of 673 cervicovaginal lavage samples from 214 women were available for HIV-1 DNA PCR testing. Overall, 13.8% of all samples were positive, with 33% of transmitting women and 24% of nontransmitting women having at least 1 positive sample (not significant).

**Placental histology.** Histopathologic results were available on the placentas of 175 infants with known infection status, including 5 sets of twins and 16 of the 19 infected infants. Twenty-four placentas (13.7%) had evidence of acute chorioamnionitis (12 category 2 and 12 category 3), whereas 151 did not (131 category 0, 16 category 1, and 4 category 4). Acute histologic chorioamnionitis (categories 2 and 3) was found significantly more often in placentas from transmitting women than in those from nontransmitting women (37.5% vs. 11.3%; *P* = .008).

There was a significant association between the clinical diagnosis of chorioamnionitis and histologic chorioamnionitis, with histologic acute chorioamnionitis present in 8 (42%) of 19 women with clinical chorioamnionitis and 18 (11%) of 161 without clinical chorioamnionitis (*P* = .0017, Fisher's one-sided exact test). However, the two were frequently discordant. The ability of clinical chorioamnionitis to predict histologic chorioamnionitis was limited, with positive and negative predictive values of 42% and 89%, respectively.

**Risk factors for transmission.** Transmitting and nontransmitting mothers are compared in table 2, and possible risk factors for transmission are presented in table 3. By univariate analysis, three factors were significantly associated with transmission: histologic chorioamnionitis, a longer duration of rupture of fetal membranes, and a history of genital warts in the mother.

A higher transmission rate was seen among women with ruptured membranes for >4 h (14.1% vs. 5.9%; *P* = .04); the 4-h cutoff was selected on the basis of previous observations [5]. A similar proportion of infected infants with early and late infections had mothers with ruptured membranes for >4 h (3/4 and 6/11, respectively). In addition to these three factors, recent cocaine use at delivery was more common among transmitting women, a finding that approached significance. Clinical chorioamnionitis was not significantly associated with transmission.

The transmission rates were similar for women of CDC classifications A (16/173, 9.2%), B (3/26, 11.5%), and C (0/5). Transmitting mothers had a lower median CD4 cell count and a

**Table 3.** Effect of possible risk factors on transmission rate—univariate analysis.

Condition	Transmission rate <sup>a</sup>		P <sup>b</sup>	Risk ratio	95% CI <sup>c</sup>
	With condition	Without condition			
Previous pregnancy	5/79 (0.063)	11/94 (0.12)	.93	0.54	0.20–1.49
Prior HIV-infected child	2/23 (0.087)	3/56 (0.054)	.46	1.62	0.29–9.08
History of STD					
Syphilis	2/44 (0.045)	17/160 (0.106)	.95	0.43	0.10–1.78
Gonorrhea	5/39 (0.128)	14/165 (0.085)	.29	1.51	0.57–3.94
Chlamydial infection	5/43 (0.116)	14/161 (0.087)	.37	1.33	0.51–3.51
Genital herpes	4/29 (0.138)	15/175 (0.086)	.28	1.61	0.57–4.51
Genital warts	8/37 (0.216)	11/167 (0.066)	.009	3.28	1.42–7.59
STD during pregnancy					
Syphilis	1/30 (0.033)	18/174 (0.088)	.95	0.29	0.04–2.08
Gonorrhea	0/5	19/199 (0.025)			
Chlamydial infection	3/17 (0.176)	16/187 (0.086)	.20	2.06	0.67–6.37
Genital herpes	3/18 (0.167)	16/186 (0.081)	.22	1.94	0.62–6.02
Cocaine detected at delivery	4/17 (0.235)	15/177 (0.085)	.058	2.78	1.04–7.43
No use of zidovudine during pregnancy	4/30 (0.133)	15/174 (0.086)	.30	1.55	0.55–4.34
No use of trimethoprim-sulfamethoxazole during pregnancy	12/151 (0.079)	7/53 (0.132)	.92	0.60	0.25–1.45
Clinical chorioamnionitis	3/21 (0.143)	16/178 (0.090)	.32	1.59	0.50–5.00
Histologic chorioamnionitis	6/24 (0.250)	10/151 (0.066)	.011	3.80	1.51–9.43
Ruptured membranes >4 h	12/85 (0.141)	7/119 (0.059)	.041	2.40	0.99–5.84
Episiotomy	2/38 (0.053)	17/166 (0.102)	.90	0.51	0.12–2.13
Vaginal delivery	15/163 (0.092)	4/41 (0.129)	.67	0.94	0.33–2.69
Laceration at delivery	4/56 (0.071)	15/147 (0.102)	.82	0.70	0.24–2.02

NOTE. STD, sexually transmitted disease.  
<sup>a</sup> Proportion.  
<sup>b</sup> Fisher's exact test, one-sided.  
<sup>c</sup> CI, confidence interval; two-sided, method of Katz et al. [26].

higher median plasma HIV-1 load at delivery, with the higher virus load approaching significance (table 2). However, there was a wide overlap between plasma virus loads of individual transmitting and nontransmitting mothers during pregnancy, and no virus load threshold for transmission is apparent (figure 1) [18]. For the group of women with virus loads in the upper quartile at delivery, the risk of transmission was 12%, not significantly different from that of 7.8% for the remaining women ( $P = .27$ , Fisher's exact test, one-sided). The frequency of cesarean section delivery was nearly identical for transmitting and nontransmitting women. The risk ratios for a number of potential risk factors for transmission are shown in table 3.

**Multivariate analysis.** The logistic regression model includes the 16 transmitting mothers and 151 nontransmitting mothers for whom all information is available. The log-likelihood ratio for the first four variables, excluding histologic chorioamnionitis, is 3.10 with 4 *df*. This ratio has approximately  $\chi^2$  distribution and is clearly not significant ( $P > .3$ ). That is, these variables together are not significant in explaining the risk of transmission. The same test for histologic chorioamnionitis alone is significant, with a log-likelihood ratio value of 7.55 with 1 *df* ( $P < .01$ ). The log-likelihood of the remaining variables added after histologic chorioamnionitis has  $P > .50$ . Hence, the only variable among the five with significant explanatory power is histologic chorioamnionitis. The odds ratio for histologic chorioamnionitis alone is 4.7 (95% confidence interval, 1.49–14.8) [29]. This value is related to the risk ratio for histologic chorioamnionitis, as shown in table 3.

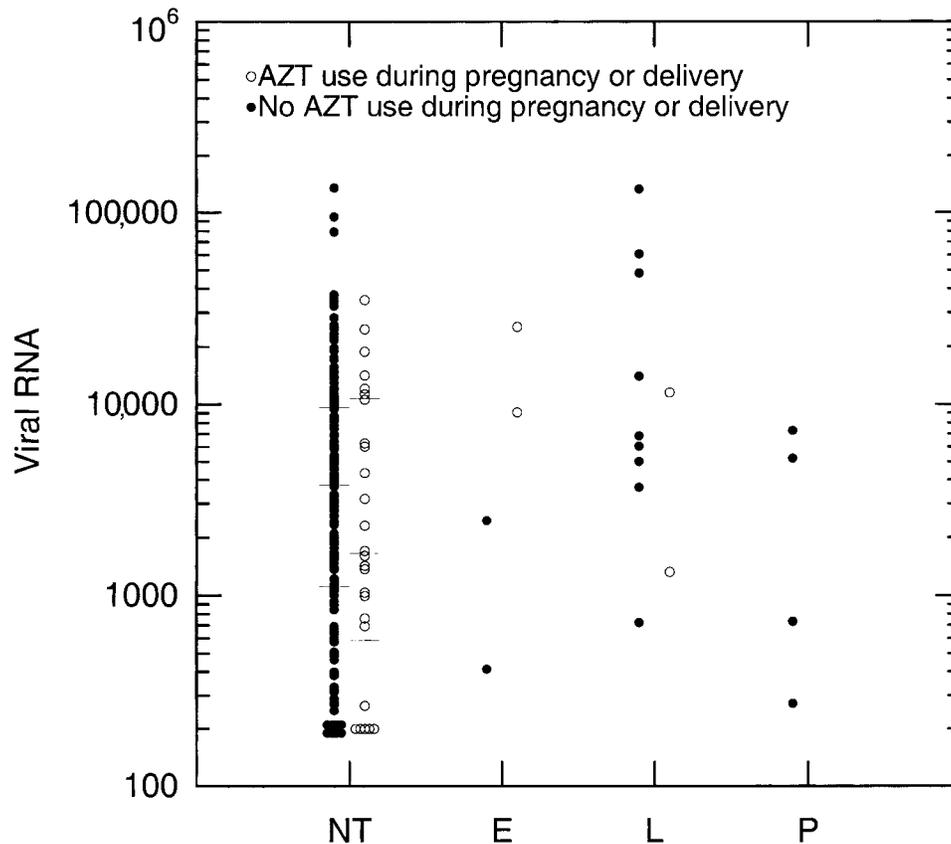
Two possible interactions between the variables in this model

were also explored. The first was an interaction between histologic chorioamnionitis and the duration of rupture of membranes. Although probably not significant, its sign is positive, suggesting that the effect of both is greater than that of either alone. In other words, histologic chorioamnionitis is more likely to be associated with transmission if prolonged rupture of membranes is also present. The second analysis was an interaction between histologic chorioamnionitis and mean plasma RNA concentration. No interaction was seen, suggesting that the virus load does not influence the association between histologic chorioamnionitis and transmission.

**Intersite variability of transmission rates.** The maternal-child transmission rates at the seven clinical sites varied widely, and the differences were statistically significant ( $P = 0.026$ ,  $\chi^2$  test). For instance, the Houston site, with 33 evaluable mothers, had no infected infants, while New Orleans, with 36 evaluable mothers, had 9 infected infants of 38 (including 2 sets of twins).

A comparison of the mothers in New Orleans with those at the other sites revealed no substantial differences in demographics, drug usage, stage of HIV-1 disease, obstetric management, or frequency of histologic chorioamnionitis. In particular, mothers in Houston and New Orleans were nearly identical in terms of race, drug usage, and disease stage (data not shown). Of note, New Orleans had the longest median duration of rupture of membranes of any site (6.0 h [interquartile range, 0.7–16 h] vs. 3.0 h [interquartile range, 0.7–9 h] for all other sites combined). However, this difference was not significant ( $P = .20$ , Wilcoxon rank sum test).

It was postulated that the variability in transmission rates



**Figure 1.** Mean plasma virus loads for transmitting and nontransmitting mothers. Geometric mean of plasma viral RNA concentrations from visits 1–4 (prepartum and intrapartum visits) was calculated for each mother. NT, nontransmitting mothers; transmitting mothers were classified as E (early), L (late), or P (timing of transmission could not be determined). Horizontal lines indicate 25th percentile, median, and 75th percentile for nontransmitting mothers. AZT, zidovudine.

could result from the occurrence of viruses with differing transmissibility at the different sites. However, sequence analysis of PCR-amplified viral RNA from 7 infected infants from New Orleans, 1 infected infant from Worcester, and 3 nontransmitting mothers from Houston did not suggest a clustering of related viral genotypes at any site. Genetic distances in these sequence viruses were comparable to a panel of contemporary representative clade B sequences from the United States (figure 2) (GenBank accession numbers for Ariel project sequences are AF112539–AF112565; accession numbers for the background control sequences are U84792–U84887) [22].

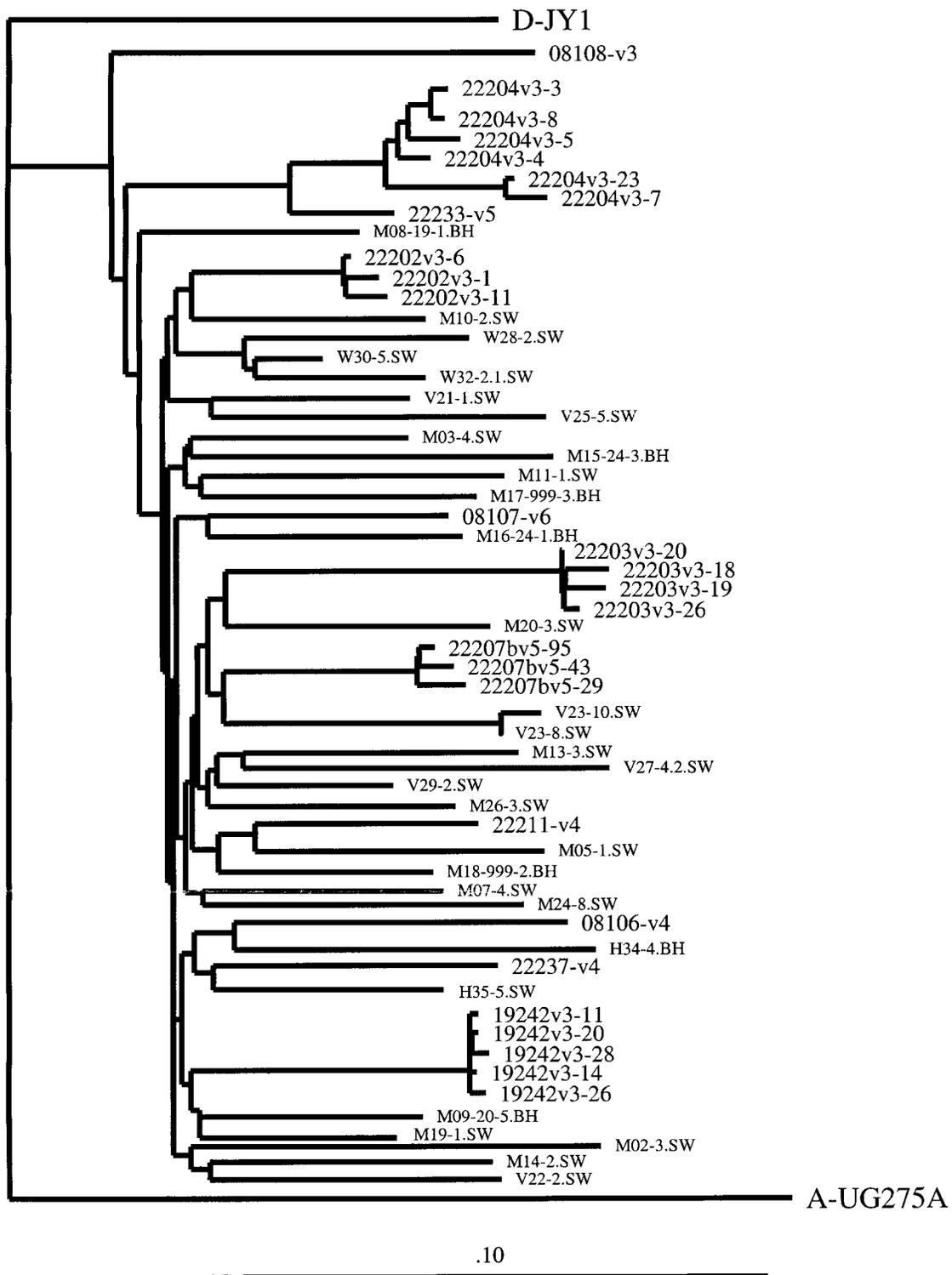
## Discussion

This is the first prospective cohort study of risk factors for maternal-child transmission of HIV-1 conducted since zidovudine was recommended to prevent maternal-child transmission. Overall, 81% of evaluable women received zidovudine during their pregnancy. We identified three factors that were significantly associated with an increased rate of transmission:

histologic evidence of chorioamnionitis, an increased duration of rupture of membranes, and a history of genital warts. In a logistic regression model, histologic chorioamnionitis was the only independent predictor of transmission, with prolonged rupture of membranes likely contributing some additional risk.

Women with histologic chorioamnionitis had a transmission rate four times that of those without chorioamnionitis. Unfortunately, clinical chorioamnionitis was a poor predictor of transmission. This is disappointing, since histologic chorioamnionitis can be identified only in retrospect, on review of the histology of the placenta and fetal membranes. Although clinical and histologic chorioamnionitis were significantly correlated, many women with histologic chorioamnionitis had no recognized symptoms. Clinical chorioamnionitis may have been underreported, since it was not specifically requested on the case report forms. Nevertheless, clinical chorioamnionitis when recognized was a poor predictor of histologic chorioamnionitis, with only 42% of women with clinical chorioamnionitis having histologic chorioamnionitis.

Although antibiotic therapy during pregnancy might prevent



**Figure 2.** Phylogenetic relationships of maternal and infant HIV-1 envelope sequences determined by neighbor-joining method. Sequences with prefixes 08, 19, and 22 are from Ariel clinical sites in Houston, Worcester, and New Orleans, respectively. Sequences 08106, 08107, and 08108 are from nontransmitting mothers; other Ariel sequences are from infected infants. Remaining sequences represent unrelated recent adult isolates from throughout United States. Scale bar represents 10% nucleotide sequence divergence.

chorioamnionitis and reduce the transmission rate, the transmission rate was slightly higher among those who received trimethoprim-sulfamethoxazole, the most common antibiotic used by participants of the study. However, this observation may be biased, because trimethoprim-sulfamethoxazole was usually administered for *Pneumocystis* prophylaxis. Thus, women receiving trimethoprim-sulfamethoxazole represent a more immunosuppressed subset of subjects, and an increased transmission rate associated with lower CD4 cell counts may have obscured any benefit afforded by trimethoprim-sulfamethoxazole in preventing chorioamnionitis and reducing transmission. However, trimethoprim-sulfamethoxazole has limited activity against many agents causing chorioamnionitis, including anaerobic bacteria and the genital *Mycoplasma* species. A prospective study of a more suitable antibiotic to prevent chorioamnionitis is needed to further address this question. Novel approaches to the diagnosis of chorioamnionitis would be important in evaluating such a strategy.

There are several ways that chorioamnionitis could directly increase the exposure of the fetus in utero to maternal virus or infected maternal cells. Maternal lymphocytes, targeting the site of inflammation, could cross into the amniotic fluid and infect the infant through the gastrointestinal or respiratory tract. Alternatively, free virus could cross into the amniotic fluid. Finally, infected cells or free virus could cross into the fetal circulation through the placenta or umbilical cord, resulting in hematogenous spread of the virus to the infant.

Prolonged rupture of membranes has been shown to be a risk factor for transmission. In a recent prospective study, the risk of transmission was shown to increase in a linear fashion with increasing duration of ruptured membranes, and rupture of membranes for  $\geq 4$  h nearly doubled the transmission rate [5]. These observations support the hypothesis that maternal-child transmission frequently occurs at birth with exposure to maternal blood or genital tract secretions. Interestingly, our site with the highest transmission rate was that with the longest overall duration of rupture of membranes. Prolonged rupture of the membranes is an important predisposing factor for the development of chorioamnionitis. This explains why prolonged rupture of membranes was not a strong independent predictor of transmission; it probably represents a marker for the presence of chorioamnionitis.

As recognized in other studies, transmitting women had more advanced HIV-1 disease, as reflected by lower CD4 cell counts and higher plasma virus loads. However, these differences did not reach significance, and importantly, there was a wide overlap in the virus loads of transmitting and nontransmitting women (figure 1). Hence, for an individual woman, the CD4 cell count and virus load does not reliably predict her risk of transmission. As reported previously, we did not identify a maternal virus load threshold below which transmission does not occur [18].

Among the sexually transmitted diseases, only a history of

genital warts was significantly associated with transmission (table 3). This association may have been due to chance alone. However, several explanations for this association can be proposed. Genital warts may represent a greater degree of sexual activity, since they are associated with an increased number of sexual partners. Indeed, Burnes et al. [30] found a higher frequency of intercourse during pregnancy among transmitting women. Mandelbrot et al. [8] noted that sexually transmitted diseases during pregnancy, including papillomavirus infections, were significantly associated with transmission by univariate analysis, but the effect was lost in multivariate analysis.

Finally, among women with genital papillomavirus infections, genital warts may be more likely to be recognized in women with more advanced immunosuppression, in whom the lesions tend to be larger. Thus, a history of genital warts may simply be a surrogate for more advanced HIV-1 disease.

Transmitting women were more likely to have cocaine detected in their urine at delivery, an observation that approaches significance. This is in agreement with other studies that found maternal illicit drug use to be an independent risk factor for transmission [5, 30, 31].

We were unable to demonstrate an association between the presence of detectable virus in the maternal genital tract before delivery and the risk of transmission. This supports the report of Nielsen et al. [32] that none of the 4 infants born to 19 mothers with positive cervicovaginal lavage samples became infected. However, the yield of virus in our cervicovaginal lavage samples by both culture and DNA PCR testing was unexpectedly low. Overall, only 14% of cervicovaginal lavage samples were positive by PCR, and 3% were positive by culture. Other studies have reported higher yields from cervicovaginal lavage samples, with 17%–67% of women positive by culture and 17%–54% by HIV-1 DNA PCR [32–35]. Although many of these reports did not include pregnant women, one study noted that the detection of cell-free virus was significantly increased during pregnancy [36]. A likely cause of our low yield was overnight shipping of the unprocessed cervicovaginal lavage samples at room temperature, allowing degradation of the sample. It is also possible that the administration of zidovudine to most women during pregnancy reduced the amount of detectable virus in the genital tract. It is interesting to note that the visit with the highest yield of HIV-1 DNA from cervicovaginal lavage samples was the 2-month–postpartum visit. This may reflect the increase in plasma virus load that was observed in this population following delivery [18]. Alternatively, it may be due to contamination with maternal blood from residual lochia following delivery. Nevertheless, further work is needed to define the relationship between virus in the maternal genital tract and infection of the infant. Optimization of the collection and processing methodologies is an important first step.

Zidovudine therapy reduces the maternal-child transmission rate, and we noted a trend toward a lower transmission rate among women who received zidovudine (table 3). The power

of this observation was limited by our low transmission rate and the small number of women who did not receive zidovudine. However, the transmission rate of 8.6% among the women who received zidovudine is nearly identical to that found among women receiving zidovudine in the ACTG 076 study [27].

We are unable to fully explain the significant differences in transmission rates at the different clinical sites. There was not a distinctive viral genotype at the site with the highest transmission rate, and it is unlikely that their population was infected with a virus with a high rate of transmissibility. Likewise, the women at that site did not have more advanced HIV-1 disease. However, the longer median duration of ruptured membranes at that site suggests that differences in obstetric practices could have influenced the transmission rate.

This study afforded us the opportunity to evaluate the performance of several virologic assays in the early detection of HIV-1 infection in exposed infants. As many as one-half of infected infants will have negative virologic tests at birth, presumably because of acquisition of infection at birth (late infections) [1]. Thus, we were interested in determining which test allowed for the earliest detection of infection. DNA PCR and PBMC culture gave similar results, although culture may be slightly more sensitive. However, the quantitative RNA PCR (virus load assay) may be the most sensitive assay, as it was positive at the first positive visit in all 11 infected infants for whom it was done. A recent report also found that the virus load assay was the most sensitive test for the early detection of infected infants [37]. Both PCR-based assays are readily available and are less expensive than HIV-1 culture.

The sensitivity of each assay in identifying infected infants was determined with only those blood samples with “detectable virus”—those obtained from infected infants once any one virologic assay was positive. The most sensitive assay was the quantitative RNA PCR, with the DNA PCR and PBMC culture only slightly less sensitive (table 1). It should be emphasized that our criteria for a positive DNA PCR assay required that samples be run in duplicate and both be positive; indeed, 6% of “detectable” samples had discordant results and were considered indeterminate.

To compare the specificities of the different virologic assays, we included all samples from the 190 uninfected infants. The false-positive rate was highest for the DNA PCR, with substantially lower rates for PBMC culture and plasma HIV-1 culture (table 1). Quantitative RNA PCR was generally not done on uninfected infants, so we cannot comment on the specificity of this assay.

Recently there have been a number of reports suggesting that exposed infants may have transient HIV-1 infection, with positive virologic tests that later revert to negative [38]. Therefore, we considered whether some of the false-positive results could actually represent transient infections. However, several observations lead us to believe that they are true false-positive results.

First, there was no clustering of the positive results at early time points or in a subset of infants. Second, many of the positive results could not be replicated in a duplicate banked sample from the same blood draw. Finally, when the positive result could be reproduced, comparison of the maternal and infant viral sequences suggested that the viruses were not related [28]. We believe that these false-positive results were caused by carryover of nucleic acid in the laboratory or the mixing of blood samples.

A limitation of this study is the small number of transmitting women, which limits the power to identify multiple risk factors for transmission. In addition, information on all variables was not available from all women. In particular, placental histologic results were available from only 84% of women. However, it is unlikely that there was a bias in the selection of placentas that were available for examination.

In conclusion, we found that among a population of HIV-1-infected pregnant women, most of whom received zidovudine, histologic chorioamnionitis and prolonged rupture of membranes were the major predictors of maternal-child transmission. A number of factors predictive of maternal-child transmission in other studies were not important in our study. However, few of the women in previous studies received zidovudine to prevent maternal-child transmission. Thus, it may be that many factors that contribute to transmission in the absence of zidovudine lose their importance in the face of zidovudine preventive therapy. Indeed, in the ACTG 076 study, the impact of a low maternal CD4 cell count in increasing the transmission rate was blunted among those women who received zidovudine [12]. However, chorioamnionitis and prolonged rupture of membranes remain important risk factors in the presence of zidovudine therapy. Interventions that prevent or treat chorioamnionitis, including the avoidance of prolonged rupture of membranes, may further contribute to a reduction in the number of HIV-1-infected infants.

#### Acknowledgments

The Ariel Project is dedicated to the memory of Elizabeth and Ariel Glazer. We thank the participating families for their commitment to the study, Catherine Wilfert for her careful review of the manuscript, Sheila Clapp for coordinating the clinical sites, David McDonald for data management, James Theiler for providing statistical advice, and the site personnel for their dedicated work.

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