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Maternal plasma human immunodeficiency virus type 1 RNA level: A determinant and projected threshold for mother-to-child transmission

(quantitative competitive PCR/vertical transmission/perinatal infection)

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ABSTRACT **To prevent mother-to-child human immunodeficiency virus type 1 (HIV-1) transmission, it is important to identify its determinants. Because HIV-1 RNA levels can be reduced by antiviral therapy, we examined the role of maternal plasma HIV-1 RNA level in mother-to-child transmission. We used quantitative competitive PCR to measure HIV-1 RNA in 30 infected pregnant women and then followed their infants prospectively; 27% of the women transmitted HIV-1 to their infants and maternal plasma HIV-1 RNA level correlated strikingly with transmission. Eight of the 10 women with the highest HIV-1 RNA levels at delivery (190,400–1,664,100 copies per ml of plasma) transmitted, while none of the 20 women with lower levels (500–155,800 copies per ml) did (P = 0.0002). Statistical analysis of the distribution of HIV-1 RNA loads in these 30 women projected a threshold for mother-to-child transmission in a larger population; the probability of a woman with a viral RNA level of ≤100,000 copies per ml not transmitting is predicted to be 97%. Examination of serial HIV-1 RNA levels during pregnancy showed that viral load was stable in women who did not initiate or change antiviral therapy. These data identify maternal plasma HIV-1 RNA level as a major determinant of mother-to-child transmission and suggest that quantitation of HIV-1 RNA may predict the risk of transmission.**

Mother-to-child transmission of human immunodeficiency virus type 1 (HIV-1) has become a major problem worldwide (1). Only 14–40% of infected pregnant women transmit HIV-1 to their infants, but the determinants of transmission are not completely understood (1). To develop strategies to prevent transmission in a broad range of infected women, it is important to identify these determinants. A recent National Institutes of Health (NIH) trial showed that the antiviral agent zidovudine (AZT) reduced the rate of transmission in a subset of women (2); the drug may have worked by lowering maternal HIV-1 load (2, 3). In fact, recent studies have shown that high maternal viral titer measured by quantitative cell culture correlates with transmission (4, 5). Because HIV-1 RNA levels in plasma can be reduced by antiviral therapy (6), we examined the role of maternal plasma HIV-1 RNA load in mother-to-child transmission, seeking to identify a determinant of transmission. We used quantitative competitive PCR (QC-PCR) (6, 7) to rigorously quantitate virion-associated HIV-1 RNA because of its advantage in reproducibly measuring a broad range of HIV-1 RNA loads in clinical specimens (6, 7). For many clinical samples, the presence of inhibitors that can interfere with reverse transcription (RT) and PCR amplification poses problems for quantitative studies. QC-PCR overcomes these problems by using a control RNA template matching the HIV-1 target sequence but differing by an internal deletion. The template is used in a competitive titration in both RT and PCR steps, providing a stringent internal control (6, 7). By using QC-PCR to measure plasma HIV-1 RNA levels in pregnant women, we identified maternal viral load as a major determinant of mother-to-child transmission. These data represent the first step toward projecting a threshold for transmission and predicting the risk of mother-to-child transmission.

MATERIALS AND METHODS

Subjects. We prospectively studied 30 HIV-1-infected women enrolled either during pregnancy or at delivery in an investigation of mother-to-child transmission at Stony Brook University Hospital in Stony Brook, NY, and St. Joseph's Medical Center in Paterson, NJ. The institutional review boards at each hospital and the New York State Department of Health approved the investigation and each woman provided informed consent for herself and her child. Five mother–child pairs participated in NIH AIDS Clinical Trial Group protocol 076 (2), a randomized, controlled trial of AZT administered to mothers during pregnancy and labor and to their infants during the first 6 wk of life to prevent mother–child HIV transmission. Separate specimens were obtained for each study. T-cell subset analyses were determined locally by flow cytometry.

We studied mother–child pairs who met the following criteria: (*i*) the mothers had HIV-1 viral load determined by both culture and QC-PCR at the time of delivery or during gestation and at delivery and (*ii*) the children born to these mothers have been followed for ≥6 mo, with their HIV-1 infection status ascertained by both culture and PCR as described (4). A 6-mo virologic follow-up period was chosen because previous studies detected HIV-1 by culture alone or by culture and PCR in 100% of infected babies by the time they were 6 mo old (8, 9).

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Abbreviations: HIV-1, human immunodeficiency virus type 1; NIH, National Institutes of Health; PBMC, peripheral blood mononuclear cell; IU, infectious unit(s); QC-PCR, quantitative competitive PCR; AZT, zidovudine; RT, reverse transcription or reverse transcriptase.

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QC-PCR to Measure Plasma HIV-1 RNA. Blood specimens were collected in citrate-treated tubes, shipped overnight at room temperature, and processed uniformly at the Wadsworth Center, where all quantitative analyses of HIV-1 including QC-PCR were performed. After centrifugation (Beckman GS-6R; 12,000 rpm, 20°C, 10 min), plasma was withdrawn and stored at ≤−80°C. To perform RT and QC-PCR of plasma virion-associated RNA, virions were pelleted from plasma by centrifugation (Sorvall FA-Micro rotor; 14,000 rpm, 10°C, 1 hr) and RNA was extracted according to Winters *et al.* (10). After extraction, glycogen (20 μg) and yeast tRNA (10 μg) were added to each sample as RNA carriers, and RNA was coprecipitated with isopropanol at −20°C overnight. The pellet was resuspended in denaturing solution (RNAgents total RNA isolation system; Promega) and reprecipitated once. RNA was then dried, dissolved in 20 μl of sterile, RNase-free water, and stored at −80°C.

RT and QC-PCR were performed as described by Piatak *et al.* (6, 7). In addition, during the RT procedure, reaction mixtures lacking RNA template and enzyme were included in each experiment as negative controls. For amplification of cDNA, primers GAG04 and GAG06 (6, 7) were used to amplify an internal fragment of a conserved region of the HIV-1 *gag* gene from both patient-derived and competing templates. Amplification was performed by using a GeneAmp PCR system 9600 thermal cycler (Perkin–Elmer) for 45 cycles (94°C for 15 sec and 60°C for 30 sec), followed by a final extension at 72°C for 10 min. Approximately 20% of the final amplified products were separated by electrophoresis in a 3% Metaphor agarose (FMC) gel. Gels were stained with ethidium bromide and the densities of product bands from both patient-derived and competing RNA were quantitated on a MasterScan interpretive densitometer using matched custom software (Scanalytics, Billerica, MA). Competition equivalence points were determined and HIV-1 RNA copy numbers were calculated as described (7). Each plasma sample was assayed at least twice and RNA levels were reproducible. The person performing the assays did not know the clinical or transmission status of the mothers.

AZT Resistance Assays. AZT-resistant virus in plasma or serum was detected by differential PCR to discern mutations at codon 215 of the HIV-1 RT gene (11); mutations at this site are often seen in clinical isolates resistant to AZT and are associated with clinical decline in patients receiving long-term AZT therapy (11, 12). For infected infants, we tested samples obtained on the same date as the first sample indicating HIV-1 infection, which was prior to AZT treatment in each case.

HIV-1 Quantitative Culture. We quantitated HIV-1 in the mothers' peripheral blood mononuclear cells (PBMCs) by performing endpoint dilution cultures in duplicate according to the NIH AIDS Clinical Trial Group procedure (4, 13, 14). Quantitative culture results and partial clinical data for mother–child pairs 1–19 (Table 1) were reported previously (4).

Statistical Methods. We used the Wilcoxon rank sum test to make comparisons between the magnitude of HIV-1 RNA level, HIV-1 titer determined by culture, $CD4^+$ counts, $CD8^+$ counts, and the presence of AZT resistance in transmitting and nontransmitting mothers. Fisher's exact test was used to compare proportions of transmitting and nontransmitting mothers. Data for factors relating to transmission were also analyzed by multiple logistic regression models to identify significant predictor variables including HIV-1 RNA level, $CD4^+$ count, $CD8^+$ count, and AZT treatment. These logistic models were used to confirm the bivariate analyses of the Wilcoxon rank sum test. We performed analyses to determine whether there was a threshold for transmission. A conservative threshold was projected by two methods: (*i*) probability methods based on distribution theory and (*ii*) logistic analysis. Confidence intervals were provided for our estimate of the probability of not transmitting at HIV-1 RNA levels below the projected threshold.

RESULTS

Patient Population. The 30 HIV-1-infected women in this study represented a broad spectrum of clinical and immunologic states (Table 1). Eight women had $CD4^+$ cell counts of <200 cells per mm^3, 3 of whom had a history of severe opportunistic infections. The remaining 22 women had $CD4^+$ counts of >200 cells per mm^3 and all were asymptomatic. Nineteen women were treated with AZT at some point during pregnancy; in most cases, the therapy was intermittent or of short duration. Seventeen women were prescribed the drug by their physicians and 2 were randomized to receive it as part of the NIH trial of AZT in pregnancy (Table 1) (2). As participants in this trial, they received intravenous AZT during labor and delivery and their infants received prophylactic AZT after delivery for 6 wk. These 2 infants were the only ones to receive AZT as newborns. None of the mothers breast-fed. Eight of the 30 women (27%) transmitted HIV-1 to their infants (Table 1).

Maternal HIV-1 Load and Transmission. To examine the role of maternal viral load in mother-to-child transmission, we quantitated HIV-1 by performing both endpoint dilution cultures of PBMCs and QC-PCR of plasma. By using quantitative culture, we found that 4 of 6 (67%) women with titers of >100 HIV-1 infectious units (IU) per 10^6 PBMCs transmitted, 3 of 12 (25%) with titers of 10–100 HIV-1 IU/10^6 PBMCs transmitted, and only 1 of 12 (8%) women with titers of <10 IU/10^6 PBMCs did (Fig. 1*A*). The relationship of viral titer to the occurrence of mother-to-child transmission was statistically significant ($P = 0.01$; Wilcoxon rank sum test). We next determined the maternal plasma HIV-1 RNA level at the

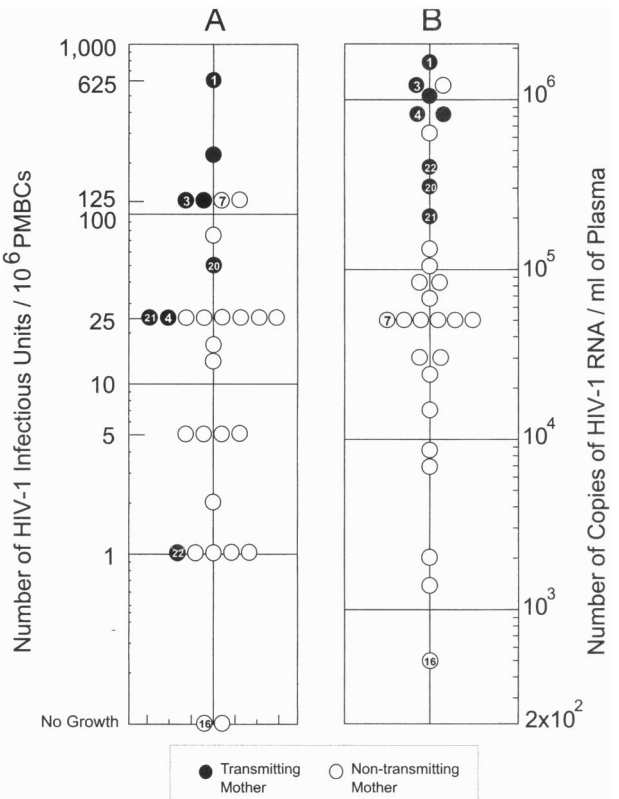

FIG. 1. Relationship of maternal HIV-1 load to mother-to-child transmission. Mothers of particular interest are identified by numbers. (*A*) HIV-1 titer measured by quantitative PBMC culture. (*B*) HIV-1 plasma RNA level determined by QC-PCR.

Table 1. Virologic and clinical characteristics of mother–child pairs

	Characteristics of mothers during pregnancy and delivery									Characteristics of children		
Pair	Age, yr/ HIV risk	Ethnic origin	CDC class	$CD4^+$ count, cells per mm^3	$CD8^+$ count, cells per mm^3	Anti-HIV therapy	Culture titer, IU/10^6 PBMCs	HIV-1 RNA, copies per ml	AZT resistance	Clinical status	Follow-up, mo	AZT resistance
						Transmission-positive families						
1	28/hetero	Caucasian	C3	160	730	—	625	1,664,100	−	P2A, B, D1	19	−
2	28/hetero	Caucasian	A3	146	334	AZT	225	1,068,700	−	P2A, E	9	−
3	36/hetero	Latina	C3	43	122	AZT	125	1,219,800	+	Died, P2A, B	16	+
4	21/hetero	Caucasian	A2	470	680	076 placebo	25	831,300	−	P2A, B	13	−
5	34/IDU	African-Am	B3	30	ND	—	125	786,100	−	Died, P2A, B	12	−
20	27/IDU	African-Am	A3	162	771	AZT	52	308,200	+	Died, P2A, B	6	+
21	25/hetero	Caucasian	A2	325	1145	AZT	25	190,400	−	P2A	12	−
22	25/IDU	Latina	A2	397	406	AZT	1	403,000	−	P2A	10	−
						Transmission-negative families						
6	29/hetero	African-Am	A2	380	513	—	5	7,500	−	Well	18	
7	29/hetero	Latina	A2	467	759	AZT	125	49,700	−	Well	17	
8	27/hetero	Caucasian	A1–A2	420	784	AZT	25	26,800	−	Well	19	
9	25/IDU	Caucasian	A2	400	1154	AZT	17	45,800	+	Well	16	
10	29/hetero	Caucasian	A2	267	748	—	25	639,300	−	Well	18	
11	36/IDU	Latina	A1	650	720	076 placebo	25	41,400	−	Well	15	
12	34/transfusion	Haitian	A3	154	514	AZT	125	1,211,900	−	Well	6	
13	29/IDU	Latina	A1	537	531	AZT	75	155,800	−	Well	19	
14	35/hetero	African-Am	A3	160	1370	AZT	15	115,200	−	Well	11	
15	33/IDU	African-Am	A2	285	635	076 AZT	1	49,100	−	Well	10	
16	36/IDU	African-Am	A1–A2	571	951	—	No growth	500	−	Well	9	
17	33/hetero	African-Am	A2	380	720	076 placebo	25	48,700	−	SCD	11	
18	28/hetero	Latina	A1	520	ND	—	1	82,700	−	Well	6	
19	29/IDU	African-Am	C3	76	450	—	5	29,100	−	Well	14	
23	32/hetero	Latina	A1	645	739	—	25	95,400	−	Well	9	
24	33/hetero	Latina	A1	645	516	AZT	5	28,900	−	Well	7	
25	33/IDU	Caucasian	A1	590	630	076 AZT	5	15,000	−	Well	8	
26	28/IDU	Latina	A1	863	711	—	5	51,400	−	Well	10	
27	27/IDU	African-Am	A2	440	787	AZT	1	1,400	−	Well	6	
28	42/IDU	Latina	A2	220	260	AZT	25	75,600	−	Well	7	
29	19/hetero	Latina	A1	728	1740	—	1	8,900	−	Well	8	
30	17/hetero	Caucasian	A1	750	490	AZT	No growth	2,200	−	Well	7	

Hetero, heterosexual transmission; 076, NIH AIDS Clinical Trial Group protocol 076; IDU, injecting drug use; African-Am, African-American; ND, not done. HIV-1 infection status of adults was described by using the 1993 Revised Centers for Disease Control (CDC) system (15) classifying individuals by both clinical status and $CD4^+$ count into nine exclusive categories. Clinical category: A, asymptomatic infection; C, severe AIDS-defining disease; B, symptomatic conditions not included in A or C. $CD4^+$ cell category: 1, counts of $\geq$500 per mm^3; 2, counts between 200 and 499 per mm^3; 3, counts of <200 per mm^3. Pediatric HIV-1 infection was described according to the CDC classification for HIV in children (16): P2A, symptomatic infection with nonspecific findings; P2B, progressive neurologic disease; P2D1, specified secondary infectious diseases listed in the CDC surveillance definition for AIDS; P2E, other diseases including thrombocytopenia; SCD, sickle cell disease.

time of delivery in these 30 women by using QC-PCR. Plasma HIV-1 RNA levels in these 30 women ranged from approximately 500 to 1,700,000 copies per ml (corresponding to 250–850,000 virion per ml) (Fig. 1*B*, Table 1). Eight of the 10 women with the highest HIV-1 RNA levels in plasma (190,400–1,664,100 copies per ml of plasma) transmitted HIV-1 to their infants, while none of the 20 women with lower levels (500–155,800 copies per ml) transmitted (Fig. 1*B*) (P = 0.0002; Fisher's exact test).

For most women, the level of plasma HIV-1 RNA correlated well with, but exceeded by an average of 10^3–10^4, the titer of cultured virus (mothers 1, 3, and 16 are examples) (Fig. 1). For a few women, however, the titers of cultured virus were not concordant with transmission. One transmitting mother (mother 22) had a low titer and 2 others (mothers 4 and 21) had titers in the middle range determined by PBMC culture; what is noteworthy is that all 3 had high levels of HIV-1 RNA (190,400–831,300 copies per ml) in their plasma (Fig. 1). Their relatively high plasma HIV-1 RNA levels appear to explain why they transmitted HIV-1 to their infants. Mother 7, by contrast, had a high viral load by culture (125 IU/10^6 PBMCs) but a plasma HIV-1 RNA level in the middle range (49,700 copies per ml) and she did not transmit to her infant (Fig. 1). Thus, plasma HIV-1 RNA levels determined by QC-PCR in this cohort demonstrated a much stronger correlation with mother-to-child transmission than titers determined by culture. The data demonstrate that maternal HIV-1 plasma RNA level measured at delivery is a major determinant of mother-to-child transmission.

Projection of a Threshold for Transmission. Eighty percent of the women in this cohort with HIV-1 RNA levels of >190,000 copies per ml of plasma transmitted to their infants and none of the 20 women with viral loads below that level transmitted (Fig. 1*B*). In our analysis seeking a possible threshold for transmission, a viral RNA level of 100,000 copies per ml of plasma provided a conservative threshold as projected by the following two methods. Since selection of mothers was not based on factors relating to transmission, probabilities regarding transmission may be estimated. The frequency of RNA levels for transmitting mothers appears to be normally distributed (mean = 808,950; median = 808,700; standard deviation = 502,383). (The distribution of the smaller RNA values for the nontransmitting mothers is not normally distributed.) Therefore, Z scores may be used for the RNA

values of the transmitting mothers. While no transmitting mothers had RNA levels of ≤100,000 copies per ml in this cohort, a larger group is projected to have 7.93% of transmitters with such levels using *Z*-score percentiles. A conservative estimate is given by applying 7.93% to the 8 transmitters in this cohort, finding that 0.634 of these theoretically have RNA levels of ≤100,000 copies per ml. Combined with the 18 nontransmitters who have levels of ≤100,000 copies per ml, the probability of being a nontransmitter if the RNA level is ≤100,000 copies per ml is 18/18.634 = 0.966 or 97%. Using the binomial distribution, the estimate of the 95% confidence interval is (0.88, 1.00).

These results are confirmed by a different method. Essentially the same results were obtained by fitting a logistic model. Here the probability of transmission was expressed as a function of RNA copy number. Excellent fit was obtained. This analysis estimates that for mothers with RNA levels of <100,000 copies per ml the probability of not transmitting is 99%, only slightly different from the 97% derived above. Although the logistic analysis shows that the statistically most optimal separation between the two groups of mothers occurs at the HIV-1 RNA level of 160,000 copies per ml (see Fig. 2), the level of 100,000 copies per ml suffices as a conservative, tentative estimate representing the order of magnitude of a threshold level. These analyses represent the first step toward identifying a threshold for transmission.

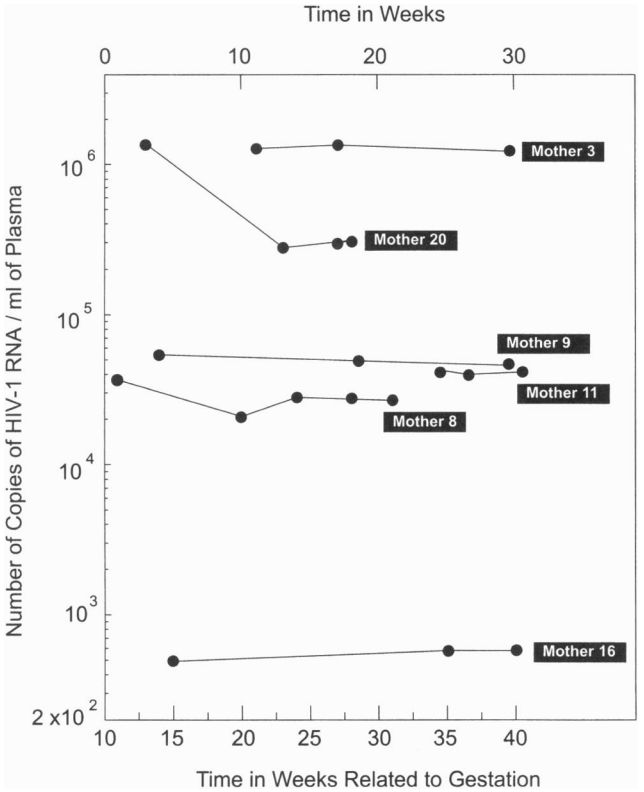

FIG. 2. Serial maternal plasma HIV-1 RNA levels. Data are expressed as copies of HIV-1 RNA per ml (●). For each woman, the first time point indicates enrollment during gestation and the last represents delivery. Mother 3, who had a diagnosis of AIDS and had taken AZT prior to pregnancy, was on treatment at enrollment and remained on it during pregnancy and until delivery. Her second sample was serum; all other samples in the study analyzed for HIV-1 RNA level were plasma. Mother 9, who was asymptomatic and had also taken AZT prior to pregnancy, was on therapy at enrollment and continued until delivery. Both mothers 3 and 9 had virus resistant to AZT. Mothers 11 and 16 were both asymptomatic and received no antiviral therapy. Mother 11 received placebo in the NIH trial of AZT in pregnancy (2). Mothers 20 and 8 are discussed in the text.

Other Factors and Mother-to-Child Transmission. Previous studies have shown a correlation between low maternal $CD4^+$ T-lymphocyte count and mother-to-child transmission (1, 4). We also documented such a correlation in this cohort ($P = 0.01$ by Wilcoxon rank sum test) but found that a low $CD4^+$ cell count did not correlate as well with transmission as did plasma HIV-1 RNA level (Table 1). Multivariate analysis examining HIV-1 RNA load and $CD4^+$ cell count as they relate to transmission demonstrated that plasma HIV-1 RNA level is a major determinant of mother-to-child transmission independent of $CD4^+$ T-cell count. In contrast to $CD4^+$ cell counts, $CD8^+$ cell counts in the transmitting mothers did not differ significantly from $CD8^+$ cell counts in the nontransmitting mothers ($P = 0.31$).

AZT Treatment, Resistance, and Transmission. In addition, we performed a statistical analysis of the relationship of AZT treatment at any time during pregnancy to both HIV-1 RNA level and mother-to-child transmission. Most women in this cohort who were prescribed AZT took it intermittently, some took it regularly, and 11 were untreated. Both AZT-treated and untreated women demonstrated a broad spectrum of viral loads (Table 1). In this heterogeneous group, use of AZT did not show a statistically significant relationship to either HIV-1 RNA level or mother-to-child transmission as determined by both univariate and multivariate analysis (all *P* values ≥ 0.37). Multivariate analyses of various factors including AZT treatment showed that HIV-1 RNA level is the variable most highly correlated with transmission. Moreover, HIV-1 RNA level cannot be joined by any other variables including AZT treatment to improve prediction probabilities for transmission.

To address whether AZT resistance contributed to the lack of correlation between AZT treatment and transmission, we assayed for resistant virus in serum or plasma obtained at delivery from all 30 women including the 8 transmitting mothers. Resistant virus was detected in 3 women; all 3 were taking AZT and 2 of the 3 had high HIV-1 RNA loads and transmitted resistant HIV-1 to their infants (mothers 3 and 20) (Table 1).

Serial HIV-1 RNA Levels. Quantitation of plasma HIV-1 RNA at delivery suggests that there is a threshold for transmission. To determine whether the probability of transmission can be predicted by measuring HIV-1 levels earlier in pregnancy, we examined the stability of plasma HIV-1 RNA levels during gestation and at delivery in part of the cohort. Stable HIV-1 titers in PBMCs have been found during pregnancy, at delivery, and postpartum by using quantitative culture (4). As a preliminary investigation, we determined HIV-1 RNA loads 3 or more times during pregnancy and at delivery in 6 women. These women were chosen because they had provided multiple samples throughout pregnancy and represented a broad range of clinical states, RNA levels, and histories of antiviral therapy. Five women were first studied at 21 weeks of gestation or earlier (Fig. 2).

In the 2 women who did not take antiviral therapy during pregnancy, viral RNA levels remained stable (mothers 11 and 16). Levels also were stable in those who had a history of AZT treatment before pregnancy and who continued treatment throughout gestation (mothers 3 and 9); both of these mothers had AZT-resistant virus detected at delivery. Antiviral therapy, however, did reduce maternal plasma HIV-1 RNA titers during pregnancy; the effect varied in different patients. Mother 20, for example, began taking AZT soon after enrollment and took it for 17 weeks, until delivery. Her plasma HIV-1 RNA level decreased from 1,335,200 copies per ml at enrollment to 308,200 copies per ml at delivery (Fig. 2). Even with this response to AZT, her virus was resistant to AZT at delivery, her HIV-1 titer remained above the projected threshold for transmission, and she transmitted HIV-1 to her infant (Fig. 1*B*). Mother 8, by contrast, took AZT for a 2-wk period during the second trimester of pregnancy; her HIV-1 RNA

level did not change significantly (Fig. 2). The serial determinations in these women suggest that measurement of maternal plasma HIV-1 titer early in pregnancy can be used to predict titer at delivery if there is no change in treatment.

DISCUSSION

Studies to date suggest that multiple factors play a role in HIV-1 mother-to-child transmission including maternal immune response, properties of the mother's virus, and obstetric factors (1, 4, 5, 17–20). By using QC-PCR, we identified maternal plasma HIV-1 RNA level as a major determinant of transmission. The viral load itself may play a crucial role in determining whether transmission occurs or it may be that high HIV-1 RNA levels are associated with other determinants of infectivity. While this study provides evidence that HIV-1 RNA level is a powerful determinant of transmission, it also supports the concept that viral titer is not the sole determinant. All 8 transmitting women in this cohort had HIV-1 RNA levels of >190,000 copies per ml but there were 2 additional women with viral loads exceeding that level who did not transmit (mothers 10 and 12) (Fig. 1*B*). Why these 2 women failed to transmit is unknown. Mother 12 had given birth previously to 4 children; 2 of them, 7 and 3 yr old, were HIV-1-infected but the other 2, 9 and 6 yr old, were uninfected. This family underscores the variability of HIV-1 mother-to-child transmission and the need to examine factors in addition to viral load that may have played a role in transmission in this cohort, particularly maternal HIV-1 phenotype, neutralizing antibodies, and genomic diversity.

The clear difference in distribution of HIV-1 RNA levels between the transmitting and nontransmitting mothers represents the first step in identifying a threshold for mother-to-child transmission. Statistical analyses projected a conservative threshold of 100,000 copies of HIV-1 RNA per ml (Fig. 1*B*). This level may be an underestimate not only because the calculation was aimed at a low threshold, but also because all of the blood samples were shipped by overnight delivery before uniform processing; this procedure may have resulted in a fractional reduction of the RNA measured in all samples (21). Although larger studies will be necessary to determine the precise threshold, the data presented here establish the principle of a threshold for mother-to-child transmission.

These data provide the scientific rationale for implementing multiple strategies to interrupt transmission by reducing viral load, including drug therapy (2), obstetric measures (1), and immunoprophylaxis (1). In addition, projection of a conservative threshold for transmission begins to point the way toward treatment goals that may be used to monitor antiviral therapy during pregnancy.

Five women in this cohort participated in the NIH clinical trial of AZT in pregnancy (2) and the 2 who were randomized to receive AZT had AZT-sensitive virus and did not transmit to their infants. In general, however, the patient population and AZT treatment schedule of our cohort differed significantly from those of the NIH trial. Most of the women in our study who took AZT did so intermittently or for short periods. Furthermore, except for the regimens of the women who actually participated in the NIH trial, AZT treatment in our cohort failed to include two components of the trial that possibly contributed to its efficacy: AZT administered to the mother during delivery and to the infant during the first 6 wk of life. Perhaps more important, our cohort differed from the subjects in the NIH trial because it included women with advanced clinical disease, low $CD4^+$ cell counts, and a history of AZT treatment, each of which is associated with AZT resistance (22). Indeed, 2 of the transmitting mothers (mothers 3 and 20) displayed these characteristics as well as high HIV-1 loads and had AZT-resistant virus detected. It is not surprising, therefore, that in this cohort AZT use at any time during pregnancy did not show a significant relationship to transmission.

Preliminary examination of serial HIV-1 RNA levels showed that viral load remained stable in pregnant women who did not initiate or change antiviral treatment. Although additional studies will be needed to quantify the precise risk of transmission associated with a particular RNA level and antiviral treatment, these data suggest that determination of plasma HIV-1 RNA level early in pregnancy may predict the risk of mother-to-child transmission.

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