Maternal antibody response at delivery and perinatal transmission of human immunodeficiency virus type 1 in African women

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Summary

Prospective cohort studies indicate that 13–45% of human immunodeficiency virus type 1 (HIV-1)-infected pregnant women transmit the virus to their infants. Although factors that influence perinatal transmission are not well understood, drug and immunotherapy trials to interrupt transmission are underway. The identification of women most at risk is essential for prevention, counselling, and medical intervention.

We assessed 70 HIV-1-infected pregnant women enrolled in a prospective study of perinatal transmission in Brazzaville, Congo. The relations between maternal health status, antibody levels to selected HIV-1 structural antigens at delivery, and infant outcome were explored. Independent of clinical stage, higher maternal antibody titres to peptides corresponding to the V3 region of gp120 and the immunodominant domain of gp41 were correlated with a higher risk of perinatal transmission. In a logistic regression model, the predicted risk of transmission for symptom-free women whose antibody titres to V3 and gp41 were lowest was 0·02, whereas it was 0·88 for symptomatic women whose antibody titres to V3 and TMSP18 were highest.

These associations may give new insight into the mechanisms of perinatal transmission and they may also provide a powerful means of identifying women who would most benefit from intervention trials to halt perinatal transmission.

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Introduction

Perinatal transmission is the main source of human immunodeficiency virus type 1 (HIV-1) infection in children. The number of infants at risk for HIV-1 perinatal transmission has increased over the past few years, and WHO estimates indicate that at the end of 1992 more than 5 million women of child-bearing age worldwide had been infected, and of these, 4 million were African.[1] However, not all HIV-1-infected pregnant women transmit the virus to their children, and studies of infants followed from birth indicate risks of transmission from 0·13 to 0·45. Maternal characteristics associated with an increased risk of perinatal transmission include advanced HIV-1 clinical stage during pregnancy, impaired immunological status, high levels of viral replication, and breast-feeding.[2-6]

The mother's antibody response to HIV-1 has also been studied as a possible factor influencing perinatal transmission. Reports published in 1989–90 suggested that mothers with antibodies against the principal neutralising domain of gp120—the V3 region—were less likely to transmit HIV-1 to their child during pregnancy, although these results have not been confirmed. Despite the mechanisms of perinatal transmission still not being understood, drug and immunotherapy trials have begun.[7] We examined maternal status at delivery and antibody response to the V3 region of gp120 and the immunodominant domain of gp41 in HIV-infected women who did and did not transmit the virus to their child.

Patients and methods

The study was carried out in Brazzaville, Congo, from March, 1987 to May, 1988, and from Aug to Dec, 1989. Pregnant women who had agreed to be tested were sequentially recruited either during their first prenatal visit or at delivery in two mother-child clinics and one maternity hospital. Women and infants were under the care of the same physician and were followed for at least 2 years after delivery.

Mother's sera were tested for antibodies to HIV-1 by enzyme-linked immunosorbent assays (ELISA) (ELAVIA, Diagnostics Pasteur, Marnes-la-Coquette, France). Positive ELISA tests were confirmed by western blot (Blot test, Du Pont de Nemours, Rockville, MD, USA). Sera were considered positive when they showed antibodies to at least two envelope glycoproteins of HIV-1. The same criteria were used to assess the serological status of infants born to seropositive mothers.

All women were given their test results and counselled. Clinical examination of mothers and infants, and blood sampling of the infants were done soon after birth, at 1 month, 3 months, and every 3 months thereafter. Mothers were classified as symptomatic if they had at least one of the WHO clinical AIDS definition criteria.[8] They were subsequently classified as transmitting based on their infants' serologically-determined or clinically-determined HIV-1 status. Infants who died before serology was interpretable (15 months, by which time infants have lost passively-transferred

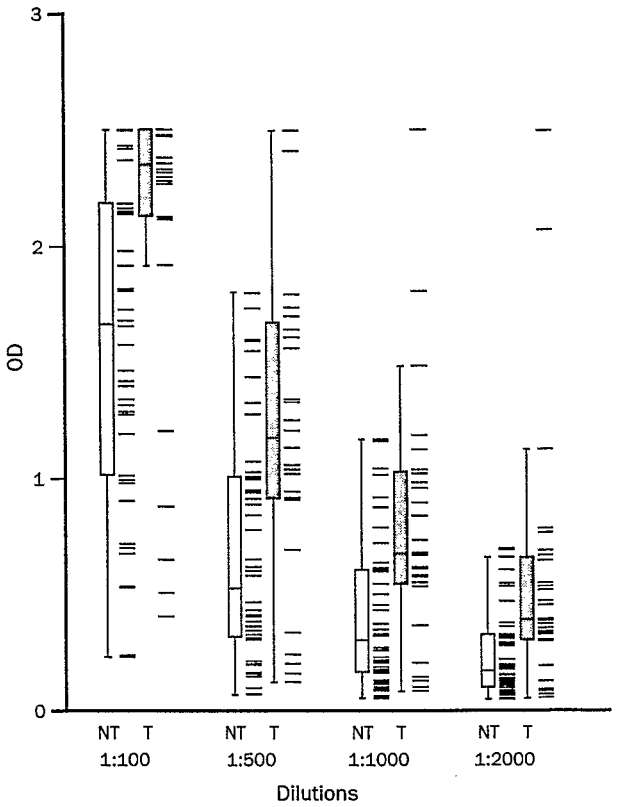

Figure 1: **One-way scatter plots of the distribution of maternal antibodies to V3Cons in transmitting and non-transmitting mothers**

Antibody levels were measured by optical density (OD) with ELISA at four dilutions of each sample. Each short horizontal line along the x axis represents one observation. Alongside each scatter plot is the corresponding box-and-whisker plot. The line in the middle of the box represents the median of the distribution; the box extends from the 25th to the 75th percentile. The lines extending above and below the box represent 1·5 times the interquartile range rolled back to where there are data. Shaded boxes indicate transmitting mothers (T) and open boxes indicate non-transmitting mothers (NT).

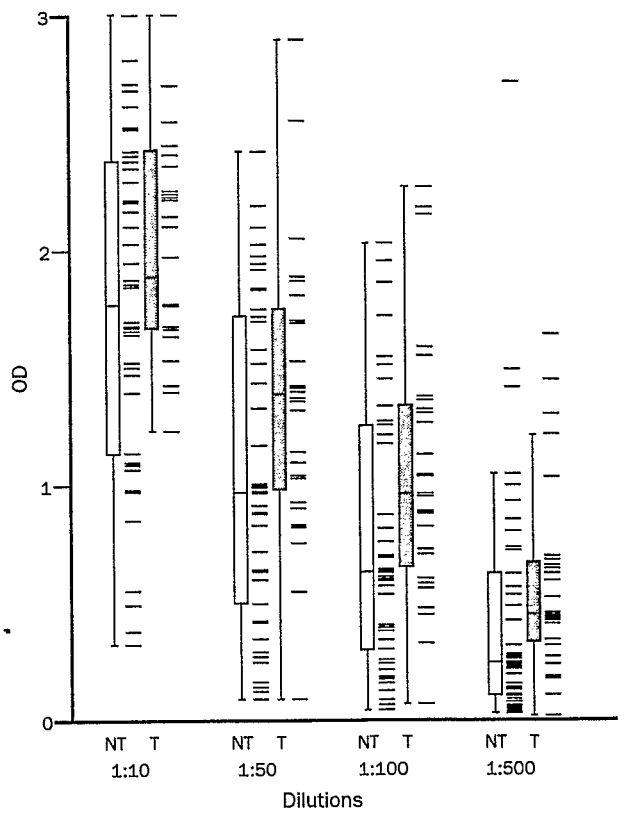

Figure 2: **One-way scatter plots of the distribution of maternal antibodies to TMSp18 in transmitting and non-transmitting mothers**

See footnote to figure 1.

maternal antibodies) were classified as infected if they either had AIDS according to the WHO case definition,[8] or if their symptoms and the circumstances of their deaths met the EEC/WHO criteria for HIV-attributable infant death.[9]

114 HIV-1-seropositive mothers were enrolled; they and their 118 infants were followed for at least 2 years. Of 10 twins delivered, 1 was stillborn. 9 infants were lost to follow-up because they moved away. The HIV-1 status of 10 infants who died before reaching 15 months was indeterminable because they did not meet the EEC/WHO criteria for HIV-1-attributable death (4 infants including 3 twins died during the neonatal period). The HIV-1-infectious status of the 99 remaining infants was established by either serological or clinical criteria. 56 were seronegative and 21 seroconverted. The other 22 infants died before serology was interpretable but had developed AIDS or met the criteria for HIV-1-attributable death. Sera from 70 of 99 mothers whose infants' HIV-1 status was known were randomly selected for further analysis. These sera were coded and investigators were blinded for both the infant's outcome and the mother's clinical status.

Maternal antibodies to the third variable domain (V3) of envelope glycoprotein gp120, the immunodominant domain of envelope glycoprotein gp41, and the core protein p24 were measured. Two peptides, V3Cons and TMSP18, were synthesised for use as antigenic sources in solid-phase ELISA. V3Cons corresponds to the consensus sequence determined by La Rosa et al.[10] TMSP18 corresponds to the highly conserved immunodominant domain of gp41.[11] Antibodies to p24 were measured with a full-length p24 recombinant protein ELISA test (Abbott Laboratories, North Chicago, IL, USA). Peptides were synthesised on an automated peptide synthesiser, purified by reverse-phase chromatography, and purified preparations characterised by a single sharp peak on high-pressure liquid chromatography and by aminoacid analysis. The peptides were used as solid-phase antigens in ELISA tests by procedures already described.[11] Antibodies to p24 were titrated with a third generation sandwich solid-phase immunoassay (HIVAB p24 [rDNA]) with a full-length recombinant p24 protein (Abbott Laboratories). Serum dilutions were 1:100, 1:500, 1:1000, and 1:2000 in the V3Cons assay, 1:10, 1:50, 1:10, and 1:500 in the TMSP18 assay, and 1:3125, 1:15625, 1:78125, and 1:390625 in the p24 assay. In each assay, endpoint antibody titres were determined by relating the absorbance of the specimen dilutions to the cut-off values corresponding to the highest dilution at which a positive result is obtained. The cut-off value for the V3Cons assay was estimated as the mean optical density of 79 negative sera plus standard deviations, and for TMSP18 as the mean optical density of negative controls[11] plus four standard deviations. Cut-off values for p24 were automatically calculated according to manufacturer's instructions with a Quantum II analyzer (Abbott Laboratories). The peptide assays were processed in one batch by a technician blinded to maternal clinical status and infant outcome.

All statistical tests were two-sided. The non-parametric Wilcoxon rank-sum test was used to compare the medians of the optical density distributions in transmitting versus non-transmitting mothers. Pearson's χ^2 and the Cochran-Armitage test for linear trend were used to evaluate associations for categorical variables. Adjusted odds ratios were obtained by logistic regression. Antibody titres for V3Cons and for TMSP18 were entered into the model as ordinal variables with values 1 to 4, because this approach best reflected the bivariate linear association between titre and risk, and provided the most precise estimates of the coefficients. The presence or absence of symptoms was entered into the model as a dichotomous variable.

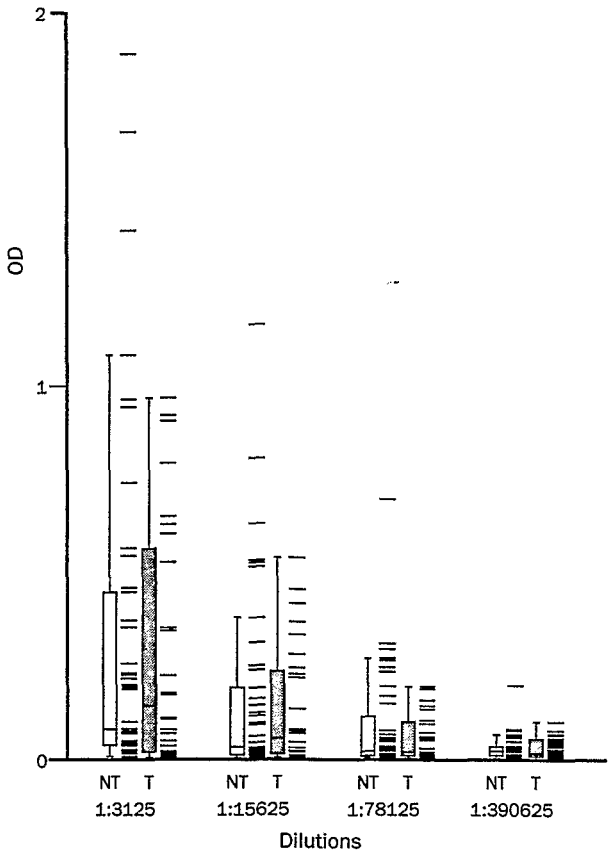

Figure 3: **One-way scatter plots of the distribution of maternal antibodies to p24 in transmitting and non-transmitting mothers**
See footnote to figure 1.

Results

In the 70 mother-child pairs studied, 43 infants (61%) were seronegative and healthy and 27 were HIV-1-infected (39%). Of infected infants, 15 were seropositive and 12 died of HIV-1/AIDS. 2 mothers had twins; 1 transmitted the virus to both of her infants and the other to neither. The HIV-1 antibody-response pattern of mothers who transmitted the virus to their infant differed from that of non-transmitting mothers. Figures 1–3 show one-way scatter plots of the distribution of maternal antibodies to V3Cons, TMSP18, and p24 in transmitting and non-transmitting mothers. All median optical densities were significantly higher for transmitting mothers at all dilutions for V3Cons ($p<0·01$) and at all dilutions except 1:10 and 1:50 for TMSP18 ($p=0·09$, 0·07, 0·04, and 0·04 at dilutions 1:10, 1:50, 1:100, and 1:500, respectively). The antibody response to p24 in the two groups of mothers did not differ.

Serum dilutions	n*	% transmitting	
V3Cons			
≤1:100	15	26·7	
1:500	11	9·1	
1:1000	13	23·1	
1:2000	31	61·3	p (trend) = 0·005
TMSP18			
≤1:10	9	11·1	
1:50	5	20·0	
1:100	21	28·6	
1:500	35	54·3	p (trend) = 0·007

*Mothers whose sera gave an endpoint positive reaction at the dilution indicated. Total = 70.

Table 1: **Risk of perinatal transmission according to mother's V3Cons or TMSP18 reactivity**

Maternal risk factor	Regression coefficient	Adjusted OR*	95% CI	p
V3Cons reactivity†	0·63	1·89	1·12–3·19	0·021
TMSP18 reactivity†	0·73	2·08	1·10–4·10	0·038
Symptoms at delivery	1·79	6·00	1·46–24·65	0·015
Regression constant	−5·22			

*Odds ratio for each unit increase in level of antibody titre. †Antibody titres were recoded into an ordinal variable to be used in the logistic regression model taking values 1 to 4 for both V3Cons and TMSP18.

Table 2: **Multivariate logistic regression model of predictors of perinatal transmission**

Table 1 shows a dose-response relation between antibody titres to V3Cons and TMSP18 and observed risk of transmission. There was again no significant difference for p24 (data not shown).

Of the 70 mothers, 1 had AIDS and 15 (23%) had symptoms of HIV-1 infection during pregnancy. The health of symptomatic women continued to deteriorate during 18-month follow-up, and 15 mothers who were well during pregnancy became symptomatic. Symptomatic women transmitted the virus two times more often than their symptom-free counterparts (95% CI: 1·1–3·4): 31·5% of 54 women without symptoms transmitted compared with 62·5% of 16 women with symptoms. Antibody titres to V3Cons and TMSP18 were only moderately correlated ($r=0·26$) and neither was associated with clinical symptoms during pregnancy. Antibody titres to gp41 were, however, higher in women who became symptomatic after delivery compared with women who remained symptom-free.

Table 2 shows logistic regression analysis with risk of perinatal transmission as the outcome. For each unit increase in antibody titre, the odds of transmission increased by a factor of 1·89 for V3Cons and 2·08 for TMSP18. The odds of transmission for women with symptoms was sixfold greater than for women without. There was no significant interaction between antibody titres to V3Cons and TMSP18 in the model ($p=0·84$). Because the proportion of women who transmitted is large, these adjusted odds ratios are not a good approximation of relative risks. However, using women's individual data in the logistic regression model allowed determination of their predicted risk of transmission. In extreme cases, for example, the model predicted that symptom-free women whose antibody titre to V3Cons was below 1:500 and to TMSP18 below 1:50 had a risk of 0·02 of transmitting HIV-1 to their infant. Conversely, the predicted risk was 0·88 for symptomatic women whose antibody titre to V3Cons was 1:2000 and to TMSP18 was 1:500.

Maternal risk factor	Regression coefficient	Adjusted OR*	95% CI	p
Serological†				
V3Cons reactivity‡	0·27	1·31	0·72–2·40	0·374
TMSP18 reactivity‡	1·11	3·04	1·01–9·16	0·049
Symptoms at delivery	1·80	6·03	1·19–30·64	0·031
Regression constant	−6·02			
Clinical§				
V3Cons reactivity‡	1·58	4·84	1·44–16·22	0·012
TMSP18 reactivity‡	0·70	2·02	0·76–5·36	0·154
Symptoms at delivery	2·56	12·96	1·08–154·83	0·043
Regression constant	−9·27			

*Odds ratio for each unit increase in level of antibody titre. †Analysis restricted to mothers of serologically diagnosed infants (n = 15). ‡Antibody titres were recoded into an ordinal variable to be used in the logistic regression model taking values 1 to 4 for both V3Cons and TMSP18. §Analysis restricted to mothers of clinically diagnosed infants (n = 12).

Table 3: **Multivariate logistic regression model of predictors of perinatal transmission**

Two additional logistic regression analyses were done to determine if the relation between maternal characteristics and risk of transmission varied depending on the diagnostic method used to assess infant HIV-1 status. One analysis included only transmitting mothers with serologically diagnosed infants, and the other only mothers with clinically diagnosed infants. The odds ratios given by these sub-analyses were similar, although their confidence intervals were larger than in the main analysis (table 3).

Discussion

We found that maternal antibody titres to V3Cons and to the immunodominant domain of gp41 had a positive dose-response relations to the risk of perinatal transmission and were independent of clinical symptoms as predictors of perinatal transmission.

Raised antibody titres to V3Cons and TMSP18 could represent an increased response to all viral antigens. However, the weak correlation between titres to V3Cons and TMSP18, along with the lack of association between transmission and antibody response to p24 (figure 3) or to the whole virus (HIV-1 ELISA; data not shown), indicates that these antibody responses are specific and do not reflect an overall heightened immune response.

Potential sources of bias that could affect the validity of our results include the inability to assess the HIV-1 status of some of the infants, that the mothers included may not be representative of HIV-1-infected women in the population, or that limitations in the clinical definition of HIV-1-related death may have led to misclassification of some infants. Selection bias in the study sample is unlikely because of 118 infants only 9 were lost to follow-up, and 10 who died did not meet the clinical criteria for HIV-1 infection. Women whose infants could not be classified were as likely to be symptomatic as those whose infants could be classified. In addition, there is no reason to consider that the relation between maternal-antibody response and infant HIV-1 outcome would have been different in women whose infants could not be classified.

Nevertheless, the stringent criteria for HIV-1-related deaths tend to cause uninfected infants dying from causes unrelated to HIV-1 to be classified more often as indeterminate than uninfected, thereby possibly generating a bias towards HIV-1 infection. However, assuming that we had included indeterminates in our sampling, we would have included 6 or 7 in our study sample, of whom a maximum of 2 or 3 would have been uninfected. Among infants who died before 15 months, exclusion of all those who did not meet the criteria for HIV-1 infection could have affected the results only if there had been an association between maternal antibody titres and infant death regardless of HIV-1 status. In fact, the analysis shown in table 3, which included all infants who died, showed results similar to the analysis that included only the infants who survived and who were diagnosed serologically. Women included in the study are indeed representative of HIV-1-infected women in the general population.

Virtually all women in Brazzaville receive prenatal care in specialised mother-child clinics and deliver in one of the five maternity hospitals of the city. Our study was conducted in these major facilities, which recruit women of low-socioeconomic and middle-socioeconomic status. Most women agreed to be tested for HIV-1 and to participate. Women whose infant's HIV-1 status could be established were randomly selected for this analysis. A comparison of the women included in the peptide analysis with those not included revealed no differences in socioeconomic status or health, particularly the presence of HIV-1-related symptoms. Moreover, the percentage of transmitting mothers in this study (38%) is within the range generally reported in Africa.[3,12]

The likelihood of misclassification of infants is also low. 12 of the 27 infected infants died before a definitive serological diagnosis could be made. Unfortunately, virus isolation or polymerase chain reaction (PCR), which can be used in developed countries for early diagnosis of HIV-1 infection in infants, was not available. Even if PCR or culture had been available, not all of the infants who died would have been classifiable since the sensitivity of these powerful techniques remains low during an infant's first weeks of life.[13] At the EEC/WHO consensus workshop,[9] stringent clinical criteria were defined for HIV-1-related death in infants born to HIV-1-positive mothers to minimise the possibility of false positives. We used this consensus definition to assess the HIV-1 status of the children in our cohort.[14] Moreover, as mentioned earlier, when transmitting mothers were separately analysed based on the clinical or serological diagnosis of their infant, the subanalyses provided similar results, so it is unlikely that misclassification would have affected our results.

Up until now, perinatal transmission of HIV-1 has been associated with clinical maternal deterioration, increased viral replication, and genotypic/phenotypic virus changes.[5,15] We tested antibodies to V3 because this immunogenic region is critical for HIV-1/host interaction since it elicits virus-strain-specific neutralising antibodies and determines virus phenotype.[10,16] Reports have examined the possible association between antibodies to gp120 and perinatal transmission,[17-22] with confusing results. Although studies have described an association between the presence of maternal antibodies and a decrease in perinatal transmission, they cannot be directly compared, since they used neither the same antigens nor the same viral strains.[17-19] Later reports have failed to confirm these findings.[21-22] The individual strain-related variation observed with neutralising assays is poorly understood, and its relation to strain-specific variation, as observed in binding assays, is unclear. Nevertheless, it is still perplexing that in most reports, investigators found either no correlation between antibodies and perinatal transmission or a negative correlation, whereas we found a positive correlation with both presence and titre of selected envelope antibodies and transmission. Our study included a larger series of transmitting mothers (27) than earlier studies, and used a consensus sequence designed to emphasise cross-reactivities within the V3 region.[10] Although neutralising antibodies have been postulated to be negatively correlated with transmission, our binding assays using the V3Cons antigen did not select for such neutralising antibodies. Significantly high neutralising responses directed to V3 are usually type-specific or variant specific. The mutation rate of the V3 region is associated with a wide range of antigen diversification. The associated broadening of antibody response to V3 may be more amenable to detection when a highly cross-reactive peptide such as V3Cons is used as an antigen source.

Mutations in V3 can modify viral growth pattern, infectivity, cytopathic effects, and cell tropism concurrently.[16-26] Transmission to the fetus may relate in part to virus strain, because isolates from infants infected perinatally have been shown to consist of a distinct subset of the maternal virus population.[27,28] The probability that a

transmissible virus will emerge presumably increases with time and rate of viral turnover. Women displaying the broadest antibody response to V3 may thus be experiencing the greatest viral turnover,[29] and may be more at risk of transmitting the virus to their infants. Antibodies to TMSP18, although not increased in symptomatic pregnant women, were higher in those who developed symptoms during the postpartum period, possibly reflecting an increased virus load (86% of women who became symptomatic during the postpartum period had titres above 1:500 compared with 35% among others; $p = 0{\cdot}001$). Furthermore, some antibodies to this region of gp41 have been shown to facilitate virus infection in vitro.[30]

The association we have observed may give new insight to our understanding of perinatal transmission, and may also provide a means for identifying which HIV-1-infected women might be most at risk of transmitting the virus to their infants. Other studies will be necessary to determine our results' application in developed countries, where HIV-1-infected women who carry their pregnancies to term are less likely to be symptomatic and are discouraged from breast-feeding. Drug and immunotherapy trials are now being initiated. Classification of mothers based on serological and clinical risk characteristics will not only help to elucidate the mechanisms of perinatal transmission but will also enhance efforts to prevent it.

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