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Evidence for Efficient Vertical Transfer of Maternal HIV-1 Envelope-Specific Neutralizing Antibodies but No Association of Such Antibodies with Reduced Infant Infection

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Abstract

Little is known about the efficiency of vertical transfer of HIV-1-specific antibodies. We compared antibody levels in plasma from 60 mother-infant pairs near the time of birth, including 14 breastfeeding transmission pairs. The Envelope binding titers were strongly correlated ($r=0.91$, $p<0.0001$) and similar (1.4-fold greater in maternal plasma) between a mother and her corresponding infant as were the neutralizing antibody (Nab) levels ($r=0.80$, $p<0.0001$; 1.3-fold higher), suggesting efficient transfer. There was no significant difference in Nab responses between transmitting and non-transmitting mothers, although there was a trend for transmitting mothers to have higher HIV-1-specific Nabs.

Keywords

Mother-to-child transfer; HIV-1 envelope; Neutralizing antibodies

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Introduction

Without any interventions to reduce transmission, ~1/3 of infants born to HIV-infected mothers will become infected. Approximately half of these infections are due to breastfeeding; the remainder occur *in utero* or during delivery.¹ The role of neutralizing antibodies (Nabs) in the setting of mother-to-child transmission (MTCT) has been extensively studied to determine if HIV-specific antibodies present at the time of exposure can protect against HIV infection. Several early studies, each relatively small, showed that non-transmitting mothers (NTM) tended to have more frequently detected and/or higher Nab responses than transmitting mothers (TM), suggesting a role for Nabs in reducing MTCT.²⁻⁴ However, additional studies reported discordant results regarding whether Nabs are important during all stages of MTCT, if at all.^{2,5,6} The association between Nabs and protection has also been somewhat dependent on the virus tested.⁵ No association was observed between infant infection risk and the breadth and potency of passively acquired antibodies in infants exposed to HIV-1 during breastfeeding.⁷ It is unclear if the results from this study in infants indicate there is simply poor vertical transfer of HIV-1-specific Nabs or that the breadth and potency of maternal Nabs are not a factor in protection against breastmilk HIV-1 transmission.

Placentally acquired antibodies reach their highest level in the infant during the third trimester. Studies in nonhuman primates have demonstrated passive transfer of HIV-1 specific Nabs^{8,9}, but little is known about the transfer of maternal HIV-1-specific Nabs in humans. One study of 35 mother/infants pairs that assessed Nabs against two lab-adapted HIV-1 strains showed a strong correlation between maternal and infants Nabs.¹⁰ However, responses to more relevant circulating variants of HIV-1 were not examined. Another study that explored the relationship between maternal and infant antibodies showed variation in antibody transfer based on antigen specificities and IgG subclasses, indicating transfer may vary by antibody type.¹¹ Thus, these studies suggest that mother-to-child transfer of HIV-1-specific Nabs may be affected by the nature of the Nabs, including antibody specificity. To address this hypothesis, we measured the levels of HIV-specific antibodies including Nabs, against three circulating HIV-1 variants that represent the dominant HIV-1 subtypes in the population under study to determine if the levels in the infant were similar to the mother, suggesting efficient transfer.

Materials and methods

Samples

Plasmas tested in the current study were from mother/baby pairs who participated in the breastfeeding arm of a randomized clinical trial done in Nairobi, Kenya.¹ Plasmas from the mothers were obtained around the time of delivery and the infant plasmas were obtained within the first week after birth, a time when passively transferred antibody levels should be high. Both women and infants were antiretroviral therapy naïve and mother/infant pairs were included in this study based on these criteria: 1) the mother breastfed, 2) the mother had a plasma sample available around delivery, 3) the infant was HIV DNA PCR negative at birth, 4) the infant was either detected as HIV positive after birth or was followed for at least 3 months if HIV negative, and 5) the infant had a plasma sample within the first week after birth.⁷ Sixty mother/infant pairs met these criteria and were included in the study. The ethical review committees of the University of Nairobi, University of Washington and Fred Hutchinson Cancer Research Center approved this study and all subjects provided written consent.

Detection of HIV-1 Envelope-specific IgG

HIV-1 Envelope-specific IgG titers were estimated using an enzyme-linked immunosorbent assay (ELISA) performed as previously outlined using a HIV subtype A gp120 protein (BL035; Immune Technology Corp., New York, NY).¹² Plasmas were diluted starting at 1:4000 followed by 2-fold serial dilutions up to a maximum dilution of 1:512000. In cases where the end point titer (EPT) was outside the range of these dilutions, additional 2-fold dilutions were made either starting with a minimum dilution of 1:1000 or diluting to maximum of 1:2048000. The EPT was defined as the reciprocal plasma dilution at which the average optical density (OD) value was greater than or equal to twice the average OD value of the background. For the purposes of analyses, in cases in which the EPT was less than the lowest dilution tested, the midpoint value (500) between the lowest dilution and zero was assigned. The EPT results represent the average of two independent duplicate assays.

Viruses & Neutralization Assay

Three HIV-1 envelopes representing two subtype A (Q461.d1 & Q842.d16) and one subtype D (QD435.A4) variants, were used to generate pseudoviruses as described.⁷ These envelopes were initially cloned from 3 viruses isolated early in infection, directly from blood of 3 Kenyan women. Neutralization assays were performed by infecting TZM-bl cells with the pseudoviruses, as described elsewhere.⁷ The median inhibitory concentration (IC50), which represented the reciprocal plasma dilution at which 50% of infection of the target cells was inhibited, was calculated as described elsewhere.¹³ The IC50 results represent the average of two independent duplicate neutralization assays.

Results

To investigate the association between maternal HIV-1-specific antibody levels and infant passive antibodies, we examined antibody levels in 60 mother-infant pairs from the Nairobi Breastfeeding Trial.¹ All of the infants were HIV-1 DNA negative at birth. Fourteen (23%) infants were detected HIV-1 positive after birth; one (7%) prior to 6 weeks (W6) of life, six (43%) at approximately W6 and the remaining seven (50%) after W6 of life. In the case of infants first positive at W6 of life, we can't differentiate peripartum from postpartum transmission for any individual case because we don't have a sample from 2 weeks of age for testing, which is the optimal method for differentiating these two routes of transmission. However, most were likely due to postpartum transmission because prior studies of this cohort comparing breast versus formula fed groups indicate that the majority of those detected as HIV-1 DNA positive by W6 were infected through breastfeeding.¹ Thus, the majority of the 14 infants acquired HIV-1 as a result of breastmilk transmission. The rest of the infants (N=46) remained uninfected during the follow-up period (mean = 21 months).

In order to assess the presence and correlation of HIV-1-specific antibodies among the mother/baby pairs, we compared the log₂ transformed EPT values within and between the mothers and corresponding infants. Because subtype A is the predominant subtype in the entire Nairobi cohort¹⁴, as well as among the 55 mothers included in this study for whom we had subtype data (66% A, 25% D, 7% C, 2% C/D recombinant), we measured maternal and infant HIV-1-specific total IgG titers against soluble subtype A gp120 (BL035) Envelope protein. This protein was isolated from early in infection of an infant from this cohort.¹³ We found that maternal and infant EPTs were correlated ($r=0.91$, $p<0.0001$, Fig.1 A) with mothers having 1.4 fold-higher EPTs (95% CI: 1.1,1.6; $p=0.001$), suggesting transfer of HIV-1 specific antibodies to levels similar to those found in the mother. Although not statistically significant, mothers and infants with subtype A viruses had higher EPTs against the subtype A protein than those with non-subtype A viruses (not shown).

We examined maternal HIV-1-specific Nab responses against a panel of three viruses including two subtype A (Q461.d1 & QD435.A4) and one subtype D (Q842.d16). The corresponding infant Nab responses had been determined as part of a prior study.⁷ The viruses tested here were among the 8 used in the prior study and included a Tier 1b (Q461.d1) and two Tier 2 (QD435.A4 & Q842.d16) viruses.⁷ The majority of maternal plasmas neutralized all three viruses: 54 (90%) neutralized QD435.A4 at greater than 50%, 52 (87%) showed activity against Q461.d1 and 51 (85%) neutralized Q842.d16. Similar levels of neutralization were seen when stratifying by subtype and there was no difference in neutralizing activity against any of the viruses for mothers with subtype A virus compared to those with non-subtype A virus (not shown).

Next, we assessed the correlation between the capacity of the mothers' and infants' plasma to neutralize the three viruses by comparing the neutralization $\log_2$ IC50s of mothers defined in this study to $\log_2$ IC50 of infants defined in the prior study⁷ using Pearson's correlation coefficient (r) and t-tests (expressed as fold-differences). We found that, on average, mothers had 1.3-fold (95% CI: 1.1, 1.5; $p=0.0096$) greater IC50 values across all viruses as compared to their infants. There was a strong linear correlation for $\log_2$ IC50s between mothers and their corresponding infants when the average IC50s of the three viruses tested were considered ($r = 0.80$, $p<0.0001$, Fig.1B). The magnitude of this correlation varied for each virus with the highest correlation between maternal and infant IC50s observed for the most neutralization sensitive variant Q461.d1 ($r= 0.81$) and the lowest correlation in $\log_2$ IC50 values was for QD435.A4 ($r= 0.48$) (Fig.1 C-E). EPTs were positively correlated with IC50s for both mothers and for infants ($r=0.59$ and 0.70 , respectively, Fig.1F). This correlation implies a relationship between the two measures, but not that all of the Nabs are directed to gp120.

The average IC50 values across the three test viruses were 1.74 times greater (95% CI: 1.06, 2.83; $p=0.030$) for TM compared to NTM (Fig.2). Correspondingly, there was a positive association between IC50 and risk of infant infection (OR=1.90 given a 2-fold greater maternal IC50, 95% CI: 1.03, 3.47; $p=0.039$). However, when controlling for $\log_{10}$ plasma viral load in linear and logistic regression models, these associations were not statistically significant (fold-difference in IC50 = 1.60, 95% CI: 0.91, 2.83, $p=0.10$; OR=1.86, 95% CI: 0.88, 3.9; $p=0.10$, respectively). This was true when we examined the average IC50 for the three viruses (Fig. 2) or the IC50 for each individual virus in relation to infant outcome (data not shown). Similarly, no association was observed when we analyzed the infant plasma Nab average IC50 values across the three viruses tested and transmission outcome for the 60 infants considered in this study (data not shown). To assess the effect of Nabs in peripartum versus postpartum transmission, we analyzed the association between IC50 and risk of infection for these two groups independently. To be conservative, we classified any infection detected within or at six weeks of age as peripartum ($N=7$) and any detected after six weeks as postpartum ($N=7$) for these analyses. We observed similar results in this subset analysis as in the entire group of 60 pairs: there were higher IC50s among TM than NTM in both groups, but these associations were not significant when controlling for maternal viral load (not shown).

Discussion

Our findings demonstrate efficient transfer of HIV-specific antibodies from mother-to-infant. Infants had only 1.4- and 1.3-fold lower HIV-1 Envelope-specific binding and Nab levels, respectively, during the first week of life compared to the levels of these antibodies in their mothers near the time of delivery. This implies that protective Nabs, if present in an HIV-infected mother, could provide infant prophylaxis, particularly late in gestation and during the early breastfeeding period, when the risk of infant infection is high¹.

The maternal Nab response against 3 heterologous HIV-1 variants was not associated with infant infection outcome after controlling for viral load, which is positively correlated with cross-reactive Nab responses and risk of transmission (reviewed in ¹⁵). However, TM showed a trend for having higher Nab levels than NTM in this analysis, a finding that is contrary to a protective role for maternal Nab in breastmilk HIV transmission. Similarly, infant Nab levels were not associated with infant infection risk, consistent with results of a prior study demonstrating a lack of association between infant infection and the breadth and potency of infant vertically acquired Nabs.⁷ These data reinforce the notion that the Nab response elicited in a typical HIV-1 infection may not be adequate to provide significant protection from MTCT. However, this finding does not preclude the possibility that exquisitely broad and potent Nabs, such as those reported in a small subset of chronically infected individuals (reviewed in ¹⁵) could provide some protection. In this context, the results presented here do suggest that in studies designed to test the potential of broad and potent antibodies to protect against MTCT, the therapeutic application of antibodies to the mothers could result in efficient transfer to the infant.

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Fig.1.

Comparison of maternal and infant HIV-1 Envelope-specific antibodies. For panels A-E, maternal and infant antibody levels are shown on the x- and y-axis, respectively. Each dot represents the data from one mother-infant pair. The line represents regression best fit line and the r is Pearson's product moment correlation coefficient. The p -values shown above each graph are based on the Student's t approximation for the distribution of the corresponding standardized test statistic. The panels include: A) Envelope binding EPTs; B) average $\log_2\text{IC}_{50}$ for the 3 viruses; C) Q461.d1 $\log_2\text{IC}_{50}$; D) QD435.A4 $\log_2\text{IC}_{50}$; E) QD842.d16 $\log_2\text{IC}_{50}$, and F) the association between EPTs and Nab average $\log_2\text{IC}_{50}$ values for both mothers (● —) and infants (◆ ---).

Ratio $IC_{50_{TM}}/IC_{50_{NTM}} = 1.74; p=0.03$
 Adj. ratio $IC_{50_{TM}}/IC_{50_{NTM}} = 1.60; p=0.10$

Fig. 2.

Comparison of the average $\log_2 IC_{50}$ values of the 46 non-transmitting (NTM) and 14 transmitting mothers (TM). The dot plots summarize the distribution of the average $\log_2 IC_{50}$ for the 3 viruses, with mean and 95% confidence intervals for the $\log_2 IC_{50}$ indicated by the horizontal bars for each. The unadjusted estimate for the fold-difference in IC_{50} and corresponding p-value are based on Welch's t-test, and the adjusted fold-difference estimate is by linear regression controlling for $\log_{10}$ viral load, with the corresponding Wald p-value.