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RISK FACTORS FOR PERINATAL TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 IN WOMEN TREATED WITH ZIDOVUDINE

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ABSTRACT

Background Maternal, obstetrical, and infant-related factors associated with the risk of perinatal transmission of human immunodeficiency virus type 1 (HIV-1) were identified before the widespread use of zidovudine therapy in pregnant women. The risk factors for transmission when women and infants receive zidovudine are not well characterized.

Methods We examined the effects of maternal, obstetrical, and infant-related characteristics and maternal virologic and immunologic variables on the risk of perinatal transmission of HIV-1 among 480 women and their infants, all of whom received zidovudine. The women and infants were participating in a phase 3 trial of passive immunoprophylaxis for the prevention of perinatal transmission.

Results In univariate analyses, the risk of perinatal transmission was associated with each of the following: decreased maternal CD4+ lymphocyte counts at base line; decreased maternal HIV-1 p24 antibody levels at base line and delivery; increased maternal HIV-1 titer at base line and delivery; increased maternal HIV-1 RNA levels at base line and delivery; and the presence of chorioamnionitis at delivery. In multivariate analyses, the only independent risk factor was the maternal HIV-1 RNA level at base line (odds ratio for transmission, 2.4 per log increase in the number of copies; 95 percent confidence interval, 1.2 to 4.7; $P=0.02$) and at delivery (odds ratio, 3.4; 95 percent confidence interval, 1.7 to 6.8; $P=0.001$). There was no perinatal transmission of HIV-1 among the 84 women who had HIV-1 levels below the limit of detection (500 copies per milliliter) at base line or the 107 women who had undetectable levels at delivery.

Conclusions Among pregnant women and their infants, all treated with zidovudine, the maternal plasma HIV-1 RNA level was the best predictor of the risk of perinatal transmission of HIV-1. Antiretroviral therapy that reduces the HIV-1 RNA level to below 500 copies per milliliter appears to minimize the risk of perinatal transmission as well as improve the health of the women. (N Engl J Med 1999;341:385-93.)

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SEVERAL studies have identified maternal, obstetrical, and infant characteristics associated with perinatal transmission of human immunodeficiency virus type 1 (HIV-1).¹⁻¹⁴ However, these studies were conducted primarily before the widespread use of zidovudine for the prevention of perinatal transmission.¹⁵⁻¹⁷ Few studies have identified risk factors for transmission among HIV-1-infected women and infants who are receiving zidovudine,¹⁸⁻²¹ yet such information is critical to the development of new interventions to reduce the risk of perinatal transmission further.

In the Pediatric AIDS Clinical Trials Group (ACTG) Study 185, a trial of passive immunoprophylaxis in which pregnant women with advanced HIV-1 disease were enrolled, prophylaxis with zidovudine was administered to all the women during and after pregnancy and to their infants after delivery. Detailed information on antenatal and obstetrical variables was collected during the trial, and laboratory assays were performed at several points during the women's pregnancies to determine maternal plasma levels of HIV-1 RNA, viral titers in quantitative cultures of peripheral-blood mononuclear cells, CD4+ lymphocyte counts,

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and quantitative HIV-1 p24 antibody levels. Thus, we were able to evaluate the independent contribution of potential risk factors for perinatal transmission of HIV-1 in a population of women and infants who received zidovudine.

METHODS

Study Design

The study was a multicenter, randomized, controlled phase 3 clinical trial conducted between October 1993 and March 1997 at 53 clinical sites in the contiguous United States and Puerto Rico. We evaluated whether prophylaxis with zidovudine combined with HIV-1 hyperimmune globulin (HIV-IG, North American Biologicals, Boca Raton, Fla.) at a dose of 200 mg per kilogram of body weight administered intravenously to the women each month during pregnancy and once to the neonates at birth would lower the risk of perinatal HIV-1 transmission more than would zidovudine and intravenous infusions of immune globulin without HIV-1 antibody (Gamimune N; Bayer, West Haven, Conn.), at a dose of 200 mg per kilogram. The preparation of HIV-1 hyperimmune globulin and the results of the phase 3 trial have been described previously.²²⁻²⁴ The study protocol and informed-consent forms were reviewed and approved by the institutional review board at each participating center. Each woman gave written informed consent for herself and (along with the father of the child, when possible) for her child or children.

We enrolled HIV-1-infected women who were 20 to 30 weeks' pregnant, who had CD4+ lymphocyte counts of no more than 500 per cubic millimeter, and who were receiving zidovudine as prescribed by their physicians. Women continued the antepartum antiretroviral regimen and received intrapartum intravenous zidovudine (a loading dose of 2 mg per kilogram followed by a continuous infusion of 1 mg per kilogram per hour). Their infants received the standard six-week course of zidovudine prophylaxis (2 mg per kilogram orally four times per day).¹⁵ Nucleoside analogues other than zidovudine and non-nucleoside reverse-transcriptase inhibitors were permitted with the approval of the protocol chair. Protease inhibitors became available only during the final year of the study; because no data were available on the safety of these drugs during pregnancy, women who were receiving protease inhibitors during pregnancy were excluded from the study. None of the women breast-fed their infants.

The women were seen monthly during pregnancy and at delivery. Quantitative culture of peripheral-blood mononuclear cells for HIV-1 was performed and blood specimens were obtained for assessment of HIV-1 RNA levels at base line, just before the third infusion of HIV-1 hyperimmune globulin or immune globulin (third trimester), and at delivery. The absolute number and percentage of CD4+ lymphocytes were assessed at base line and just before the third infusion. Chorioamnionitis was diagnosed clinically by a physician; specific histopathological diagnosis was not part of the protocol. The women were also evaluated 6 weeks and 3, 6, 12, and 18 months after delivery.

Infants were seen at weeks 1, 2, 6, and 12; every 4 weeks from week 16 through week 24; every 12 weeks from week 24 through week 60; and for a final evaluation at week 78 (approximately 18 months). Peripheral-blood mononuclear cells were obtained from the infants at birth and at 6, 24, and 48 weeks of age for quantitative cultures. A second, confirmatory culture was performed for all infants who had a positive culture. The infants' status with respect to HIV-1 infection was based on the results of the HIV cultures. The results of all virologic and serologic assays were reviewed in a blinded fashion by a subgroup of the study team for the final determination of infection status. Pregnancies resulting in multiple births were counted only once in the assessment of infection status: transmission was considered to have occurred if any of the infants were infected and not to have occurred if none were infected.

We calculated that 400 women were needed in each treatment group for the study to have a power of 80 percent to detect a 50 percent reduction in the rate of perinatal transmission of HIV-1 with the use of HIV-1 hyperimmune globulin, assuming that the rate of transmission in the group given intravenous immune globulin was at least 15 percent. This assumption was based on the more advanced stage of disease and the prior use of antiretroviral drugs among the women enrolled in this study as compared with those enrolled in ACTG Protocol 076.^{15,23} However, at the first planned interim analysis in March 1997, the overall rate of transmission was only 4.8 percent (4.7 percent in the group that received HIV-1 hyperimmune globulin and 4.8 percent in the group that received intravenous immune globulin), well below the percentage on which the initial calculations of power and sample size were based.²³ Given the unexpectedly low overall rate of transmission, an estimated treatment effect that appeared to be much less than 50 percent, and the large increase in the sample size that would be required to address the original hypothesis with adequate power, enrollment in the study was discontinued on March 25, 1997.²³

Laboratory Assays

Quantitative microculture of peripheral-blood mononuclear cells and lymphocyte phenotyping were performed in study laboratories according to standard methods.^{25,26} The titer of HIV-1 in cultures of peripheral-blood mononuclear cells was expressed as the number of infectious units per million cells. Flow cytometry was performed on EDTA-treated whole blood within 30 hours after collection.

For assays of HIV-1 RNA, within 30 hours after collection, plasma was separated from fresh whole blood that had been treated with acid-citrate-dextrose, stored at -70°C , and shipped overnight on dry ice to a central repository.²⁷ HIV-1 RNA was measured with a nucleic acid sequence-based amplification assay according to the manufacturer's instructions (Organon Teknica, Durham, N.C.). The lower limit of detection was 500 copies per milliliter. All specimens for an individual patient were assayed in a batched fashion whenever possible. All assays were performed by a single laboratory participating in the Division of AIDS Virology Quality Assurance program.

The plasma HIV-1 p24 antibody level was determined with the use of an enzyme immunoassay according to the manufacturer's instructions (Abbott Laboratories, Chicago). Plasma samples from the patients were incubated with polystyrene beads coated with recombinant p24 antigen. HIV-1 p24 antibodies were quantified on the basis of the end-point titer in serial dilutions (1:1 to 1:390,625) of plasma. Results are expressed as reciprocal titer units.

Statistical Analysis

Possible risk factors for perinatal transmission were evaluated with chi-square and logistic-regression analysis. The HIV-1 RNA level, the HIV-1 titer, the CD4+ lymphocyte count, and the HIV-1 p24 antibody level in the mother were evaluated as both categorical and continuous variables; virologic values and antibody titer were log-transformed for analyses.

Results of HIV-1 RNA and p24 antibody assays that were below the limit of detection were assigned values that were one half the limit of detection (e.g., 250 copies per milliliter for HIV-1 RNA and 0.5 reciprocal titer unit for p24 antibody). Goodness-of-fit tests indicated that both the univariate and multivariate models fit the data reasonably well when the assigned values were used. Logistic-regression analysis was used to test whether the model for the probability of transmission differed significantly with the inclusion of such values. Since no significant difference was found, we used the assigned values in continuous-variable analyses for subsequent analyses.

Repeated-measures analysis was used for longitudinal comparisons of prognostic markers for the risk of transmission. Variables significantly associated with the risk of perinatal transmission in univariate analyses were included in multivariate logistic-regression models. Goodness of fit was evaluated with the use of the Hos-

mer-Lemeshow method²⁸ and the Schwarz criterion²⁹; none of the models evaluated showed a significant lack of fit ($P > 0.08$ by the Hosmer-Lemeshow method). Collinearity was evaluated with the use of condition indexes and variance decomposition.^{28,30,31}

RESULTS

Study Population

A total of 501 women were enrolled in the study; 4 were lost to follow-up before delivery, resulting in a study population of 497 women. There were 505 live-born infants, including 9 sets of twins and 487 singletons, and 1 stillborn infant. The infection status could not be determined for the stillborn infant and 16 live-born infants (3.4 percent); 6 infants died during the neonatal period, and 10 were lost to follow-up before the age of six months, the time at which definitive infection status could be ascertained. Therefore, the final study population consisted of 480 mother-infant pairs. Overall, 24 infants were infected (5.0 percent; 95 percent confidence interval, 3.1 to 6.9 percent).

There were no significant differences between the group that received HIV-1 hyperimmune globulin and the group that received intravenous immune globulin with respect to base-line maternal, obstetrical, and infant characteristics (data not shown) or rates of HIV-1 transmission (4.1 percent [95 percent confidence interval, 1.6 to 6.5 percent] vs. 6.0 percent [95 percent confidence interval, 2.9 to 9.0 percent], $P = 0.34$). Since the rates of perinatal HIV-1 transmission and the clinical and laboratory characteristics were similar in the two groups, data for all women were combined in all subsequent analyses of risk factors.

Base-line titers of HIV-1 peripheral-blood mononuclear cells were available for 444 of the 480 women (92.5 percent), levels of HIV-1 RNA were available for 479 (99.8 percent), and p24 antibody levels were available for 476 (99.2 percent). At base line, the mean CD4+ lymphocyte count was 310 per cubic millimeter (median, 315), the mean HIV-1 titer was 77.5 infectious units per million (median, 8.1), the mean HIV-1 RNA level was 38,346 copies per milliliter (median, 8000), and the mean HIV-1 p24 antibody level was 19,219 reciprocal titer units (median, 114). Antiretroviral therapy was started before the current pregnancy in 116 women (24 percent). During the pregnancy, 6 women (1 percent) received antenatal treatment with a single nucleoside analogue other than zidovudine and 68 (14.2 percent) received antenatal treatment with two (66 women) or three (2 women) nucleoside analogues. Only 27 women (5.6 percent) had their regimens changed between base line and delivery; most regimens were switched from monotherapy with zidovudine to therapy with a combination of nucleoside analogues.

Univariate Analysis of Risk Factors

The maternal CD4+ lymphocyte count at base line was significantly associated with the risk of transmis-

sion of HIV-1 (Tables 1 and 2). The mean CD4+ lymphocyte count was significantly lower at base line and during the third trimester among the women who transmitted the infection to their infants than among those who did not transmit the infection ($P < 0.001$) (Table 3).

The HIV-1 titers at base line and at delivery were also significantly associated with the risk of transmission (Tables 1 and 2). The mean titer was significantly higher at base line, during the third trimester, and at delivery among the women who transmitted the infection than among those who did not ($P < 0.001$) (Table 3).

The HIV-1 RNA levels at base line and at delivery were significantly associated with the risk of transmission (Tables 1 and 2). HIV-1 RNA levels were generally stable during pregnancy ($r = 0.75$ for the comparison of base-line levels and levels at delivery, $P < 0.001$). For the 451 women (94.0 percent) for whom levels were measured at both base line and delivery, the levels did not change appreciably (≤ 0.5 log) in 276 (61.2 percent), the levels increased by at least 0.5 log in 55 (12.2 percent), and the levels decreased by at least 0.5 log in 120 (26.6 percent). There was no association between a change in HIV-1 RNA levels and the risk of transmission ($P = 0.34$).

There were no significant associations between the age at which the first positive HIV-1 culture was obtained in infected infants (< 48 hours vs. ≥ 1 week) and the maternal HIV-1 RNA level, either at base line ($P = 0.35$) or at delivery ($P = 0.56$). Although transmission of infection was observed at all detectable levels of HIV-1 RNA, there were no instances of perinatal transmission among the 84 women who had levels below the limit of detection at base line and the 107 women who had undetectable levels at delivery (Table 1). The upper limit of the 95 percent confidence interval for the risk of perinatal transmission among women with undetectable HIV-1 RNA levels at base line was 3.5 percent, and for those with undetectable levels at delivery, it was 2.8 percent.

The HIV-1 p24 antibody levels at base line and at delivery were also associated with the risk of transmission (Tables 1 and 2). The mean HIV-1 p24 antibody levels were significantly lower at base line, during the third trimester, and at delivery among the women who transmitted infection to their infants than among those who did not ($P = 0.001$) (Table 3).

At base line, antibody levels were similar among the women who received HIV-1 hyperimmune globulin and those who received immune globulin and were lower among women who transmitted the infection than among those who did not. Antibody levels did not change significantly during the study in the immune-globulin group. HIV-1 hyperimmune globulin was specifically manufactured to contain high levels of HIV-1 p24 antibody,^{19,22} and therefore at delivery, the women who received this treatment

TABLE 1. RESULTS OF CATEGORICAL UNIVARIATE ANALYSIS OF RISK FACTORS FOR PERINATAL TRANSMISSION OF HIV-1 INFECTION.*

RISK FACTOR	NO. OF WOMEN	NO. OF INFANTS INFECTED (%)	P VALUE
Maternal			
Cigarette smoking during pregnancy			
Yes	148	9 (6.1)	0.47
No	331	15 (4.5)	
Unknown	1		
Alcohol use during pregnancy			
Yes	87	7 (8.0)	0.17
No	391	17 (4.3)	
Unknown	2		
Hard-drug use during pregnancy†			
Yes	58	4 (6.9)	0.52
No	420	20 (4.8)	
Unknown	2		
Diagnosis of sexually transmitted disease during pregnancy‡			
Yes	134	6 (4.5)	0.74
No	346	18 (5.2)	
Treatment group			
HIV-1 hyperimmune globulin	246	10 (4.1)	0.34
Immune globulin	234	14 (6.0)	
Antiretroviral use			
Begun before pregnancy	116	7 (6.0)	0.56
Begun during pregnancy	364	17 (4.7)	
Type of antiretroviral therapy			
Single nucleoside analogue	412	22 (5.3)	0.56
Combination of nucleoside analogues	68	2 (2.9)	
CD4+ lymphocyte count at base line			
<200/mm ³	109	14 (12.8)	0.001
≥200/mm ³	371	10 (2.7)	
HIV-1 titer at base line			
<10 IUPM	267	10 (3.7)	0.16
10 to 49.99 IUPM	113	7 (6.2)	
≥50 IUPM	64	6 (9.4)	
Unknown	36	1	
HIV-1 titer at delivery			
<10 IUPM	278	8 (2.9)	0.005
10 to 49.99 IUPM	83	7 (8.4)	
≥50 IUPM	71	8 (11.3)	
Unknown	48	1	
HIV-1 RNA at base line			
<500 copies/ml	84	0	0.01
≥500 copies/ml	395	24 (6.1)	
Unknown	1		
HIV-1 RNA at delivery			
<500 copies/ml	107	0	0.006
≥500 copies/ml	344	23 (6.7)	
Unknown	29	1	
HIV-1 p24 antibody at base line			
<5 RTU	120	14 (11.7)	0.001
≥5 RTU	356	10 (2.8)	
Unknown	4		
HIV-1 p24 antibody at delivery			
<5 RTU	54	8 (14.8)	0.003
≥5 RTU	397	15 (3.8)	
Unknown	29	1	

had mean HIV-1 p24 antibody levels that were more than 1 log reciprocal titer unit higher than those in the women who received immune globulin. Interestingly, although lower antibody levels at delivery were associated with higher rates of transmission in both treatment groups, the women who transmitted the infection in the group that received HIV-1 hyperimmune globulin had higher mean antibody lev-

els at delivery than the women who did not transmit the infection in the group that received immune globulin (3.4 log vs. 2.3 log reciprocal titer units).

The effect of HIV-1 hyperimmune globulin and immune globulin on the association of HIV-1 p24 antibody levels and the risk of transmission was evaluated in models that included treatment as a covariate, as well as a term for possible interactions between

TABLE 1. CONTINUED.

RISK FACTOR	NO. OF WOMEN	NO. OF INFANTS INFECTED (%)	P VALUE
Obstetrical			
Diagnosis of chorioamnionitis			
Yes	19	3 (15.8)	0.06
No	461	21 (4.6)	
Time from rupture of membranes to delivery			
<4 hr	275	12 (4.4)	0.41
≥4 hr	180	11 (6.1)	
Unknown	25	1	
Mode of delivery			
Vaginal	352	20 (5.7)	0.42
Nonelective cesarean	92	4 (4.3)	
Elective cesarean§	36	0	
Infant			
Gestational age at birth			
<37 wk	82	6 (7.3)	0.27
≥37 wk	398	18 (4.5)	
Birth weight¶			
<2500 g	64	6 (9.4)	0.11
≥2500 g	416	18 (4.3)	
Break in infant's skin during labor			
Yes	38	4 (10.5)	0.11
No	442	20 (4.5)	

*Other nonsignificant risk factors included maternal age (P=0.50), maternal weight at base line (P=0.77), unexplained vaginal bleeding during pregnancy (P=0.29), antenatal invasive obstetrical procedures (percutaneous umbilical-cord blood sampling, chorionic-villus sampling, cerclage placement, or amniocentesis) (P=0.61), placenta previa or abruptio placentae (P=1.00), preterm labor requiring tocolytic therapy (P=0.41), and premature rupture of the membranes (≥24 hours before delivery) (P=0.35). Women with missing values were not included in the analyses. IUPM denotes infectious units per million cells, and RTU reciprocal titer units.

†Hard-drug use was defined as the use of cocaine, heroin, or injection drugs.

‡Sexually transmitted diseases were gonorrhea, chlamydia, syphilis, chancroid, and genital herpes.

§Elective cesarean delivery was defined as operative delivery before the onset of labor and rupture of the membranes.

¶For twin births, the lower of the two birth weights was used.

||This category included breaks in the infant's skin that occurred at birth, during blood sampling, during placement of electrodes on the scalp, and during placement of an internal-pressure transducer.

HIV-1 p24 antibody levels and treatment with HIV-1 hyperimmune globulin. There was no evidence of a modifying effect of HIV-1 hyperimmune globulin on the relation between p24 antibody levels and the risk of transmission of HIV-1.

Among clinical and obstetrical variables, only the presence of chorioamnionitis was significantly associated with a higher risk of transmission (Tables 1 and 2). Twenty-four percent of patients had received zidovudine therapy before the current pregnancy, and this factor was not associated with the risk of transmission (P=0.56).

Multivariate Analysis of Risk Factors

Separate multivariate models containing variables significantly associated with the risk of transmission of HIV-1 infection in the univariate analyses were developed for the variables present at base line and for those present at delivery. These included the CD4+

TABLE 2. RESULTS OF UNIVARIATE ANALYSIS OF CONTINUOUS RISK FACTORS FOR PERINATAL TRANSMISSION OF HIV-1 INFECTION.

RISK FACTOR	ODDS RATIO (95% CI)*	P VALUE
At base line		
CD4+ lymphocyte count (per 100-cell decrement)	1.6 (1.2–2.2)	0.002
HIV-1 titer (per log increment)	1.7 (1.1–2.6)	0.009
HIV-1 RNA (per log increment)	3.5 (1.9–6.5)	<0.001
HIV-1 p24 antibody (per log decrement)	1.5 (1.1–2.0)	0.006
At delivery		
HIV-1 titer (per log increment)	1.8 (1.2–2.6)	0.003
HIV-1 RNA (per log increment)	4.1 (2.2–7.6)	<0.001
HIV-1 p24 antibody (per log decrement)	1.5 (1.2–1.9)	0.003
Presence of chorioamnionitis	3.9 (1.1–14.5)	0.04

*The odds ratio is for the odds of perinatal transmission of HIV-1 infection. CI denotes confidence interval.

TABLE 3. MEAN CD4+ LYMPHOCYTE COUNTS, TITERS OF HIV-1, HIV-1 RNA LEVELS, AND p24 ANTIBODY LEVELS AMONG WOMEN WHO TRANSMITTED HIV-1 INFECTION TO THEIR INFANTS AND THOSE WHO DID NOT.*

VARIABLE	BASE LINE		THIRD TRIMESTER		DELIVERY		P VALUE†
	PERINATAL TRANSMISSION	NO PERINATAL TRANSMISSION	PERINATAL TRANSMISSION	NO PERINATAL TRANSMISSION	PERINATAL TRANSMISSION	NO PERINATAL TRANSMISSION	
	mean (95 percent confidence interval)						
CD4+ lymphocyte count (per mm ³)	218 (160–276)	315 (302–328)	222 (161–283)	329 (315–343)	ND	ND	<0.001
Titer of HIV-1 (IUPM)‡	1.3 (0.9–1.7)	0.7 (0.6–0.8)	1.5 (1.1–1.9)	0.7 (0.6–0.8)	1.3 (0.9–1.7)	0.7 (0.6–0.8)	<0.001
HIV-1 RNA (copies/ml)‡	4.6 (4.3–5.0)	3.8 (3.7–3.9)	4.7 (4.4–5.1)	3.7 (3.7–3.8)	4.6 (4.2–4.9)	3.6 (3.6–3.7)	<0.001
HIV-1 p24 antibody (RTU)‡	1.2 (0.5–1.9)	2.2 (2.1–2.4)	1.9 (1.4–2.5)	2.9 (2.7–3.0)	2.1 (1.5–2.7)	3.0 (2.9–3.1)	0.001

*ND denotes not determined, IUPM infectious units per million cells, and RTU reciprocal titer units.

†P values are for the repeated-measures analysis of the overall comparison of differences between the women who transmitted the infection to their infants and those who did not, with adjustment for longitudinal patterns and correlations over time within individual women.

‡Values are expressed on a logarithmic (base 10) scale.

TABLE 4. RESULTS OF MULTIVARIATE ANALYSIS OF RISK FACTORS FOR PERINATAL TRANSMISSION OF HIV-1 INFECTION.

RISK FACTOR	ADJUSTED ODDS RATIO (95% CI)*	P VALUE
At base line		
CD4+ lymphocyte count (per 100-cell decrement)	1.2 (0.9–1.7)	0.21
HIV-1 titer (per log increment)	1.2 (0.7–1.9)	0.55
HIV-1 RNA (per log increment)	2.4 (1.2–4.7)	0.02
HIV-1 p24 antibody (per log decrement)	1.3 (0.9–1.7)	0.14
At delivery		
HIV-1 titer (per log increment)	1.2 (0.7–2.0)	0.56
HIV-1 RNA (per log increment)	3.4 (1.7–6.8)	0.001
HIV-1 p24 antibody (per log decrement)	1.3 (1.0–1.7)	0.08
Presence of chorioamnionitis	4.4 (1.0–20.6)	0.06

*The odds ratio is for the odds of perinatal transmission of HIV-1 infection. Each value was adjusted for the other risk factors in the model. CI denotes confidence interval.

lymphocyte count, the viral load as measured by HIV-1 titers and HIV-1 RNA levels, HIV-1 p24 antibody levels, and the presence or absence of chorioamnionitis.

In the analysis of base-line variables, the HIV-1 RNA level was the only variable that remained independently associated with the risk of transmission. Similarly, in the analysis of variables at delivery, the HIV-1 RNA level was again the only variable significantly associated with the risk of transmission (Table 4). In the multivariate analysis of the variables present at delivery, the presence of chorioamnionitis was not significantly associated with the risk of transmission after adjustment for the other variables included in the model (odds ratio, 4.4; 95 percent confidence interval, 1.0 to 20.6; P=0.06). In a model that

included the HIV-1 RNA levels, both at base line and at delivery, the levels at delivery remained significantly associated with the risk of transmission (odds ratio, 2.5; 95 percent confidence interval, 1.1 to 5.8; P=0.03) but the levels at base line did not (odds ratio, 2.0; 95 percent confidence interval, 0.8 to 4.9; P=0.11).

Because HIV-1 RNA levels were correlated with the CD4+ lymphocyte count (r=−0.37, P<0.001), HIV-1 titers (r=0.48, P<0.001), and HIV-1 p24 antibody levels (r=−0.15, P<0.001), we assessed the effect of multicollinearity on the multivariate analyses. No significant effect was observed (data not shown).

After a review of both the univariate and multivariate logistic-regression analyses, the univariate model containing HIV-1 RNA levels at delivery was the strongest predictor of the risk of transmission of HIV-1 infection.²⁹

DISCUSSION

In this large cohort of women and infants who received zidovudine, multivariate analyses were used to adjust for a variety of virologic and immunologic markers as well as antenatal, obstetrical, and infant-related characteristics. In univariate analyses, general and HIV-1-specific immunosuppression (as measured by the CD4+ lymphocyte count and the HIV-1 p24 antibody level), the presence of chorioamnionitis, and elevated cell-associated or plasma viral load (as measured by viral titers and HIV-1 RNA levels) were each associated with an increased risk of HIV-1 transmission. However, in multivariate analyses, only HIV-1 RNA levels at base line and at delivery were independently associated with the risk of transmission.

Only a few of the published studies of HIV-1 transmission among women and infants who were receiving zidovudine have included an assessment of

viral load with HIV-1 RNA assays,^{5,20,32,33} and none have controlled for all the covariates that we did. In findings similar to ours, among the women who received zidovudine in ACTG Protocol 076, HIV-1 RNA levels at base line and delivery were the only variables for which there was a significant, albeit small, association with the risk of perinatal transmission³²; parallel findings were reported for the Ariel Project cohort, in which a majority of women received zidovudine.²⁰ Furthermore, HIV-1 RNA levels at delivery were independently associated with the risk of perinatal transmission in women receiving zidovudine in a trial of a short course of perinatal zidovudine in Thailand.³³

Although infusions of HIV-1 hyperimmune globulin did not affect the risk of perinatal transmission of HIV-1 in our study, HIV-1 p24 antibody levels at base line and at delivery were associated with the risk of transmission in univariate analyses. However, in multivariate analyses that included the CD4+ lymphocyte count and viral load, this association was no longer present. In addition, perinatal transmission occurred despite an increase in HIV-1 p24 antibody levels at delivery in the women who received HIV-1 hyperimmune globulin, and antibody levels at delivery in the women who transmitted infection in the group that received HIV-1 hyperimmune globulin were higher than those in the women who did not transmit infection in the group that received immune globulin. Thus, the HIV-1 p24 antibody level may be a surrogate for overall maternal immune response and stage of disease rather than an independent predictor of the risk of transmission of the virus.

Several studies conducted among pregnant women who did not receive zidovudine, primarily in Africa, and a recent analysis by the Ariel Project, in which 81 percent of the women studied received zidovudine, have described an association between clinical or histologically confirmed chorioamnionitis and the risk of transmission of HIV-1 infection.³⁴⁻³⁸ In the Ariel Project, histologically confirmed chorioamnionitis, found in 13.7 percent of women, was the only independent predictor of the risk of transmission in multivariate analyses.³⁸ The diagnosis of clinical chorioamnionitis was a poor predictor of histologic findings; only 42 percent of women with clinical symptoms had histologically confirmed chorioamnionitis. In our study, chorioamnionitis was associated with the risk of transmission in univariate analyses but not in multivariate analyses. However, because histologic data from the placental examination were not collected, the determination of chorioamnionitis was based on the physician's diagnosis, and the prevalence of chorioamnionitis was lower (4.0 percent) than in the other studies (10 to 26 percent). Therefore, our ability to detect a significant association between the presence of chorioamnionitis and the risk of transmission of HIV-1 in multivariate analyses was limited. However,

a nonsignificant trend for an association was retained ($P=0.06$). Chorioamnionitis is associated with placental inflammation and immune-cell activation and breaches in the placental barrier, which allow passage of virus or infected lymphocytes from the mother to the fetus. The presence of such conditions could increase the risk of transmission even in women with low levels of HIV-1 in their blood.

The use of zidovudine before pregnancy was associated with an increased risk of transmission in a study conducted in France³⁹; however, such an association was not observed in our study, in which 24 percent of women were receiving zidovudine before pregnancy. The duration of zidovudine therapy is probably a surrogate for viral resistance; there are conflicting data regarding the association between resistance to zidovudine and the risk of perinatal transmission.^{40,41} In ACTG Protocol 076, most of the infants who became infected in the zidovudine group were infected with zidovudine-sensitive strains,⁴⁰ and the results of another study suggested that zidovudine-sensitive virus may be preferentially transmitted by women who have mixed populations of zidovudine-sensitive and zidovudine-resistant virus.⁴² We did not evaluate the susceptibility to zidovudine of the strains identified in our subjects. The data from our study are consistent with current recommendations to offer zidovudine therapy to all infected pregnant women, regardless of their treatment history, in an effort to reduce the risk of perinatal transmission.¹⁷

There were no instances of perinatal transmission among the women who had undetectable levels of HIV-1 RNA at the time of delivery. However, the upper limit of the 95 percent confidence interval for the risk of transmission was 3.5 percent among women who had undetectable levels at base line and 2.8 percent among those with undetectable levels at delivery. Other studies have reported perinatal transmission among women with HIV-1 RNA levels below the limit of quantitation who were receiving zidovudine.^{5,20,32} Thus, we cannot conclude that there is a threshold for viral load below which there is no risk of perinatal transmission. Therapy with zidovudine to reduce the risk of transmission of HIV-1 should be recommended to all infected pregnant women regardless of their HIV-1 RNA levels.¹⁷

The HIV-1 RNA level at delivery was the strongest predictor of the risk of transmission in our study. However, we could not address whether a treatment-related reduction in HIV-1 RNA levels during pregnancy will further decrease the risk of perinatal transmission. The Public Health Service recommends that antiretroviral treatment of HIV-1-infected pregnant women should follow the same standards used for infected women who are not pregnant and for men.^{17,43} Thus, combination antiretroviral therapy is recommended for the treatment of HIV-1-infected women with HIV-1 RNA levels above 10,000 to 20,000 cop-

ies per milliliter.⁴³ Although further studies are needed, our data strongly suggest that the use during pregnancy of antiretroviral regimens that reduce the HIV-1 RNA levels to below the limit of detection may reduce the risk of perinatal transmission of the virus as well as improve the health of the women.

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APPENDIX

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