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MATERNAL VIRAL LOAD, ZIDOVUDINE TREATMENT, AND THE RISK OF TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 FROM MOTHER TO INFANT

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ABSTRACT

Background and Methods A placebo-controlled trial has shown that treatment with zidovudine reduces the rate at which human immunodeficiency virus type 1 (HIV-1) is transmitted from mother to infant. We present data from that trial showing the number of infected infants at 18 months of age and the relation between the maternal viral load, the risk of HIV-1 transmission, and the efficacy of zidovudine treatment. Viral cultures were obtained, and HIV-1 RNA was measured by two assays in samples of maternal blood obtained at study entry and at delivery.

Results In 402 mother-infant pairs, the rate of transmission of HIV-1 was 7.6 percent (95 percent confidence interval, 4.3 to 12.3 percent) with zidovudine treatment and 22.6 percent (95 percent confidence interval, 17.0 to 29.0 percent) with placebo ($P < 0.001$). In the placebo group, a large viral burden at entry or delivery or a positive culture was associated with an increased risk of transmission (the transmission rate was greater than 40 percent in the highest quartile of the RNA level). In both groups, transmission occurred at a wide range of maternal plasma HIV-1 RNA levels. Zidovudine reduced plasma RNA levels somewhat (median reduction, 0.24 log). Zidovudine was effective regardless of the HIV-1 RNA level or the CD4+ count at entry. In the zidovudine group, however, after we adjusted for the base-line HIV-1 RNA level and CD4+ count, the reduction in viral RNA from base line to delivery was not significantly associated with the risk of transmission of HIV-1.

Conclusions A high maternal plasma concentration of virus is a risk factor for the transmission of HIV-1 from an untreated mother to her infant. The reduction in such transmission after zidovudine treatment is only partly explained by the reduction in plasma levels of viral RNA. To prevent HIV-1 transmission, initiating maternal treatment with zidovudine is recommended regardless of the plasma level of HIV-1 RNA or the CD4+ count. (N Engl J Med 1996;335:1621-9.)

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TWO years ago, the Pediatric AIDS Clinical Trials Group (ACTG) Protocol 076 study demonstrated that zidovudine dramatically reduced the risk of maternal-infant transmission of human immunodeficiency virus type 1 (HIV-1).¹ The availability of a treatment that could prevent a fatal pediatric infection from being transmitted led the Public Health Service to develop guidelines for the use of zidovudine during pregnancy.² However, the mechanism by which zidovudine reduced the risk of HIV-1 transmission was unknown. Maternal zidovudine therapy may have reduced the circulating viral load and diminished the exposure of the fetus to the virus in utero and at the time of delivery. Alternatively, or in addition, thera-

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peutic concentrations of the drug in the infant, both as a fetus and as a newborn, may have provided protection during and after exposure to the virus. We updated our earlier analysis and assayed stored samples of maternal blood collected at study entry and at delivery to determine whether the maternal viral burden predicted either the risk of HIV-1 transmission or the efficacy of zidovudine treatment.

METHODS

Study Subjects

ACTG 076 was a randomized, double-blind, placebo-controlled study that enrolled HIV-1-infected women between 14 and 34 weeks of pregnancy who had CD4+ T-lymphocyte counts exceeding 200 cells per cubic millimeter and no indications for antiretroviral therapy.¹ The women were randomly assigned to receive either zidovudine or placebo. In the zidovudine group, maternal therapy both ante partum and intra partum was combined with six weeks of therapy for the newborn.¹

The present study included all mothers eligible for the original study¹ who had delivered live infants by January 4, 1994. Mothers who delivered infants thereafter were excluded from this study because their infants could not have completed six weeks of blinded treatment by the time the study was unblinded.

Laboratory Methods

Blood samples were collected from the mothers before they entered ACTG 076 and at the time of delivery in tubes containing heparin (72 percent of samples), acid citrate dextrose (25 percent), or EDTA (3 percent). The specimens were transported at ambient temperature and processed on the day of collection. Peripheral-blood mononuclear cells were isolated by Ficoll-Hypaque density-gradient separation, and qualitative cultures of mixed lymphocytes were performed by previously published methods.³

The cultures were assayed for p24 antigen at 7, 14, 21, and 28 days by a commercially available enzyme-linked immunosorbent assay (Abbott, Chicago). A quality-assurance program evaluated the performance of each participating laboratory.^{3,4} A culture was scored as positive if there was more than 30 pg of HIV-1 p24 antigen per milliliter of supernatant and if that level doubled within 28 days.³ Some laboratories performed quantitative microcultures of peripheral-blood mononuclear cells (21 percent of all cultures) using established procedures.⁵

Aliquots of plasma collected in tubes containing heparin were stored at -70°C . All the specimens were shipped on dry ice, then thawed and divided into aliquots for testing by each of two methods: a second-generation quantitative branched-chain DNA (bDNA) signal-amplification assay (Chiron, Emeryville, Calif.)⁶ and a quantitative reverse-transcription-polymerase-chain-reaction (PCR) amplification assay (Amplicor HIV Monitor Test, Roche Molecular Systems, Branchburg, N.J.).⁷ In the reverse-transcription-PCR assay, the plasma specimens collected in heparin required pretreatment with heparin lyase I. The bDNA and reverse-transcription-PCR assays were performed by the manufacturers of the assay kits; when the volume of a specimen was small, it was tested only by the latter assay.

In each assay, the HIV-1 RNA copy number was assessed by using the manufacturer's reference standards. The lower limit of sensitivity of the bDNA assay varied with the volume of the specimen; the limit was 500, 1000, and 10,000 copies of RNA per milliliter for volumes of 1, 0.5, and 0.05 ml, respectively. The lower limit of sensitivity of the reverse-transcription-PCR assay was approximately 400 copies per milliliter, but the limit varied among samples, since the test result for each specimen was based on the reverse-transcription-PCR amplification of an internal RNA standard that was added to each specimen. The assays dif-

fered in the range of the RNA concentration over which the detection signal was linear.^{6,7} Recent data suggest that of the two, the bDNA assay has more variability in the low range (below 3000 copies per milliliter).⁸ The upper limit of this linear range was 750,000 RNA copies per milliliter for the reverse-transcription-PCR assay and 1.6 million RNA copies per milliliter for the bDNA assay.^{6,7}

Statistical Analysis

The updated comparison of treatment groups with respect to the efficacy of zidovudine treatment was based on the difference between groups in the percentage of eligible live-born infants who had HIV-1 infection at 18 months of age, expressed as a simple ratio with a binomial 95 percent confidence interval. Twins were counted as a single infant; discordant twins (one of whom was infected but the other not) were counted as a single infected infant. Infants were defined as infected if assays for HIV-1 were positive on two separate occasions or if a condition considered to define the presence of the acquired immunodeficiency syndrome (AIDS) was noted.⁹ An uninfected infant was defined as one who did not meet this definition and who met at least one of the following two criteria: (1) two or more negative serologic tests between the ages of 6 and 18 months or a single negative serologic test after the age of 18 months,⁹ or (2) two negative HIV cultures after the age of 4 weeks, one of which was performed at the age of 24 weeks or thereafter. Infants with a single positive culture but for whom there were not enough data for classification as uninfected were classified as infected; all other infants were considered to have indeterminate data and were excluded from all the analyses.

Specimens of maternal blood collected more than four days after the start of the study treatment or more than four days after delivery were excluded from study. RNA measurements were analyzed on a logarithmic scale (base 10) to reduce the tendency of the data to become more variable with increasing levels of RNA. We excluded bDNA measurements if the coefficient of variation between duplicate measurements exceeded 30 percent. Specimens in which HIV-1 RNA was undetectable were assigned values equal to half the reported detection limit of the assays of those specimens.

HIV-1 RNA levels were compared between groups by the two-sample Wilcoxon test. Nonparametric confidence intervals with at least 95 percent confidence were calculated around the median values.¹⁰ Changes in RNA levels from study entry to delivery were calculated as $\log(\text{delivery RNA}) - \log(\text{entry RNA})$. Tests for trend were used to compare transmission rates according to the quartile of the RNA measurements in each study group.¹¹ Associations between prognostic factors were assessed with Spearman rank correlations. Logistic-regression analyses were used to assess whether virologic and immunologic measures predicted the risk of HIV-1 transmission and to estimate the proportion of the treatment effect that the measures explained.¹²

RESULTS

Four hundred twenty-five mothers (211 in the zidovudine group and 214 in the placebo group) who were randomized by December 20, 1993, gave birth to 433 infants (417 singleton infants and 16 twins) by January 4, 1994. Among these 425 mother-infant pairs, there were 402 (198 in the zidovudine group and 204 in the placebo group) for which there were sufficient data for the infant's infection status to be classified.

Fifteen infants in the zidovudine group and 46 infants in the placebo group were infected with HIV-1. An estimated 7.6 percent (95 percent confidence interval, 4.3 to 12.3 percent) of the infants in

the zidovudine group were infected, as compared with 22.6 percent (95 percent confidence interval, 17.0 to 29.0 percent) of those in the placebo group ($P < 0.001$).

The characteristics of the study cohort have been reported previously.¹ Not all the mothers had blood specimens obtained at entry and at delivery for virologic testing. Of the 402 mother–infant pairs, 333 (163 in the zidovudine group and 170 in the placebo group) had at least one plasma HIV-1 RNA measurement obtained at entry by either assay that could be evaluated. Of these mother–infant pairs, 286 (139 in the zidovudine group and 147 in the placebo group) had paired plasma RNA measurements obtained at entry and at delivery that could be evaluated. There were no significant differences between either of these subgroups and the entire cohort with respect to the characteristics of the mother, the delivery, or the newborn, nor were there differences between the two subgroups of mothers and their counterparts who did not have measurements that could be evaluated.

Predictive Variables at Study Entry

The zidovudine group was compared with the placebo group with respect to plasma HIV-1 RNA levels before the study treatment began (Fig. 1). The median RNA levels at entry were similar. In both groups there was transmission from mothers with entry RNA levels spanning virtually the entire range of values.

The plasma RNA measurements made by the reverse-transcription–PCR assay were highly correlated with those made by the bDNA assay ($r = 0.79$, $P < 0.001$), but the two types of measurements differed in several respects. There were fewer bDNA results available for study, because of the larger specimen volume required. With the bDNA assay the copy numbers tended to be lower; the values differed by a median of 0.34 log (a 2.2-fold difference in copy number) in specimens with RNA detectable by both assays. The proportion of specimens with undetectable copy numbers was higher with the bDNA assay (24.1 percent) than with the reverse-transcription–PCR assay (11.6 percent).

RNA levels at entry were compared between moth-

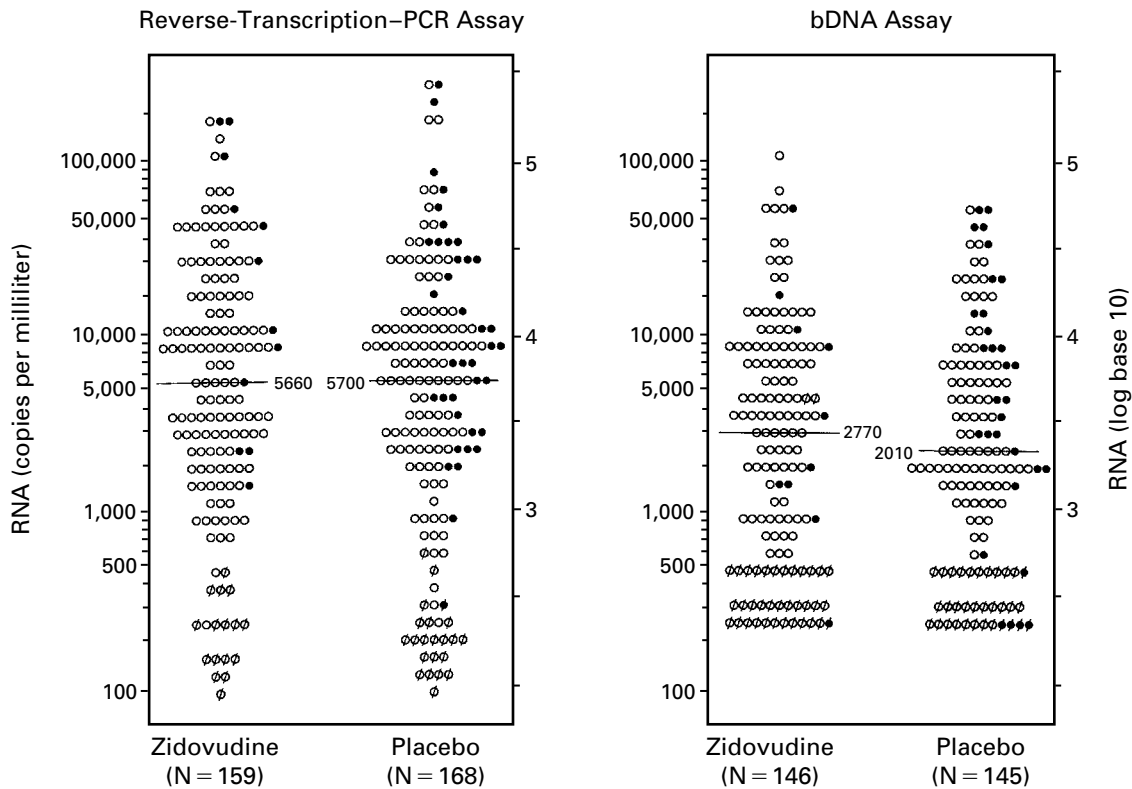


Figure 1. Plasma HIV-1 RNA Measurements at Entry, According to Treatment Group and Transmission Status. RNA measurements obtained by the reverse-transcription–PCR and bDNA assays are shown on a logarithmic scale and as log-transformed values. Solid circles denote mothers who transmitted HIV-1 to their infants, open circles mothers who did not transmit HIV-1, and circles with slashes mothers with RNA levels below the limit of sensitivity of the assay (these values are plotted at half the limit of sensitivity reported for the specimen in question). The horizontal lines indicate the median values in each group.

TABLE 1. MEDIAN PLASMA HIV-1 RNA LEVELS AT STUDY ENTRY, ACCORDING TO WHETHER MATERNAL-INFANT TRANSMISSION OF THE VIRUS OCCURRED.*

ASSAY AND STUDY GROUP	HIV-1 TRANSMISSION		NO TRANSMISSION		P VALUE
	NO. OF WOMEN STUDIED	MEDIAN (95% CI)	NO. OF WOMEN STUDIED	MEDIAN (95% CI)	
Reverse-transcription-PCR assay					
Zidovudine	12	19,330 (2100-108,310)	147	4650 (3340-8320)	0.03
Placebo	38	8,320 (4350-26,770)	130	5370 (2890-6770)	0.003
bDNA assay					
Zidovudine	10	2,620 (920-16,310)	136	2770 (1870-3900)	0.45
Placebo	31	4,680 (2200-11,130)	114	1850 (1540-2280)	0.008

*Medians are reported with nonparametric 95 percent confidence intervals (CI) as described in the Methods section. P values were calculated by the two-sample Wilcoxon test.

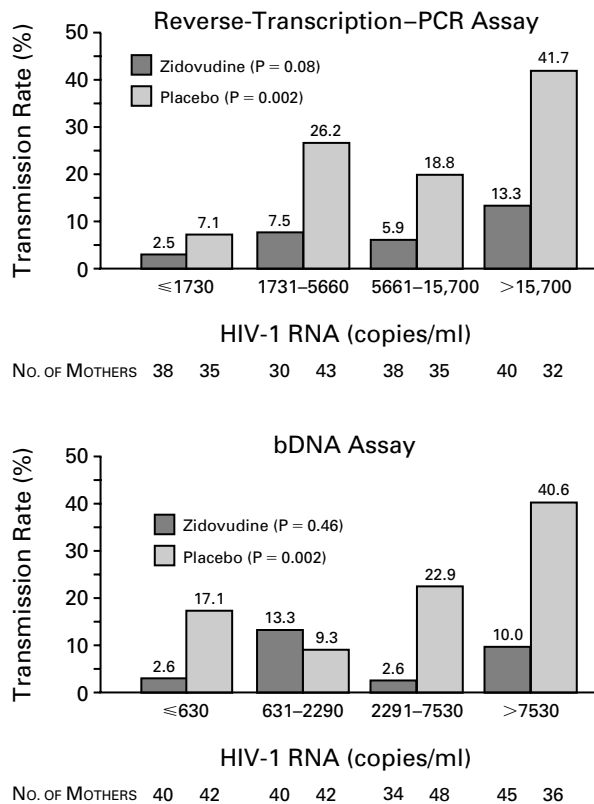


Figure 2. Transmission of HIV-1 in the Study Groups, According to Quartile of the Plasma HIV-1 RNA Level Measured at Entry. The transmission rates for the mothers studied by each assay are shown, with P values for each group calculated by the test for trend among RNA quartiles. The number of mothers studied in each subgroup is shown below the graphs.

ers who transmitted HIV-1 to their infants and those who did not (Table 1). By the reverse-transcription-PCR assay, in both study groups the mothers who transmitted the virus were found to have higher median RNA levels at entry. By the bDNA assay, those in the placebo group who transmitted the virus were found to have higher median RNA levels than those who did not, but in the zidovudine group no significant difference was detected.

Quartile analyses (Fig. 2) and logistic-regression models (Table 2) were used to assess how well the base-line variables predicted the risk of viral transmission. When the mothers were grouped according to the quartile in which their RNA values as measured by the reverse-transcription-PCR assay fell (Fig. 2), the transmission rates were higher among the mothers in both treatment groups who had higher HIV-1 RNA levels at entry. In the placebo group, the transmission rate was greater than 40 percent for the highest RNA quartile. In each quartile, however, the transmission rate was substantially lower in the zidovudine group than in the placebo group. The analyses of RNA quartiles as measured by the bDNA assay yielded similar results, except that in the zidovudine group no significant trend was detected in rates of transmission.

Results of HIV-1 cultures performed before treatment were available for 337 mothers. In the placebo group, the transmission rate was lower among mothers with negative cultures than among mothers with positive cultures (9.6 percent [5 of 52] vs. 29.8 percent [36 of 121], $P=0.004$); no significant association was observed in the zidovudine group (Table 2). Mothers with positive cultures had higher median RNA levels (by either test) than mothers with negative cultures ($P<0.001$).

Immunologic data obtained at entry into the study were available for 400 mothers. Transmission rates in

TABLE 2. LOGISTIC-REGRESSION MODELS OF VIROLOGIC AND IMMUNOLOGIC DATA OBTAINED IN THE MOTHERS AT ENTRY INTO THE STUDY AND AT THE TIME OF DELIVERY, ACCORDING TO STUDY GROUP.

VARIABLE*	ZIDOVUDINE GROUP†	PLACEBO GROUP‡	TREATMENT EFFECT‡
odds ratio (95 percent confidence interval)			
Univariate analysis, values at entry			
HIV-1 RNA			
By RT-PCR	2.29§ (1.17–4.46)	1.84¶ (1.26–2.69)	0.25 (0.12–0.50)
By bDNA	1.26 (0.65–2.44)	1.80§ (1.15–2.81)	0.26 (0.12–0.55)
Positive HIV-1 culture	2.42 (0.50–11.8)	3.98¶ (1.46–10.8)	0.21 (0.10–0.45)
CD4+ cells			
Count	1.12 (0.88–1.43)	1.19§ (1.02–1.40)	0.28 (0.15–0.52)
Percentage	1.23 (0.93–1.63)	1.33¶ (1.08–1.64)	0.27 (0.14–0.51)
CD8+ cells			
Count	1.04 (0.91–1.18)	1.03 (0.97–1.11)	0.28 (0.15–0.53)
Percentage	1.04 (0.83–1.30)	1.25§ (1.07–1.46)	0.26 (0.15–0.51)
Multivariate analysis, values at entry			
RNA (by RT-PCR)	2.27§ (1.13–4.56)	1.69§ (1.13–2.52)	
CD4 percentage	1.00 (0.71–1.42)	1.37§ (1.05–1.78)	0.24 (0.12–0.49)
RNA (by bDNA)	1.16 (0.57–2.36)	1.58 (0.98–2.53)	
CD4 percentage	1.13 (0.79–1.61)	1.45¶ (1.10–1.93)	0.25 (0.11–0.54)
Univariate analysis, values at delivery			
HIV-1 RNA			
By RT-PCR	1.95§ (1.02–3.71)	2.21 (1.45–3.36)	0.30¶ (0.15–0.63)
By bDNA	1.11 (0.52–2.36)	1.80¶ (1.16–2.79)	0.25 (0.11–0.55)
Positive HIV-1 culture	6.78 (0.85–54.3)	3.85§ (1.28–11.6)	0.2 (0.14–0.58)
Change in RNA**			
By RT-PCR	0.49 (0.15–1.63)	0.52§ (0.27–0.99)	0.28¶ (0.12–0.62)
By bDNA	0.58 (0.14–2.45)	0.72 (0.36–1.43)	0.25¶ (0.10–0.60)

*RT-PCR denotes reverse transcription–polymerase chain reaction.

†These odds ratios show the increase in the risk of transmission associated with every fivefold (0.69 log) increase in the RNA level, every decrease of 100 cells per cubic millimeter in the CD4+ count, every increase of 100 cells per cubic millimeter in the CD8+ count, every absolute decrease of 5 percentage points in the CD4 percentage, every absolute increase of 5 percentage points in the CD8 percentage, and a positive as compared with a negative HIV-1 culture.

‡These odds ratios compare the zidovudine group with the placebo group, using a logistic-regression model that also included the individual predictor (univariate analyses) or all specified predictors (multivariate analyses). None of the models had a significant interaction of predictor with treatment.

§P<0.05.

¶P<0.01.

||P<0.001.

**These odds ratios are for a decrease in RNA by a factor of 5 (a decrease of 0.69 log) in a multivariate logistic-regression model that also included the RNA level and the CD4 percentage at entry. Odds ratios (not shown) for these pretreatment variables were similar to those in the multivariate analysis shown above.

the placebo group increased as CD4+ cell counts decreased (Fig. 3); no significant trend was detected in the zidovudine group. In each subgroup determined on the basis of the CD4+ count, the transmission rate was lower in the zidovudine group than in the placebo group. The presence of a lower proportion of CD4+ cells and a higher proportion of CD8+ cells was associated with a higher risk of transmission in the placebo group, but not in the zidovudine group (Table 2). CD4+ counts at entry were negatively correlated with plasma RNA levels at entry (reverse-transcription–PCR assay, $r = -0.38$; bDNA assay, $r = -0.31$; $P < 0.001$ for both).

We used logistic-regression models to assess wheth-

er the pretreatment variables predicted the risk of HIV-1 transmission, either individually (in a univariate analysis) or in combination (in a multivariate analysis), and to determine whether the effect of zidovudine treatment persisted after adjustment for base-line predictors (Table 2). In the zidovudine group, the pretreatment RNA level as measured by the reverse-transcription–PCR assay was the only significant predictor, whether or not other base-line variables were included in the model. In the placebo group, the RNA level as measured by the reverse-transcription–PCR assay and the proportion of CD4+ cells (as a percentage of all lymphocytes) were the strongest predictors before study entry;

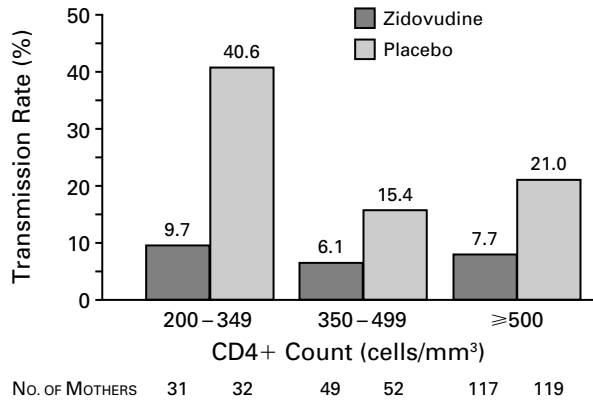


Figure 3. Rates of HIV-1 Transmission in the Study Groups, According to CD4+ Cell Count at Entry.

The number of mothers studied in each subgroup is shown below the figure.

both remained significant in the multivariate model. Of the two measures of viral load, the plasma RNA level and positivity for HIV-1 by culture, the RNA level (determined by either assay) was the stronger predictor of the risk of transmission; when both measures were included in the model, only the RNA level remained significant. Treatment with zidovudine was associated with a significantly reduced risk of transmission even after adjustment for the variables that were predictive before treatment.

Viral Load and Effect of Zidovudine Treatment

In assessing whether RNA levels explained the effect of zidovudine treatment, we focused on the larger set of data obtained by the reverse-transcription-PCR assay. Among mothers for whom there were plasma RNA measurements obtained at both study entry and delivery, those in the zidovudine group had median RNA values 1.7 times (0.24 log) lower at delivery than the values in the placebo group (median reduction, 0.28 vs. 0.04 log copies per milliliter; $P < 0.001$). There was a larger median reduction (0.34 log) among zidovudine-treated mothers whose RNA levels at entry exceeded 1000 copies per milliliter than among their counterparts in the placebo group (0.8 log), but the net reduction was similar (0.26 log).

Most specimens obtained at delivery were collected within one (92 percent) or two (97 percent) days after delivery; the median changes in RNA levels were similar when the analysis was restricted to these narrower intervals. The median time between the sample collections (at entry and at delivery) was 13 weeks; there was no relation in either group between the change in RNA and the time between the collections. The proportion of positive cultures at delivery as compared with entry did not differ between treatment groups; in most mothers (75.5 per-

cent), the results of the cultures were concordant, and those who had discordant cultures were divided equally in both groups between those with negative cultures at entry but positive cultures at delivery and those with the reverse.

We used logistic-regression models to assess whether measures of viral load at delivery or changes in RNA levels from the base-line values predicted the risk of transmission (Table 2), explained the effect of zidovudine treatment, or both. In the zidovudine group, a higher absolute level of RNA at delivery as measured by the reverse-transcription-PCR assay was the only significant predictor of an increased risk of transmission (odds ratio, 1.95; $P < 0.05$). In the placebo group, a higher absolute level of RNA at delivery as measured by either assay was significantly associated with a higher risk of transmission (odds ratio with reverse-transcription-PCR assay, 2.21; with bDNA assay, 1.80; $P < 0.01$ for both), as was an increase in RNA from entry to delivery as measured by the reverse-transcription-PCR assay and adjusted for the base-line RNA value and the percentage of CD4+ cells (odds ratio, 0.52; $P < 0.05$). A negative culture at delivery was associated with a lower risk of transmission. A significant effect of zidovudine treatment remained after adjustment for each predictive variable. The estimated proportion of the treatment effect that was explained by each measure of viral load at delivery was 10.8 percent (95 percent confidence interval, -0.5 to 31.5 percent) for RNA as measured by the reverse-transcription-PCR assay, 6.7 percent (no stable 95 percent confidence interval could be determined) for RNA as measured by the bDNA assay, and 6.3 percent (95 percent confidence interval, 0.5 to 18.2 percent) for a positive HIV-1 culture of peripheral-blood mononuclear cells. The change in the RNA level (after adjustment for the base-line RNA level and proportion of CD4+ cells) accounted for approximately 16.6 percent (95 percent confidence interval, 3.9 to 41.2 percent) of the effect of zidovudine treatment when the reverse-transcription-PCR assay was used and 9.2 percent (95 percent confidence interval, -3.3 to 30.4 percent) of the effect when the bDNA assay was used.

DISCUSSION

The initial report of the controlled trial of the efficacy of zidovudine to prevent maternal-infant transmission of HIV-1 included 363 mother-infant pairs for whom at least one viral culture from the infant was available.¹ We now present analyses based on clinical, virologic, and serologic data obtained after an 18-month follow-up in 402 mother-infant pairs. The revised estimates of the proportions of infected infants in the zidovudine and placebo groups strongly support our earlier report¹ and are consistent with published reports of uncontrolled studies.¹³⁻¹⁵

In the placebo group, an increased maternal load

of HIV-1 at entry or delivery, as measured by either the bDNA assay or the reverse-transcription-PCR assay in plasma or by culture of peripheral-blood mononuclear cells, was associated with an increased risk of maternal-infant transmission of HIV-1. In the zidovudine group, only an increased viral load at entry or delivery as measured by the reverse-transcription-PCR assay was significantly associated with the risk of transmission. This finding is in accordance with data obtained in small U.S. cohorts.¹⁶⁻¹⁸ In contrast to these reports,^{16,17} however, we found that transmission occurred at lower absolute levels of plasma RNA and across the entire range of measurable RNA levels, as well as from some mothers with undetectable plasma viremia. Although the rates of transmission decreased as RNA levels decreased, our data do not suggest that there is an absolute plasma RNA level below which transmission does not occur.

The effect of zidovudine treatment was seen with all plasma HIV-1 RNA levels in samples obtained at study entry. Overall, zidovudine treatment was associated with only a small reduction in circulating levels of plasma RNA. Neither the change in the plasma RNA level from entry to delivery nor a critical level of plasma RNA at delivery accounted for the substantially reduced transmission rate in the zidovudine group. Only a small part of the treatment effect could be explained by the observed RNA measurements, which is further evidence that the protective effect of zidovudine results at least in part from a mechanism other than the reduction of the maternal plasma viral burden.

Explanations need to be considered for the apparent lack of association between the observed RNA levels and the effect of zidovudine treatment. The possibility of a prophylactic effect both in utero and intra partum, during and after exposure, is raised. A recent case-control study of health care workers with percutaneous exposures suggests that zidovudine use may be protective after exposure to HIV-1.¹⁹ Moreover, the plasma RNA level is only one measure of viral activity. The timing of transmission (either in utero or intra partum), maternal infection with a zidovudine-resistant strain of virus, or other maternal factors ante partum or intra partum may all have influenced the success of zidovudine therapy and the risk of transmission.

We used both the bDNA and the reverse-transcription-PCR assays to assess the influence of the viral load on the risk of vertical transmission. Confirming our results by two independent quantitative methods was considered important because no other placebo-controlled trials of antiretroviral agents in the United States are likely, given that zidovudine has been accepted for use in preventing perinatal transmission.² Since the two assays had not been developed when the ACTG 076 trial began, specimens

were not always collected by methods optimal for the performance of these assays.

In general, the results obtained by both assays were in agreement. The higher absolute values obtained from the reverse-transcription-PCR assay, as well as the stronger observed association with the risk of transmission, underscore the problems with interpreting these comparisons. First, since the reverse-transcription-PCR assay requires a smaller volume of plasma than the bDNA assay, specimens whose volume was limited were tested only by the reverse-transcription-PCR assay. The statistical power of the study to allow conclusions based on the results of the bDNA assay was therefore more limited. Second, there are no standard references for use in calibrating the results of the two assays. The copy number obtained with the bDNA assay tended to be lower than that obtained by the reverse-transcription-PCR assay. Among specimens found to have detectable RNA by both techniques, the RNA copy numbers measured differed by a median factor of two. Third, the assays have different performance characteristics. Many specimens tested had relatively low RNA copy numbers. The nonlinear performance of the bDNA assay in specimens with low copy numbers may in part explain the weaker association observed with the risk of transmission. Finally, the performance of an assay can be affected by the handling and processing of specimens. The stored specimens were collected in heparin, which had to be removed with heparin lyase I before the reverse-transcription-PCR assay could be performed. The choice of anticoagulant has been shown to interfere with quantification by both the reverse-transcription-PCR and the bDNA assays; plasma samples collected in heparin may yield values up to 38 percent lower than those of specimens collected in EDTA.⁶ The relatively low median RNA levels reported may partly be due to delays in separating plasma from whole blood, which result in increasing degradation of RNA with time.²⁰ Despite the variations that may have occurred in the collection and handling of specimens, there was no evidence that the median RNA copy number differed significantly according to study site or over time.

Statistical considerations limit the conclusions that can be drawn from the study. The small number of women in the zidovudine group who transmitted HIV-1 to their infants limited the statistical power of the study to detect an association between changes in the RNA level and the risk of transmission. The estimated proportions of the treatment effect that are explained by this measure may have been attenuated by errors of measurement in reporting the RNA results.²¹

In other studies, advanced maternal immunosuppression, as evidenced by a low CD4+ cell count,^{22,23} has been associated with an increased risk of perina-

tal HIV-1 transmission. In the placebo group, lower base-line CD4+ counts, lower percentages of CD4+ cells, and higher percentages of CD8+ cells were all associated with an increased risk of transmission. In the zidovudine group, no immunologic predictors were significantly associated with this risk. The effect of zidovudine treatment was seen in all groups regardless of the CD4+ count at study entry.

In our study there was HIV-1 transmission with a wide range of plasma RNA levels and CD4+ counts at entry, the treatment effect was seen in all RNA subgroups, and the reduction in plasma RNA from entry to delivery did not account for the success of zidovudine therapy. Therefore, our data strongly support the current practice of offering zidovudine to all HIV-1-infected pregnant women and their newborns, regardless of the maternal plasma viral burden and CD4+ count. Even when alternative antiretroviral drugs are indicated to treat the mother's disease, the regimen of zidovudine that has been proved to prevent perinatal transmission of HIV-1 must be considered.

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APPENDIX

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